



VKM Report 2022: 17

Benefit and risk assessment of fish in the Norwegian diet

Scientific Opinion of the Steering Committee of the Norwegian Scientific Committee for Food and Environment

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Preparation of the opinion

The Norwegian Scientific Committee for Food and Environment (Vitenskapskomiteen for mat og miljø, VKM) appointed a project group to draft the opinion. The project group consisted of six VKM members, five VKM staff and eight external experts. Three referees commented on and reviewed the draft opinion. The Scientific Steering Committee assessed and approved the final opinion.

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The authors have contributed to the opinion in a way that fulfils the authorship principles of VKM (VKM, 2019). The principles reflect the collaborative nature of the work, and the authors have contributed as members of the project group and/or the Scientific Steering Committee

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Competence of VKM experts

Persons working for VKM, either as appointed members of the Committee or as external experts, do this by virtue of their scientific expertise, not as representatives for their employers or third-party interests. The Civil Services Act instructions on legal competence apply for all work prepared by VKM.

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Summary

The assignment and premises

In 2006, VKM published "A comprehensive assessment of fish and other seafood in the Norwegian diet". The assessment supported the recommendation from Norwegian Health authorities to eat more fish both for dinner and on sandwiches. In an update of the assessment in 2014 VKM concluded that "the benefits from fish consumption clearly outweigh the negligible risk presented by current levels of contaminants and other undesirable substances in fish". Because of new knowledge available, the Norwegian Food Safety Authority requested a new benefit and risk assessment of fish in the Norwegian diet in 2019. In the assignment, they asked VKM to answer the following questions:

Which health consequences will it have for the Norwegian population if they:

- Continue with the same fish consumption levels as of today
- Increase the consumption of fish to match the recommendations given by the Norwegian Directorate of Health

The recommendation for fish consumption is to eat fish for dinner two to three times a week, and also use fish as bread spread. This amounts to 300-450 grams of prepared fish a week for adults, and at least 200 grams should be fatty fish like salmon, trout, mackerel or herring.

There has been a change in the premises for this benefit and risk assessment of fish as compared to the previous assessments; the tolerably weekly intakes for the contaminants PCDD/Fs and DL-PCBs (polychlorinated biphenyls), and PFASs (perfluorinated alkylated substances) were markedly reduced in 2018 and 2020, respectively. Based on exposure assessments from European Food Safety Authority (EFSA), exceedance of the tolerable weekly intakes of these contaminants in the Norwegian population was expected. It was therefore anticipated that a benefit and risk assessment of fish would be an even more complex task than previous assessments, and that high confidence in the evidence would be critical. Applying as systematic, transparent, and intelligible process, based on established guidelines, to ensure high quality and confidence in the results, has been important.

A protocol for this benefit and risk assessment was published in February 2020 after a public consultation, and the work has been conducted according to the protocol with a few very minor deviations (see Appendix IV, Chapter 18).

A comprehensive systematic literature review and weight of evidence analyses

This benefit and risk assessment of fish is based on an extensive systematic literature review to evaluate the epidemiological evidence for associations between fish consumption and health outcomes. The review covers both primary studies and previous systematic reviews and meta-analyses. The health outcomes included are non-communicable diseases or conditions common in the Norwegian population for which fish, or compounds in fish (nutrients or contaminants) have an established or hypothesized role. The literature review includes CVD, CHD, myocardial infarction, stroke, heart failure, heart fibrillation, venous thrombosis, neurodevelopment in children, mental disorders in children (e.g., ASD and ADHD), cognition and cognitive decline in adults (including Alzheimer's and dementia), depression in adults, type 2 diabetes, weight/overweight in children and adults, bone health, birth outcomes such as preterm birth, small for gestational age, low birth weight, birth weight (continuous), birth length and head circumference (continuous), asthma and allergy (especially in children), multiple sclerosis, rheumatoid arthritis, cancer (only from the report from the World Cancer Research Fund (WCRF) from 2018, which VKM found to be sufficiently comprehensive), vaccine response, and semen quality/male fertility.

For several of these outcomes, there were too few studies to draw a conclusion, or the studies did not fulfill VKM's criteria for inclusion or high quality. A summary of all the health outcomes for which there were sufficient evidence to draw a conclusion, is given in Table 10.1-1 in the assessment (Chapter 10). For health outcomes that are also common causes of death, the epidemiological evidence has been summarised separately for studies of incidence (the risk of developing disease) and mortality.

For the systematic literature review, searches were performed in relevant databases, and primary studies were selected based on predefined inclusion and exclusion criteria in pairwise blinded selections. The included primary studies were then quality assessed before data extraction and calculation of pooled estimates. The pooled estimates were compared to previous meta-analyses when available, and finally a weight of evidence process based on the criteria defined by the WCRF was performed. The weighting was based on the following factors: the results from published evidence of fish intake and health outcome, heterogeneity between studies, evidence for biological plausibility, and dose-response relationship. The evidence categories used by WCRF are: "convincing", "probable", "limited, suggestive", and "limited, no conclusion" or "substantial effect on risk unlikely", see Box 2 in Chapter 3.1.6.5 for details. According to WCRF, "convincing", "probable" and "substantial effect on risk unlikely", evidence is classified as strong evidence. Only evidence judged to be strong is usually used as the basis for recommendations.

In this benefit and risk assessment, no associations are categorized as "convincing". The associations for the following outcomes are categorized as "probable": All-cause mortality, mortality from CVD, CHD, stroke, and myocardial infarction, and incidence of CHD, stroke, dementia and Alzheimer's disease in adults, and preterm birth and low birth weight. Only health outcomes where the evidence for an association between fish intake and the outcome

was judged to be “probable”, are included in the quantitative assessment as described below.

For the subgroups fatty fish and lean fish, our systematic literature review did not conclude with a strong association between intake and any health outcome, and consequently our quantitative benefit and risk assessment of fish consumption is based on total fish, and not subgroups of fish. All weight of evidence conclusions for total fish, fatty fish, lean fish, and nutrients and contaminants are given in Table 10.2.3-1.

This benefit and risk assessment consists of a quantitative assessment of benefits and risks from fish consumption, a semi-quantitative benefit assessment of nutrients in fish, and a semi-quantitative risk assessment of contaminants in fish.

Quantitative benefit and risk assessment of fish intake

The overall aim of the quantitative assessment was to estimate the effect on disease incidence and mortality in the Norwegian population as a result of eating fish in other quantities than the currently consumed amount. The systematic literature review and weight of evidence is the basis for the quantitative benefit and risk assessment of fish intake. There was no strong evidence for an impact of children’s fish consumption on any health outcomes in the children (neurodevelopment, mental health challenges, overweight/obesity, asthma and allergy), and consequently the quantitative analysis only includes adults. Moreover, there was no strong evidence for an adverse effect of fish intake on any of the included health outcomes. Consequently, the quantitative modelling only contains beneficial effects.

A modelling approach is used to estimate how changes in fish intake may affect disease incidence and mortality at the population level. The modelling is based on the current mean fish intake from Norkost 3 (2010-2011), two fish intake-scenarios mirroring the range of the recommendation for fish intake, and an additional scenario with fish intake below the recommended intake of fish. The fish intake scenarios are simple constructed scenarios in which all participants in the food dietary surveys are assigned a fixed daily intake of fish and a fixed amount of fatty and lean fish (see Table 9.1-1). In both scenario 2 and 3, the amount of fatty fish is kept steady at 200 grams per week, and only the amount of lean fish is increased from scenario 2 to scenario 3.

Dose-response relationships were calculated for fish intake and the health outcomes based on relative risk found in the quality assessed meta-analyses identified in VKM’s systematic literature review. Occurrence of disease in the Norwegian population are publicly available numbers from national health registries when attainable, or based on published studies.

Quantitative estimations were conducted for adult women and men separately for all outcomes, except coronary heart disease (CHD) incidence, where both sexes were combined. Quantitative estimation for preterm birth was naturally only done for women.

For men it was estimated that a decrease in the current mean intake at 350 grams per week to 300 grams per week would increase the annual numbers of incident cases or deaths for the outcomes included, except for Alzheimer's disease and CVD mortality. An increase from the current mean fish intake to 450 grams per week, would decrease the annual numbers of incident cases or deaths for all outcomes except for CVD mortality. The decrease was most prominent for incidence of CHD, stroke, and dementia (see Table 9.2.6-1 in Chapter 9).

For women it was estimated that an increase from the current mean intake at 238 g per week to 300 g per week, would give a small decrease in annual numbers of incident cases or deaths for all outcomes. The decrease was largest for dementia and preterm birth. When changing from current fish intake to 450 grams per week, a decrease in CHD incidence was estimated, in addition to dementia, stroke and preterm birth (see Table 9.2.6-2 in Chapter 9).

Overall, the results for men and women suggest that increasing intake of fish towards the upper range of recommended intake will reduce number of cases of stroke and CHD, non-communicable diseases that are important contributors to the burden of disease in Norway. Likewise, increasing intake of fish towards recommended intake is estimated to reduce the number of new cases of dementia and Alzheimer's disease, both cognitive disorders which are increasing in the population as the proportion of elderly is increasing. The modelling indicated that an increase in fish intake from the current level to the recommended level would only have a very small impact on CVD and CHD mortality in both men and women. This is due to the dose-response curve used for these outcomes in the quantitative analysis, which was flat for fish intake higher than 300 grams per week.

Quantitative estimation for preterm birth (women only) was included in the modelling, but not low birth weight, as the underlying cause of low birth weight appeared to be preterm birth in studies of maternal fish intake during pregnancy.

The evidence is also graded "probable" for stroke mortality, and myocardial infarction mortality but no dose-response meta-analysis was found that included studies of stroke- or myocardial infarction mortality only. These outcomes could therefore not be included in the quantitative modelling separately.

Semi-quantitative assessment of nutrient intake and contaminant exposure from fish

The quantitative modelling of benefits and risks from fish consumption does not include all critical health outcomes relevant for the contaminants due to limited available data. Also, the quantitative modelling approach with incidence and mortality as common metrics could not be applied to contaminants and nutrients relevant for fish consumption due to limitations in available models for these compounds. The evaluation of all nutrients and contaminants relevant for fish intake is therefore performed using a semi-quantitative approach. In the semi-quantitative assessment, the term "current intake" refers to intake reported in the

national dietary surveys Norkost 3 (2010-2011), Ungkost 3 (2015-2016, Småbarnskost 3 and Spekost 3 (2019).

Nutrients

Fish is an important source in the diet for LC n-3 fatty acids (eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA)), vitamin D, iodine, selenium, and vitamin B₁₂. More than 20% of the total intake of these nutrients are derived from fish in the Norwegian diet. Especially for LC n-3 FA, vitamin D and iodine there are very few natural sources in the diet but fish.

For nutrients in fish and potential associated health outcomes, VKM conducted a second comprehensive systematic literature review. Except for including only systematic reviews and meta-analyses, not primary studies, the review process, and the weighting of evidence criteria were similar to those for fish intake and health outcomes. A wide range of combinations of nutrients and health outcomes were evaluated, mostly the same as for fish with exception of the immune related diseases.

In summary, a strong association ("probable") was found for LC n-3 FA and CVD mortality, CHD mortality, CVD incidence (only for doses >1 g LC n-3 FA per day), CHD incidence, myocardial infarction incidence and birth weight (continuous). The results from the weight of evidence analyses for LC n-3 FA and health outcomes support the conclusions from the weight of evidence analyses of fish and the same health outcomes. For vitamin D a strong association ("probable") was found for all-cause mortality and bone fracture/fall (based on NNR, 2012). No strong associations were found for neither iodine, selenium nor vitamin B₁₂.

For the semi-quantitative benefit assessment of nutrients in fish, VKM used average requirements (AR) which are based on established knowledge of an association between iodine and goiter, selenium and Keshan disease, and vitamin B₁₂ and pernicious anemia. For vitamin D the critical endpoint for setting an AR is bone health (fractures). No AR is available for the LC n-3 FAs. However, we have compared the intakes of EPA plus DHA with an adequate intake based on cardiovascular health set by EFSA (2010). For LC n-3 FAs, vitamin D, iodine, selenium, and vitamin B₁₂ VKM have performed a semi-quantitative benefit assessments based on percentages in the population with intakes below the AR.

The semi-quantitative assessment of the LC n-3 FAs EPA plus DHA shows that at current fish intake levels, 18% of women of childbearing age (18-45 years) and 10% of adult men and women (18-70 years) have intakes below the adequate intake. In the fish scenarios, in which all participants in the food dietary surveys are assigned a fixed daily intake of fish, all adults have estimated intakes of EPA plus DHA above the adequate intake.

At current fish intake levels, all included age groups have a relative high proportion of individuals with an intake of vitamin D below the AR. The scenario estimations indicate that increasing intake of fish from the current intake to the recommended intake would lead to a moderate increase in vitamin D intake at the population level but may be of special

importance for those with a very low dietary intake of vitamin D, where even a small increase may be of substantial importance (from e.g., 67% of 13-year-old girls with intakes below AR with current fish intake reduced to 50% in fish scenario 3, and for women of childbearing age, the intake in the 5th percentile increases from 2.3 µg/day with current fish intake to 5.4 µg/day in fish scenario 3).

For iodine, groups at highest risk of low intakes are young girls and women of childbearing age, and at current intake 34% of 13-year-old girls and 19% of women of childbearing age had an intake below the AR. Increasing the fish intake to the upper range of the recommended intake would cause all age groups and genders to have iodine intakes above the AR except for 1-year-olds.

For selenium, young girls and women of childbearing age have the lowest intakes, and at current intake 7% of women of childbearing age and 71% of 9-year-old girls have an intake below AR. Increasing intake of fish to the recommended intake would reduce the proportion having a probability of inadequate selenium intake to null for most age groups in both genders. With current fish consumption no specific age groups are at risk of having vitamin B₁₂ intake below the AR.

Contaminants

Fish may also contain a variety of contaminants. Due to markedly reduced tolerable weekly intakes (TWIs) for PCDD/F and DL-PCBs, and PFASs, VKM was especially requested to include these contaminants. Additionally, VKM evaluated an extensive list of possibly relevant substances for inclusion in this assessment. After a stepwise selection process, focusing on “concern in relation to fish intake” and “exposure close to existing health-based guidance values” methyl mercury is also included.

For contaminants in fish, VKM’s evaluation of adverse health outcomes is based on the hazard characterization and the corresponding TWIs published by EFSA (EFSA 2012; 2018; 2020). For methyl mercury, many new publications have assessed the association between exposure and different endpoints since EFSA’s risk assessment of in 2012. However, based on results from a review of systematic reviews for methyl mercury, VKM decided to use the TWI from 2012 without any updating.

VKM have performed semi-quantitative risk assessments of PCDD/F and DL-PCB, PFASs, and methyl mercury based on percentages in the population with exposures above the TWIs set by EFSA. The critical endpoint for PCDD/Fs and DL-PCBs is reduced sperm concentration after prenatal exposure during pregnancy, and postnatal exposure via breastfeeding and other food intake during childhood. For PFASs the present assessment is restricted to the same four PFASs that are covered by the TWI: PFOA, PFNA, PFHxS and PFOS. The critical effect for the sum of these four PFASs is effects on the immune system, measured as a decreased vaccination response in children after pre- and postnatal exposure. Various associations between serum levels of PFAS and several outcomes was evaluated by EFSA, but given that the effects on the immune system occur at lower exposure, other effects are

not considered in the present assessment. The critical effect for methyl mercury is neurodevelopmental effects in prenatally exposed children.

The semi-quantitative risk assessment of contaminants shows that at the current level of fish intake, a high proportion (96 – 100%) of the Norwegian population exceed the TWI of 2 pg TEQ/kg bw/week set for PCDD/Fs and DL-PCBs. The adult population have a mean estimated exposure that is 2.3-times the TWI at current level of fish intake. Fish contribute 39% to PCDD/F and DL-PCB exposure in the adult population, of which lean species (< 5% fat) contribute 6%, fatty species (> 5% fat) 28% and liver and roe 5% to the total PCDD/F and DL-PCB exposure. If the fish intake is increased to recommended intake, the mean exposure to PCDD/Fs and DL-PCBs is increased for all age groups. VKM applied exposure assessments for PCDD/F and DL-PCBs that are conservative (probable overestimates).

The adult population have a mean estimated PFAS exposure that is 1.7-times the TWI at current level of fish intake. The proportion of the population exceeding the TWI in different age groups is 44 to 100%. For all age groups, fish is the main contributor to sum of these four PFASs (about 38% for adults). Lean and fatty fish contribute approximately equally across age groups, with a little higher contribution from lean fish in adults. Increasing the fish intake up to the higher range of recommended intake will cause an increase in the proportion exceeding the TWI, leading to an exceedance for all adults. Children have high estimated exposures both in the current situation and in the calculated scenarios, ranging from 1.5 times the TWI in the current situation for nine-year-olds to 4.8 times the TWI for two-years-old in scenario 3. Exposure estimates for PFAS are uncertain due to high level of detection in the analytical methods.

Fish and other seafood is practically spoken the only source of methyl mercury in Norway. With the current fish intake in Norway, only a small proportion of the population was estimated to exceed the TWI for methyl mercury. VKM applied a conservative approach, assuming that all mercury in fish and shellfish is methyl mercury (probable overestimate). With altered fish intake in the scenarios, the estimated mercury intake decreases in general. This is partly because the scenarios are based on the most consumed species, which are low in mercury. Furthermore, the high fish intake scenario represents a decrease in fish consumption for high fish consumers. In summary, the proportion exceeding the TWI for methyl mercury is either zero or very low for all age groups in all three scenarios.

Summary of conclusions and answer to the terms of reference

Sixty-two percent of Norwegian women, and 58% of the men report that they have two or more meals of fish per week. As shown in the quantitative assessment, a reduction in the weekly fish intake from the current mean intake among adult men and women to 150 grams per week, results in an increase in annual numbers of incident cases or deaths estimated for all outcomes included in the quantitative modelling (CVD mortality, CHD mortality, all-cause mortality, incidence of CHD, stroke, dementia, Alzheimer's disease and preterm birth). Overall, this indicates that a low fish consumption is a potential health risk, and that optimal beneficial health effect of fish intake is not obtained at current fish intake levels.

Mathematical modelling indicates that increasing intake of fish to recommended intakes, and especially towards the upper range of recommended intake, 450 grams per week in scenario 3 will reduce the probability of having stroke and CHD, non-communicable diseases that are important contributors to the burden of disease in Norway. Increasing intake of fish towards recommended intake is also estimated to reduce the number of new cases of dementia and Alzheimer's disease, both cognitive disorders which are increasing in the population as the proportion of elderly is increasing. The proportion of the population with an intake below AR for selenium and iodine will also be reduced. The low intake of vitamin D will not necessarily be rectified by increasing fish intake alone, but increasing the fish intake and especially fatty fish intake could be of importance for those with the lowest vitamin D intakes. In conclusion, all age groups would benefit from increasing from current intake to recommended intake of fish.

On the other hand, increasing fish intake towards recommended intake would increase intake of PCDD/Fs and DL-PCBs, and PFASs to a level where almost everyone in all age groups would exceed the TWIs. For adults the increase in exceedance would be moderate, i.e., from 2.3 times the TWI to 2.8 times the TWI in scenario 3 for PCDD/Fs and DL-PCBs, and from 1.7 times the TWI to 1.9 times the TWI in scenario 3 for PFASs. The contribution of the critical effects linked to PCDD/Fs and DL-PCBs, and PFASs exposure (reduced sperm concentration and decreased vaccine response in children, respectively) to the combined death and disability burden has not been estimated. However, male infertility accounts for a minor part of the burden of disease in Norway. A reduced response to vaccination in children is commonly used as a marker of a reduced immune response. But the general applicability of reduced response to vaccination as a marker of reduced immune response, as well as the size and severity of the potential increase in infection risk from a reduced immune response, is not known. Moreover, there are many dietary sources of these contaminants, so even though a reduction of fish intake probably will cause some reduction in the exposure, it may not suffice to get an exposure below the TWIs.

VKM concludes that fish intake is beneficial and protective against several health outcomes that present important public health challenges in Norway. For these outcomes, the evidence is graded "probable" which is considered strong evidence according to the WCRF grading system. The evidence for beneficial effects of fatty fish intake was weaker than for total fish intake. However, the evidence was substantiated by strong evidence ("probable") for beneficial effects of LC n-3 FAs on several of the same health outcomes as for fish.

VKM's conclusion is based on a systematic review and weight of evidence analyses of associations between fish intake, fatty fish intake and health outcomes, and a quantitative assessment of fish intake and health outcomes with incidence rates and mortality as common metrics. Additionally, we have conducted systematic literature reviews for nutrients, and included semi-quantitative assessments of the nutrients LC n-3 FA, vitamin D, iodine, selenium, and vitamin B₁₂ and of the contaminants PCDD/Fs and DL-PCBs, PFASs, and methyl mercury, all substances of which fish intake is an important source.

The outcomes included in the quantitative assessment are generally chronic non-communicable diseases affecting the older age groups (except for preterm birth). However, these diseases may have a long latency period. Also, dietary behaviour tends to track from young age into adulthood. These factors support that recommended fish intake already in young age may be of importance for intake later in life and for later health benefit.

VKM concludes that the benefits from increasing fish intake to the recommended two to three dinner courses per week (corresponding to 300-450 grams, including at least 200 grams fatty fish in adults) outweigh the risks for all age groups.

Keywords: VKM, benefit, risk, RBA, systematic review, weight of evidence, fish, nutrients in fish, omega-3, EPA, DHA, DPA, selenium, iodine, vitamin B₁₂, vitamin D, contaminants in fish, dioxins, PCDD/F, DL-PCB, PFAS, methyl mercury, fish consumption, health effects, coronary heart diseases, CHD, cardiovascular diseases, CVD, mortality, neurodevelopment, cognitive functioning, cognitive decline, bone health, immunology and allergy, fertility, anthropometric outcomes, birth outcomes, diabetes, , Norwegian Scientific Committee for Food and Environment

Sammendrag (Norwegian summary)

Oppdraget og premissene

I 2006 publiserte VKM «En helhetlig vurdering av fisk og annen sjømat i norsk kosthold». Vurderingen støttet anbefalingen fra norske helsemyndigheter om å spise mer fisk, både til middag, og som pålegg. I en oppdatering av vurderingen i 2014 konkluderte VKM med at «fordelene ved fiskekonsum klart oppveier den ubetydelige risikoen som dagens nivåer av kontaminanter og andre uønskede stoffer i fisk gir». I 2019 ønsket Mattilsynet igjen en ny nytte- og risikovurdering av fisk i norsk kosthold, på grunn av ny tilgjengelig kunnskap. I oppdraget ber Mattilsynet VKM om å svare på følgende spørsmål:

Hvilke helsemessige konsekvenser vil det få dersom den norske befolkningen:

- fortsetter med dagens konsum av fisk?
- øker konsumet av fisk opp til det inntaket Helsedirektoratet anbefaler?

Kostrådet fra Helsedirektoratet er å spise fisk til middag to til tre ganger i uken, og også bruke fisk som pålegg. Det utgjør 300 - 450 gram tilberedt fisk i uken for voksne. Minst 200 gram bør være fet fisk som laks, ørret, makrell eller sild.

Premissene for denne nytte- og risikovurderingen av fisk er endret, sammenlignet med tidligere vurderinger. Det tolerable ukentlige inntaket (TWI), også kalt tålegrense på norsk, for stoffgruppene PCDD/F og DL-PCB-er og PFAS-er ble betydelig redusert i henholdsvis 2018 og 2020. På bakgrunn av eksponeringsberegninger fra det europeiske mattrygghetsorganet EFSA, var det forventet at tålegrensen for disse stoffene ville overskrides også i den norske befolkningen. VKM antok derfor at denne nye nytte- og risikovurderingen av fisk ville bli mer kompleks enn tidligere, og at det ville være nødvendig med en systematisk kunnskapsoppsummering av både positive og mulig negative helseeffekter knyttet til det å spise fisk. Det har vært viktig å bruke en systematisk, transparent og tydelig framgangsmåte basert på etablerte retningslinjer for å sikre høy kvalitet og tillit til resultatene.

Det ble utarbeidet en protokoll for nytte- og risikovurderingen som ble sendt på offentlig høring. Protokollen ble publisert i februar 2020. Arbeidet er utført etter protokollen, med noen mindre avvik (se vedlegg IV, kapittel 18).

Omfattende systematiske kunnskapsoppsummeringer og gradering av den samlede evidensen

Denne nytte- og risikovurdering er basert på flere omfattende kunnskapsoppsummeringer, og gradering av den samlede evidensen for sammenhenger mellom fiskekonsum og helseutfall basert på epidemiologiske studier. Gjennomgangen dekker både enkeltstudier,

tidligere systematiske oversiktsartikler og metaanalyser. Helseutfallene som er inkludert er folkesykdommer, eller tilstander som er vanlige i den norske befolkningen, som antas å ha en sammenheng med fisk eller stoffer i fisk (næringsstoffer eller kontaminanter).

Litteraturgjennomgangen omfatter hjerte- og karsykdom, koronar hjertesykdom, hjerteinfarkt, slag, hjertesvikt, hjerteflimmer, venetrombose, nevrologisk utvikling hos barn, mentale lidelser hos barn (som autisme og ADHD), kognisjon og kognitiv svikt hos voksne (inkludert demens og Alzheimers sykdom), depresjon hos voksne, type 2 diabetes, vekt/overvekt og fedme hos barn og voksne, benhelse, fødselsutfall som for tidlig fødsel, liten for gestasjonsalder, lav fødselsvekt, fødselsvekt (kontinuerlig), fødselslengde og hodeomkrets (kontinuerlig), astma og allergi særlig hos barn, multippel sklerose, reumatoid artritt, kreft (kun hentet fra World Cancer Research Fund sine konklusjoner fra 2018, siden VKM vurderte denne til å være dekkende), vaksinerespons og sædkvalitet/mannlig fertilitet.

For mange av disse utfallene var det ikke nok studier til å konkludere, eller studiene oppfylte ikke VKMs kriterier for kvalitet. Oppsummering av alle helseutfallene det var grunnlag for å konkludere på er gitt i tabell 10.1-1 i vurderingen (kapittel 10). Det epidemiologiske evidensgrunnlaget er oppsummert separat for studier av insidens (risikoen for å utvikle sykdom) og dødelighet.

For VKMs systematiske litteraturgjennomganger ble det utført søk i flere relevante databaser. Enkeltstudier og systematiske oversiktsartikler eller metaanalyser ble valgt ut etter forhåndsdefinerte inklusjons- og eksklusjonskriterier. Utvelgelsen ble gjort parvis og blindet. Enkeltstudiene som ble inkludert ble deretter kvalitetsvurdert, før data ble hentet ut og sammenstilt.

VKM har beregnet sammenslåtte effektestimater for relativ risiko for ulike helseutfall for å kunne sammenligne med estimater fra tidligere publiserte metaanalyser der hvor slike var tilgjengelige. Til slutt ble den samlede evidensen gradert basert på kriterier definert av WCRF. Graderingen er basert på følgende faktorer: Resultater fra publiserte studier av fiskeinntak og helseutfall, heterogenitet (variasjon i resultater) mellom studier, evidens for biologisk mekanisme, og for en dose-respons sammenheng. Kategoriene WCRF benytter er: «overbevisende», «sannsynlig», «begrenset, antydende» og «begrenset, ingen konklusjon», eller «vesentlig effekt på risiko usannsynlig», se boks 2 i kapittel 3.1.6.5 for detaljer. Ifølge WCRF er kategoriene "overbevisende", "sannsynlig" og «vesentlig effekt på risiko usannsynlig» klassifisert som sterk evidens. Vanligvis brukes kun evidens som karakteriseres som sterk som grunnlag for kostråd.

I denne nytte- og risikovurderingen er det ingen helseutfall hvor den samlede evidensen for en sammenheng med fiskeinntak er gradert som "overbevisende". Sammenhengen er gradert som «sannsynlig» for at inntak av fisk reduserer total død, død av hjerte- og karsykdom, koronar hjertesykdom, slag og hjerteinfarkt. Sammenhengen er også gradert som «sannsynlig» for at fisk reduserer risiko for å utvikle koronar hjertesykdom, slag, demens og Alzheimers sykdom, samt reduserer risiko for tidlig fødsel og lav fødselsvekt. Kun helseutfall der evidensen for en sammenheng mellom fiskeinntak og utfallet er gradert som «sannsynlig», er inkludert i VKMs kvantitative analyse som er beskrevet under.

Vi fant færre studier av fet fisk og mager fisk enn av total fisk i våre systematiske litteraturgjennomganger, noe som ga et svakere evidensgrunnlag. Den kvantitative nytte- og risikovurdering av fiskekonsum er derfor kun basert på totalt inntak av fisk. Graderingen av evidensen for sammenhenger mellom total fisk, fet fisk, mager fisk, næringsstoffer og kontaminanter, og alle de inkluderte helseutfallene, er gitt i tabell 10.2.3-1.

Denne nytte- og risikovurderingen består av en kvantitativ analyse av nytte og risiko ved fiskekonsum, en semikvantitativ nyttevurdering av næringsstoffer i fisk, og en semikvantitativ risikovurdering av kontaminanter i fisk.

Kvantitativ nytte- og risikovurdering av fiskeinntak

Det overordnede målet med den kvantitative analysen var å beregne effekten på sykdomsforekomst og dødelighet i den norske befolkningen som følge av å endre fiskeinntaket. Beregningene er gjort for helseutfall hvor det var et sterkt evidensgrunnlag for en sammenheng med inntak av fisk. Det ble ikke funnet noe sterkt evidensgrunnlag for at barns fiskeinntak har effekt på helseutfallene hos barna (nevrologisk utvikling, mentale lidelser, overvekt/fedme, astma og allergi), og den kvantitative analysen inkluderer derfor bare voksne. Ettersom det ikke var sterk evidens for negative helseeffekter av økt inntak, er det bare helseutfall med sterk evidens for gunstige effekter av økt inntak som inngår i den kvantitative analysen.

Vi bruker modellering for å beregne hvordan endringer i fiskeinntak kan påvirke forekomst av sykdom og dødelighet på populasjonsnivå. Modelleringen tar utgangspunkt i «dagens inntak» av fisk som refererer til gjennomsnittlig inntak av fisk i den siste nasjonale kostholdsundersøkelsen Norkost 3 (2010-2011). Dagens inntak sammenlignes med to scenarioer som tilsvarende kostrådet om 2-3 middagsporsjoner fisk (scenario 2 tilsvarende 300 gram fisk i uken, og scenario 3 tilsvarende 450 gram fisk i uken), og i tillegg et scenario for inntak av fisk som er lavere enn kostrådet (scenario 1, 150 gram fisk i uken).

Scenarioene for fiskeinntaket er enkle, konstruerte scenarioer der alle deltakerne i kostholdsundersøkelsene tildeles et fast, daglig inntak av fisk, og en fast mengde fet og mager fisk (se tabell 9.1-1). I scenario 2 og 3 er mengden fet fisk 200 gram per uke, slik at det kun er mengden mager fisk som økes fra scenario 2 til scenario 3.

Relativ risiko for ulike helseutfall ved ulike inntaksnivåer av fisk er basert på kvalitetsvurderte metaanalyser av dose-respons sammenhenger fra VKMs systematiske litteraturgjennomgang. Forekomsttall for de ulike helseutfallene i den norske befolkningen er basert på offentlig tilgjengelige tall fra nasjonale helseregistre når dette finnes, eller basert på publisert forskning.

Beregningene ble gjort for voksne kvinner og menn separat for alle utfall, unntatt koronar hjertesykdom, der forekomsttallene var samlet for begge kjønn. Beregninger på for tidlig fødsel inkluderte naturlig nok bare kvinner.

For menn er det anslått at en reduksjon i inntaket av fisk fra dagens inntak på 350 gram per uke til 300 gram per uke, vil øke årlig antall nye tilfeller og/eller dødsfall for de inkluderte helseutfallene, unntatt Alzheimers sykdom og hjerte- og kardød. En økning fra dagens fiskeinntak til 450 gram per uke, vil redusere antall nye tilfeller og/eller dødsfall for alle helseutfall, bortsett fra samlet hjerte- og kardød. Reduksjonene er størst for nye tilfeller av koronar hjertesykdom, demens og slag (se tabell 9.2.6-1 i kapittel 9).

For kvinner er det anslått at en økning fra dagens inntak på 238 gram per uke til 300 gram per uke, ville gi en liten reduksjon i årlig antall nye tilfeller og/eller dødsfall for alle helseutfall. Reduksjonen er størst for demens, samt for tidlig fødsel. Ved en økning fra dagens fiskeinntak til 450 gram per uke, var det også en nedgang for nye tilfeller av koronar hjertesykdom i tillegg til demens, slag og for tidlig fødsel (se tabell 9.2.6-2 i kapittel 9).

Resultatene fra den kvantitative analysen for menn og kvinner tyder på at økt inntak av fisk mot det øvre området i kostrådet (450 gram fisk i uken) vil redusere antall tilfeller av slag og koronar hjertesykdom. Dette er folkehelsesykdommer som er viktige bidragsytere til sykdomsbyrden i Norge. Likeledes anslås det at økt inntak av fisk opp mot anbefalt inntak, vil redusere antall nye tilfeller av demens og Alzheimers sykdom. Begge disse tilstandene er kognitive lidelser som øker som følge av en aldrende befolkning. Modelleringen indikerte at en økning i fiskeinntaket fra dagens nivå til det anbefalte nivået vil ha svært liten innvirkning på hjerte- og kardødelighet samlet sett, og dødelighet av koronar hjertesykdom hos både menn og kvinner. Årsaken til dette er at dose-responskurven som er brukt i den kvantitative analysen for disse utfallene var flat for inntak av fisk over 300 gram per uke.

For tidlig fødsel ble inkludert i den kvantitative modelleringen for kvinner, men ikke lav fødselsvekt, da den underliggende årsaken til lav fødselsvekt så ut til å være for tidlig fødsel, i studiene som hadde sett på mors fiskeinntak under svangerskapet.

Evidensen for død av hjerteinfarkt og slag er også gradert som "sannsynlig", men ettersom det ikke ble funnet publiserte metaanalyser av dose-respons sammenhenger basert på studier av død av disse utfallene, ble død av hjerteinfarkt og slag kun inkludert som del av samlet hjerte- og kardødelighet.

Semikvantitativ vurdering av næringsstoffer og kontaminanter fra fisk

Den kvantitative nytte- og risikovurderingen av fisk inkluderer ikke spermiekonsentrasjon og vaksinerespons som er de kritiske endepunktene for to av de inkluderte kontaminantene. Dette er fordi det ikke ble funnet studier av god kvalitet med fisk som eksponering for disse utfallene i vår litteraturgjennomgang. Det finnes heller ingen modeller som muliggjør å inkludere kontaminantene og næringsstoffene i den kvantitative analysen. Evalueringen av alle næringsstoffene og kontaminantene er derfor gjort med en semikvantitativ tilnærming. I de semikvantitative analysene refererer «dagens inntak» av fisk til inntak rapportert i de nasjonale kostholdsundersøkelsene Norkost 3 (2010-2011), Ungkost 3 (2015-2016), Småbarnskost 3 og Spedkost 3 (2019).

Næringsstoffer

Fisk er en viktig kilde i kostholdet for langkjedede n-3 fettsyrer (eikosapentaensyre (EPA), dokosapentaensyre (DPA) og dokosaheksaensyre (DHA)), vitamin D, jod, selen og vitamin B₁₂. Mer enn 20 prosent av det totale inntaket av disse næringsstoffene i kosten kommer fra fisk. Særlig de langkjedede n-3 fettsyrene, vitamin D og jod har svært få andre naturlige kilder i kosten.

VKM har også gjort omfattende systematiske kunnskapsoppsummeringer for næringsstoffer i fisk og mulige sammenhenger med helseutfall. Metoden for litteraturgjennomgangen, og graderingen av evidensen, var de samme som for fisk. For næringsstoffene er det imidlertid bare inkludert systematiske oversiktsartikler og metaanalyser, ikke enkeltstudier. Et bredt spekter av næringsstoffer og helseutfall ble evaluert. Helseutfallene var stort sett de samme som for fisk, med unntak av de immunmedierte sykdommene.

Kort oppsummert er det funnet en sterk sammenheng ("sannsynlig") mellom inntak av langkjedede n-3 fettsyrer og hjerte- og kardødelighet, dødelighet fra koronar hjertesykdom, utvikling av hjerte- og karsykdom (kun for doser >1 gram langkjedede n-3 fettsyrer per dag), koronar hjertesykdom, hjerteinfarkt, samt effekter på fødselsvekt (kontinuerlig). Konklusjonene for graderingen av evidensen for sammenhenger mellom langkjedede n-3 fettsyrer og helseutfall støtter konklusjonene fra fisk og de samme helseutfallene.

For vitamin D er det funnet en sterk sammenheng ("sannsynlig") for samlet dødelighet og benbrudd/fall (basert på Nordiske næringsrekommandasjoner, 2012). Ingen sterke sammenhenger ble funnet for verken jod, selen eller vitamin B₁₂. VKM brukte gjennomsnittlig behov (average requirements, AR) i den semikvantitative nyttevurderingen av næringsstoffer i fisk. Behovsfastsettelse er basert på etablert kunnskap om en sammenheng mellom jod og struma, selen og Keshan-sykdom, og vitamin B₁₂ og pernisiøs anemi. Det kritiske endepunktet for behovsfastsettelse for vitamin D er beinhelse (benbrudd). Det er ingen behovsfastsettelse tilgjengelig for de langkjedede n-3 fettsyrene. Vi har imidlertid sammenlignet inntaket av summen av EPA og DHA med et adekvat inntak (AI) basert på kardiovaskulær helse som er satt av EFSA (2010). VKM har gjort en semikvantitativ nyttevurdering av langkjedede n-3 fettsyrer, vitamin D, jod, selen og vitamin B₁₂ basert på andeler i befolkningen som har et inntak under henholdsvis gjennomsnittlig behov og adekvat inntak.

Den semikvantitative vurderingen av summen av de langkjedede n-3 fettsyrene EPA og DHA viser at 18 prosent av kvinner i alderen 18-45 år og ti prosent av voksne menn og kvinner (18-70 år) med dagens inntak av fisk ligger under adekvat inntak for disse fettsyrene. I fiskescenarioene, der vi anslår at alle deltakerne i kostholdsundersøkelsene har et bestemt daglig inntak av fisk, får alle voksne et estimert inntak av EPA pluss DHA som er over det adekvate inntaket.

Ved dagens inntak av fisk er det en relativt høy andel personer som har et inntak av vitamin D under gjennomsnittlig behov. Dette gjelder for alle aldersgrupper. Beregningene for fiskescenarioene tyder på at det å øke inntaket av fisk fra dagens nivå til det som er

anbefalt, vil føre til en moderat økning i vitamin D-inntaket på populasjonsnivå. En slik økning kan være av særlig betydning for de med svært lavt inntak av vitamin D fra kosten. For disse kan selv en liten økning være av vesentlig betydning. F.eks. har 67 prosent av 13-årige jenter et inntak under gjennomsnittlig behov med dagens fiskeinntak. Det ble estimert at dette ble redusert til 50 prosent i scenario 3. For kvinner i alderen 18-45 år øker inntaket for de med det 5 prosent laveste inntaket fra 2,3 µg/dag med dagens fiskeinntak til 5,4 µg/dag i scenario 3.

Unge jenter og kvinner i alderen 18-45 år har høyest risiko for lavt inntak av jod. Ved dagens inntak av fisk hadde 34 prosent av 13-årige jenter, og 19 prosent av kvinner i aldersgruppen 18-45 år, et inntak under gjennomsnittlig behov. Det ble estimert at å øke inntaket av fisk opp til det øvre området av det anbefalte inntaket (450 gram fisk i uken) ville føre til at alle aldersgrupper og kjønn, bortsett fra 1-åring, får et inntak av jod som er over gjennomsnittlig behov.

For selen er det unge jenter og kvinner i alderen 18-45 år som har lavest inntak. Ved dagens inntak har 71 prosent av 9-årige jenter, og 7 prosent av kvinner i alderen 18-45 år, et inntak under gjennomsnittlig behov. Å øke inntaket av fisk opp til anbefalingene ble estimert å føre til at alle har et inntak over gjennomsnittlig behov for selen. Med dagens fiskekonsum er det ingen spesifikke aldersgrupper som står i fare for å ha et inntak av vitamin B₁₂ som er under gjennomsnittlig behov.

Kontaminanter

Fisk kan også inneholde en rekke kontaminanter. VKM ble spesielt bedt om å inkludere PCDD/F og DL-PCB-er, og PFAS-er, på grunn av en betydelig reduksjon i tålegrensene for disse stoffene. VKM vurderte også en omfattende liste over andre stoffer som det kunne være relevant å inkludere. Etter en trinnvis utvelgelsesprosess med fokus på «bekymring i forhold til fiskeinntak» og «eksponering nær en eksisterende helsebasert referanseverdi», ble også metylkvikksølv inkludert.

For kontaminanter i fisk er VKMs vurdering av ugunstige helseutfall basert på farekarakteriseringen og de tilhørende tålegrensene som EFSA har publisert (EFSA 2012; 2018; 2020). For metylkvikksølv har det kommet mange nye publikasjoner som har vurdert sammenhengen mellom eksponering og ulike endepunkter etter at EFSA publiserte sin risikovurdering i 2012. Basert på en gjennomgang av systematiske oversikter for metylkvikksølv bestemte imidlertid VKM seg for å bruke tålegrensen fra 2012.

VKM har utført semikvantitative risikovurderinger av PCDD/F og DL-PCB-er, PFAS-er og metylkvikksølv basert på andeler i befolkningen som har eksponeringer over de tålegrensene som er fastsatt av EFSA. Det kritiske endepunktet for PCDD/F og DL-PCB-er er redusert spermiekonsentrasjon etter eksponering via mor under graviditet, og eksponering via amming etter fødsel, i tillegg til eget matinntak i barndommen. For PFAS-er er vurderingen begrenset til de samme fire PFAS-ene som dekkes av tålegrensen: PFOA, PFNA, PFHxS og PFOS. Den kritiske effekten for summen av disse fire PFAS-ene er effekter på

immunsystemet, målt som redusert vaksinerespons hos barn etter eksponering før og etter fødsel. EFSA vurderte også ulike sammenhenger mellom serumnivåer av PFAS-er og flere andre utfall, men fordi effektene på immunsystemet oppstår ved lavest eksponering, er ikke andre effekter vurdert i denne nytte- og risikovurderingen. Nevroutviklingseffekter hos barn er den kritiske effekten for metylkvikksølv. Effekten ses hos barn som er eksponert via mor i svangerskapet.

Den semikvantitative risikovurderingen av kontaminanter viser at en høy andel (96 – 100 prosent) av den norske befolkningen overstiger tålegrensen på 2 pg TEQ/kg kroppsvekt/uke satt for PCDD/F og DL-PCB ved dagens inntak av fisk. Den voksne befolkningen har en gjennomsnittlig eksponering som er beregnet til å være 2,3 ganger høyere enn tålegrensen ved dagens inntak av fisk. Fisk bidrar med 39 prosent av eksponering til PCDD/F og DL-PCB i den voksne befolkningen. Av dette bidrar mager fisk med 6 prosent, fet fisk med 28 prosent og lever og rogn med 5 prosent. Hvis fiskeinntaket økes til anbefalt inntak, økes gjennomsnittlig eksponering for PCDD/F og DL-PCB-er for alle aldersgrupper. VKM har brukt eksponeringsberegninger for PCDD/F og DL-PCB-er som er konservative (sannsynlige overestimer).

Den voksne befolkningen har en gjennomsnittlig beregnet PFAS-eksponering som er 1,7 ganger høyere enn tålegrensen ved dagens inntak av fisk. Andelen som overskrider tålegrensen er fra 44 til 100 prosent i de ulike aldersgruppene. For alle aldersgrupper er fisk den viktigste bidragsyteren til summen av disse fire PFAS-ene (ca. 38 prosent for voksne). Mager og fet fisk bidrar omtrent likt på tvers av aldersgrupper, med litt høyere bidrag fra mager fisk hos voksne. Det er estimert at å øke inntaket av fisk til det høyeste anbefalte inntaket vil føre til en økning i andelen som overskrider tålegrensen, noe som vil gjøre at alle voksne overskrider. Barn har høye estimerte eksponeringer, både i dagens situasjon og i de beregnede scenariene, fra 1,5 ganger tålegrensen i dagens situasjon for 9-åringer, til 4,8 ganger tålegrensen for 2-åringer i scenario 3. Eksponeringsberegninger for PFAS er usikre på grunn av høyt deteksjonsnivå i analysemetodene.

Fisk og annen sjømat er praktisk talt den eneste kilden til metylkvikksølv i Norge. Med dagens inntak av fisk er det estimert at kun en liten andel av befolkningen overskrider tålegrensen for metylkvikksølv. VKM brukte en konservativ tilnærming og antok at alt kvikksølv i fisk og skalldyr er metylkvikksølv (sannsynlig overestimat). Ved endret fiskeinntak i scenarioene vil det estimerte kvikksølvinntaket generelt synke. Dette er blant annet fordi scenarioene er basert på de mest spiste fiskeartene. Disse inneholder relativt lite kvikksølv. Videre representerer det høyeste scenarioet (scenario 3) en nedgang i fiskekonsumet for personer som spiser velig mye fisk. Oppsummert er andelen som overskrider tålegrensen for metylkvikksølv enten null, eller svært lav, for alle aldersgrupper i alle de tre scenarioene.

Oppsummering av konklusjoner og svar på oppdraget

62 prosent av norske kvinner, og 58 prosent av mennene, oppgir at de spiser to eller flere fiskemåltider i uken. Modellering tyder på at en reduksjon i det ukentlige inntaket av fisk til 150 gram per uke, vil medføre en økning i antall årlige tilfeller, eller dødsfall, for alle

helseutfall som inngår i modelleringen (død av hjerte- og karsykdom samlet, død av koronar hjertesykdom og total død, samt nye tilfeller av koronar hjertesykdom, slag, demens, Alzheimers sykdom og for tidlig fødsel). Effekten var størst på hjerte- og karsykdom, kognitiv svikt og for tidlig fødsel. Beregningene er en indikasjon på at et lavt inntak av fisk er en mulig helserisiko, og at man ved dagens fiskeinntak går glipp av gunstige effekter av å spise fisk.

Basert på modellering vil et økt inntak av fisk opp til anbefalt inntak, og spesielt mot det øvre området av anbefalt inntak som er 450 gram per uke, redusere nye tilfeller av slag og koronar hjertesykdom i befolkningen. Dette er folkehelsesykdommer som bidrar mye til sykdomsbyrden i Norge. Økt inntak av fisk opp mot anbefalt inntak ansås også å redusere antall nye tilfeller av demens og Alzheimers sykdom. Begge disse er kognitive lidelser som øker som følge av en aldrende befolkning.

Andelen av befolkningen med et inntak av jod og selen under gjennomsnittlig behov vil også reduseres ved økt inntak av fisk. Det lave inntaket av vitamin D vil ikke nødvendigvis rettes opp ved økt fiskeinntak alene, men å øke fiskeinntaket, og spesielt inntaket av fet fisk, vil kunne ha betydning for de med lavest vitamin D-inntak. I sum vil alle aldersgrupper, basert på semikvantitative vurderinger av næringsstoffer, være tjent med å øke inntaket av fisk fra dagens inntak til anbefalt inntak.

Samtidig vil økt inntak av fisk til anbefalt inntak føre til at eksponeringen for PCDD/F og DL-PCB, og PFAS-er øker til et nivå der nesten alle i alle aldersgrupper vil overskride tålegrensene. For voksne vil økningen i overskridelse være moderat, dvs. fra 2,3 ganger tålegrensen ved dagens fiskeinntak til 2,8 ganger tålegrensen i scenario 3 for PCDD/F og DL-PCB, og fra 1,7 ganger tålegrensen til 1,9 ganger tålegrensen i scenario 3 for PFAS-er. I hvor stor grad de kritiske effektene knyttet til eksponering for PCDD/F og DL-PCB og PFAS-er, henholdsvis redusert spermiekonsentrasjon og redusert vaksinerespons hos barn, bidrar til infertilitet og sykdomsbyrde er ikke beregnet, men mannlig infertilitet utgjør en relativt liten del av sykdomsbyrden i Norge. En redusert respons på vaksinasjon hos barn brukes ofte som en markør for redusert immunrespons. Hvor god markør vaksinerespons er for dette utfallet, samt i hvilken grad en eventuell redusert immunrespons vil medføre økt risiko for infeksjoner, er ikke kjent. Det er dessuten mange kilder til disse kontaminantene i det norske kostholdet, så selv om et lavere inntaket av fisk sannsynligvis vil føre til en viss reduksjon i eksponeringen, er det trolig ikke tilstrekkelig til å få eksponeringen ned under tålegrensene.

VKM konkluderer med at det å spise fisk er gunstig, og at fisk beskytter mot flere helseutfall som er viktige folkehelseutfordringer i Norge. For disse utfallene er evidensen gradert som "sannsynlig", noe som karakteriseres som sterk evidens i henhold til WCRFs kriterier. Evidensen for gunstige effekter av å spise fet fisk er svakere enn for det totale inntaket av fisk. Evidensen blir imidlertid underbygget av sterk evidens («sannsynlig») for beskyttende effekter av de langkjedede n-3 fettsyrene på flere av de samme helseutfallene som for fisk.

VKMs konklusjon er basert på systematiske kunnskapsoppsummeringer, og gradering av evidens for sammenhenger mellom inntak av total fisk eller fet fisk og helseutfall ved bruk av

etablerte kriterier. Vurderingen inkluderer også en kvantitativ estimering av effekter av fiskeinntak på helseutfall med insidensrater og dødelighet som felles måleenhet. I tillegg har vi gjennomført systematiske kunnskapsoppsummeringer for næringsstoffer, og inkludert semikvantitative vurderinger av næringsstoffene langkjedede n-3 fettsyrer, vitamin D, jod, selen og vitamin B₁₂, og av kontaminantene PCDD/F og PCB-er, PFAS-er, og metylkvikksølv.

Generelt er utfallene som inngår i den kvantitative analysen kroniske, folkehelsesykdommer som rammer eldre aldersgrupper (unntatt for tidlig fødsel). Disse sykdommene har imidlertid lang latenstid. Kostvaner har også en tendens til å etablere seg i ung alder og vedvare i voksen alder. Disse faktorene støtter at anbefalt inntak av fisk allerede i ung alder kan ha betydning for inntak av fisk senere i livet, og for senere helsegevinst.

VKM konkluderer med at fordelene ved å øke inntaket av fisk opp til de anbefalte to til tre middagsmåltidene per uke (tilsvarende 300-450 gram, inkludert minst 200 gram fet fisk hos voksne) oppveier risikoen. Dette gjelder for alle aldersgrupper.

Abbreviations and definitions

Abbreviations

ADHD	Attention Deficit/Hyperactivity Disorder
AMSTAR	A MeasSurement Tool to Assess systematic Reviews
AR	average requirement
ASD	Autism spectrum disorder
ATSDR	Agency for Toxic Substances and Disease Registry
BFR	Brominated flame retardants
BMI	body mass index
bw	body weight
CHD	coronary heart disease
CI	confidence interval
CINAHL	Cumulative Index to Nursing and Allied Health
CNS	central nervous system
CVD	cardiovascular disease
DHA	docosahexaenoic acid
DL-PCB	dioxin-like PCB
DPA	docosapentaenoic acid
DRI	dietary reference intake
EAR	Estimated average requirement
EAR	estimated average requirement
EFSA	The European Food Safety Authority
EMBASE	Excerpta Medica Database
EPA	eicosapentaenoic acid
EU	The European Union
HBGV	Health based guidance value
HCH	Hexachlorocyclohexane
IARC	International Agency for Research on Cancer
IOM	Institute of Medicine

IQ	intelligence quotient
LB	lower bound
LBW	low birth weight
LC n-3 FA	long chain n-3 fatty acid
LOD	level of detection
LOQ	level of quantification
MC	Monte Carlo (simulations)
MD	mean difference
MEDLINE	Medical Literature Analysis and Retrieval System Online
MI	myocardial infarction
MoBa	Norwegian Mother, Father and Child Cohort Study
MoE	Margin of Exposure
MRL	Minimal Risk Level
NASEM	National Academies of Science, Engineering and Medicine
NNR	Nordic Nutrition Recommendations
NOS	Newcastle–Ottawa Scale
OIM	Observed individual mean
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzo-p-dioxins
PCDF	polychlorinated dibenzofurans
PFAS	Poly- and perfluoroalkyl substances
PICO	Population Intervention Comparison Outcome
POP	Persistent organic pollutant
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PROSPERO	International Prospective Register of Systematic Reviews
PsychINFO	Psychological Information Database
PTB	preterm birth
PUFA	polyunsaturated fatty acid
RBA	Risk and benefit assessment
RCT	randomized controlled trial
RI	recommended intake

RTI	respiratory tract infection
SACN	Scientific Advisory Committee on Nutrition, UK
SCF	Scientific Committee on Food
SD	Standard deviation
SGA	small for gestational age
SRR	Summary Relative Risk
T2DM	type 2 diabetes mellitus
TEF	Toxic equivalence factor
TEQ	Toxic equivalent quantity
ToR	Terms of Reference
TSH	thyroid stimulating hormone
TWI	tolerable weekly intake
UB	upper bound
UIC	urinary iodine concentration
UL	tolerable upper intake level
WCRF	World Cancer Research Fund
WHO	World Health Organization

Definitions

Bioaccumulation

The gradual accumulation of substances, such as chemicals, in an organism. Bioaccumulation occurs when an organism absorbs a substance at a rate faster than that at which the substance is lost or eliminated by catabolism and excretion.

Biomagnification

The process by which a toxin or contaminant build up within predators such that each level of the food chain has a greater concentration of the substance.

Dietary reference value

An umbrella term for a set of nutrient reference values (e.g. AR, AI, RI and UL)

Congeners

Chlorinated organic compounds that share the same molecular backbone (such as biphenyls, dibenzodioxins and dibenzofurans backbones) but which have a variable chlorination substitution pattern on this backbone. Examples of compound groups that each contain many congeners are polychlorinated biphenyls (PCBs, 209 congeners), polychlorinated dibenzodioxins (75 congeners), and dioxin-like PCBs (12 congeners).

Consumers only

A term that refers to a calculated value based on data from only those who reported consumption of the specific food item.

Current fish intake

The term “current intake” is used for intake from the most recent national dietary surveys as reported by the respondents, i.e. Norkost 3: 2010-2011, Ungkost 3: 2015/2016, Spedkost 3 and Småbarnskost 3: 2019

Dioxin-like

A description used for compounds that have chemical structures, physico-chemical properties, and toxic responses similar to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD).

Food group

A food group is a collection of foods that share similar nutritional properties and/or have the same usage. The food groups used in this assessment are grouped according to the KBS food groups (food composition database, University of Oslo)

Habitual intake

The long-term mean intake of foods or nutrients for the group or individuals under study

I²

A statistical measure of between-study heterogeneity used in meta-analysis.

Limit of detection (LOD)

A limit of detection is the lowest concentration of a substance that can be detected with a certain degree of confidence using a validated analytical method.

Limit of quantification (LOQ)

The limit of quantification is the lowest concentration of a substance that can be quantified with a required certainty using a validated analytical method.

Lower bound (LB) estimate

Lower bound estimates are calculated by setting analytical results below the limit of detection (LOD) or limit of quantification (LOQ) of the analytical method to zero. The LB estimate represents an underestimate of the true value.

Mixed model

The mixed model is a statistical model containing fixed and random effects. Mixed models allow estimation of day-to-day variation in the modelled exposure for each survey participant and of clustered variation between survey participants, and simulation of long-term chronic exposure. The model is used to correct for day-to-day variation in the modelled exposure for each survey participant, and for variation between survey participants.

Observed individual means (OIMs)

Observed individual means are arithmetic mean intakes for each individual over the dietary survey days, often used as estimates of individual chronic exposure.

Prepared fish fillet

Fish fillet that is ready to eat, without inedible parts, either with or without heat-treatment, according to product.

Tolerable weekly intake (TWI)

The maximum intake of contaminants in food that can be consumed weekly over a lifetime without risking adverse health effects (EFSA glossary).

Toxic equivalency factor (TEF)

A value representing the relative toxicity of PCDDs, PCDFs and DL-PCBs in relation to TCDD, which is the most toxic compound in this category. The TEF approach for PCDD/Fs and DL-PCBs is based on a common, receptor-mediated mechanism of action for these compounds. To include a compound in a TEF scheme, the following criteria should be met: the compound should show structural relationship to the PCDDs and PCDFs; it should bind to the aryl hydrocarbon receptor; it should elicit dioxin-specific biochemical and toxic responses; it should be persistent and accumulate in the food-chain (WHO, 2000).

Toxic equivalent (TEQ)

A weighted quantity measure based on the toxicity of each PCDD, PCDF and DL-PCB relative to TCDD. TEQ for each PCDD, PCDF and DL-PCB is calculated by multiplying the concentration of each congener with its corresponding TEF. The resulting concentration in TEQ for each congener can be summarised as they express TCDD-like toxicities on a common scale.

Upper bound (UB) estimate

Upper bound estimates are calculated by setting analytical results below the LOD or LOQ equal to the LOD or LOQ for the analytical method. The UB estimate represents an overestimate of the true value.

Women of childbearing age

In this assessment this term refers to women aged 18 to 45 years.

Background as provided by the Norwegian Food Safety Authority

Fish contain nutrients that are positive for our health. At the same time, it contains varying levels of undesirable substances that can have a negative effect on health. Undesirable substances can be found in different levels in most types of food. A risk-benefit assessment assesses both the nutrients and the undesirable substances and evaluate if it in total gives a more positive effect to eat certain foodstuff than not, and possibly how much one should eat to achieve optimal use of the positive health effects.

A risk-benefit assessment of fish has been conducted two times earlier by VKM. The reports were published in 2006 and 2014. In 2006 VKM pointed out that consumption of fish had positive effects on public health, especially because of the content of polyunsaturated fatty acids and vitamin D. VKM also found that mainly mercury, dioxins and dioxin-like-PCBs posed a potential risk when consuming fish in Norway. In 2014, VKM concluded that the health benefits by eating fish clearly outweighed the risk of negative health effects from the exposure to undesirable substances from fish. According to the committee it was well documented that fish protects against cardiovascular disease. Further on, the report concludes that fish contribute to a positive development of the neural system in the foetus and in breastfed infants, and that they can miss out on these effects if the mother does not eat enough fish (i.e. less than one dinner portion per week).

The role of NFSA is to warn the population against foods that can contain too high levels of substances that can give negative health effects. In addition, the NFSA contributes in the work to develop regulations and maximum levels (MLs) for contaminant in foodstuffs, which also is a means to protect the population. The Norwegian Directorate of Health (NDH) gives advice on diet that describes what one should eat to get the best possible health effects from our diet.

After 2014, several new data relevant for a risk- benefit assessment of fish, has become available. The Institute of Marine Research (IMR) has on commission from NFSA and others collected occurrence data for undesirable substances and nutrients in fish species that we did not have sufficient data on in earlier assessments. The Department of Nutrition at the University of Oslo has, in collaboration with the Norwegian Institute of Public Health (NIPH), NDH and NFSA, completed diet studies of children and adolescents (4-, 9-, and 13-year-olds) in 2015-2016. In addition to more data available, the general knowledge has also increased. Several tolerable weekly intakes (TWIs) for undesirable substances have been revised by EFSA. The most important ones were published in 2018 and are summarized below:

In November 2018, EFSA published a new risk assessment of the substance group dioxins and dioxin-like-PCBs in food and feed. In this assessment, EFSA concluded that the tolerable weekly intake level for this substance group should be lowered from 14 to 2 pg/kg body weight/week. The new tolerable intake protects against reduced sperm concentration. In the assessment EFSA also suggested that the WHO-TEF-value (which describes the

relative toxicity of the substances in the group compared with the most toxic substance of dioxins, 2,3,7,8-TCDD) for PCB-126 probably is too high and should be revised. A revision will probably take at least one year. It is therefore important that the risk- benefit assessment in fish can adjust to possible new WHO-TEF-values.

In December 2018, EFSA published a risk assessment of the perfluoroalkylated substances, PFOS and PFOA in food. Also, in this assessment EFSA concluded that the health-based guidance values should be lowered for both substances. For PFOS the TWI level was lowered from 1050 to 13 ng/kg body weight/week. The new TWI protects against risk of increased cholesterol in adults, and reduced effect of vaccines in children. For PFOA, the TWI was reduced from 10500 to 6 ng/kg body weight/week. The new tolerable intake protects against increased cholesterol. The conclusions in the assessment are provisional until a second assessment of other PFAS is ready. It is therefore important that the risk- benefit assessment of fish in the Norwegian diet can be adjusted to possible changes in the PFAS TWI when the second assessment is published.

With regard to the new knowledge available, NFSA suggest that there is a need for a new risk- benefit assessment of fish in the Norwegian diet.

Terms of reference as provided by the Norwegian Food Safety Authority

The Norwegian Food Safety Authority (NFSA) asks the Norwegian Scientific Committee for Food and Environment (VKM) to conduct a risk- benefit assessment for fish consumption in the Norwegian diet. In the assignment we ask VKM to answer the following questions:

Which health consequences will it have for the Norwegian population if they:

- Continue with the same consumption levels as of today
- Increase the consumption of fish to match the recommendations given by the Norwegian Directorate of Health (NDH)¹

VKM decides which substances and scenarios that should be included to conduct a relevant risk-benefit assessment of fish consumption. The decisions need to be justified in the assessment. The assessment of dioxins and dioxin-like-PCBs must be done in a manner that allow for later adjustments if/when the toxic equivalency factor (TEF-) values is revised. Perfluoroalkylated substances (PFAS) should also be assessed in a manner that makes it possible to adjust the assessment to new health-based guidance values (tolerable intakes²).

Data gaps and insufficient data (e.g. too high limit of quantification, LOQ) should be made visible in the assessment, this information will be useful for planning future data collection.

The risk- benefit assessment should be delivered in English with a Norwegian summary.

The assignment was updated in May 2022, after an agreement between NFSA and VKM on leaving out the ToR regarding health consequences for the Norwegian population if they reduce the consumption of fish and replaces parts or all of it with other foods in the diet. Nutrients and contaminants have several important sources other than fish, and through the work it became clear that a sophisticated approach is needed taking many aspects related to

¹ "Eat fish for dinner two to three times a week. Also use fish as spread on bread. The advice equals 300-450 grams of fish filets during the week. At least 200 grams should be fatty fish like salmon, trout, mackerel or herring. Six portions of fish used as bread spread equals approximately one portion of dinner." Matportalen.no (downloaded 09.04.19).

² Tolerable intake (which is a health-based guidance value) describes the maximum intake of substances in food, such as nutrients or contaminants, that can be consumed daily or weekly over a lifetime without risking adverse health effects.

dietary changes into consideration. This was not possible with the time and resources available.

1 Introduction

Fish and fish products are important basic foods with long traditions in the Norwegian diet and a natural part of any meals, both as dinner and as spread on bread (bread spreads). Fish is an important source of protein, and of marine long chain n-3 fatty acids (in this report named LC n-3 FA) and a variety of vitamins and minerals, but fish may also contain contaminants.

In 2006, VKM published "A comprehensive assessment of fish and other seafood in the Norwegian diet". The assessment supported the general Norwegian recommendation to eat more fish both for dinner and on sandwiches. In 2014, VKM published an update of the benefit and risk assessment of fish published in 2006 (VKM, 2014). In the 2014 assessment, VKM concluded that "the benefits clearly outweigh the negligible risk presented by current levels of contaminants and other undesirable substances in fish". VKM stated that adults, including pregnant women, may miss the beneficial effects if they consume less than one serving of fish per week. The beneficial effects were related to reduced risk of cardiovascular diseases, cardiac mortality, and to improved neurodevelopmental outcomes from maternal fish consumption (VKM, 2014).

In 2018 and 2020; new tolerable weekly intakes (TWI) for the dioxins and dioxin-like PCBs (referred to in this report as PCDD/F and DL-PCBs), and perfluorinated substances (referred to as PFASs) were markedly reduced by the European Food Safety Authority (EFSA) (EFSA, 2018; EFSA, 2020). It was expected that the intake of these contaminants in the Norwegian population, like the populations in other European countries, will exceed the new TWIs. From previous opinions, it was anticipated that the food group fish is the most significant contributor to these contaminants in the Norwegian diets, but also to vitamin D, iodine and LC n-3 FA, all nutrients with very few natural sources in the diet, and intakes generally known to be scarce in the Norwegian population.

A new benefit and risk assessment of fish was warranted. To provide high confidence of the evidence, we have conducted systematic literature reviews of the associations between fish intake and a wide range of health outcomes relevant to public health. We aimed to identify scientific literature for all relevant beneficial and adverse health outcomes related to fish in the diet and have weighted the overall evidence for each of these outcomes in accordance with international criteria. In addition, we have assessed positive health effects from nutrients in fish, and adverse health effects from contaminants in fish.

A protocol for this benefit and risk assessment was published in February 2020 after a public consultation. As described in the protocol, we have followed a tiered approach as suggested by EFSA's Guidance on human health risk-benefit assessment of foods (EFSA, 2010) and the later more refined Benefit-Risk Analysis for Foods (BRAFO) tiered approach for benefit and risk assessment of foods by Hoekstra and colleagues (Hoekstra et al., 2012). A quantitative modelling with incidence and mortality as common metrics is used to estimate how changes in fish intake from current intake to three constructed intake scenarios may change disease incidence and mortality. The three constructed scenarios include two where the fish intake is

based on the recommendations as given in the mandate, and one which expands beyond the mandate, where the fish intake is lower than the recommended intake.

1.1 Scope

The scope of this benefit and risk assessment of fish in the Norwegian diet is to give the Norwegian Food Safety Authority (NFSA) a scientifically based answer to the two questions asked in the revised terms of reference. The answers are based on systematic reviews and weight of evidence for fish and relevant health outcomes, and internationally established methods for quantitative modelling with incidence and mortality as common metrics.

1.2 Delimitations

VKM have done the following delimitations in the present benefit and risk assessment:

- The exposure assessment of fish intake includes fish and fish products, and not other seafood
- The systematic literature review of associations between fish intake and health outcomes does not include risk factors or biomarkers considered to be intermediate in the disease process, e.g., changes in blood pressure or blood lipids, which are important risk factors for endpoints such as stroke, coronary heart diseases and mortality (outcomes that are included in this opinion). However, as semen quality is the critical endpoint for the tolerable weekly intake for PCDD/Fs and DL-PCBs, and vaccination response is the critical endpoint for PFAS, it was necessary to include these intermediate risk factors in addition to fertility
- VKM could not include the single compounds (nutrients and contaminants) in fish in the quantitative benefit and risk assessment modelling due to limitations in available methodology. However, nutrients and contaminants are treated at a lower tier, in a semi-quantitative manner, since NFSA had specifically requested that the assessment should cover the new TWIs set by EFSA
- Lastly, fish consumption also has impact on fish biodiversity and on the environment. This will also impact the weighing of benefits and risks of fish in the Norwegian diet in a broader perspective, but this is outside the scope of this assessment

1.3 How to read the assessment

As this benefit and risk assessment of fish is very comprehensive, to guide the reader, we have listed the main content in the chapters below:

Chapter 2: description of the current recommendations for fish intake, which nutrients and contaminants have been included for this benefit and risk assessment of fish, and the established reference values for these compounds (i.e., average requirements for nutrients and tolerable weekly intakes for contaminants).

Chapter 3: description of methods for systematic literature reviews of primary studies and previous systematic reviews, including description of inclusion criteria for health outcomes,

search strategies, study selection, study quality assessment, and the meta-analysis methods and weight of evidence criteria used for this report.

Chapter 4: presents results of systematic literature review and weight of evidence analyses for associations between fish intake and health outcomes. Included health outcome categories are CVD and mortality (all-cause, and cause-specific), neurodevelopment and cognition/mental health, birth outcomes, type 2 diabetes, bone health, anthropometry, and immune-related outcomes.

Chapter 5: results of systematic literature review and weight of evidence analyses for the associations between nutrients in fish and selected health outcomes, with a main focus on marine n-3 fatty acids and vitamin D, but also including iodine, selenium and vitamin B₁₂.

Chapter 6: description of adverse effects of the contaminants in fish, summarised from the published EFSA opinions on dioxins and DL-PCBs, PFASs and methyl mercury, with focus on the critical effects used to determine the TWIs.

Chapter 7: description of food databases (i.e., concentration data for the included nutrients and contaminants) and dietary survey data used for calculating fish intake and intake of nutrients and contaminants in fish.

Chapter 8: presentation of estimated current fish intake in the Norwegian population and estimated current intakes of included nutrients and contaminants from the total diet, and the contribution to the intake resulting from fish consumption.

Chapter 9: presentation of

- 1) the results of the scenarios for fish consumption
- 2) quantitative benefit and risk assessment of fish and selected health outcomes (related to the weight of evidence conclusions for fish in Chapter 4 and fish consumption estimates from Chapter 8)
- 3) semi-quantitative benefit assessment of nutrients in fish (related to average requirements described in Chapter 2, nutrient intake estimates from Chapter 8, and the weight of evidence conclusions for nutrients in Chapter 5)
- 4) semi-quantitative risk assessment of contaminants in fish (related to tolerable weekly intakes described in Chapter 2 and contaminant intake estimates from Chapter 8).

Chapter 10: comparison of benefits and risks presented in Chapter 9.

Chapter 11: outline of uncertainties in all the elements relevant for the conclusions in this benefit and risk assessment of fish.

Chapter 12: conclusions and answer to the terms of references.

Chapter 13: data gaps and needs for further research that have been revealed during the preparation of the opinion, including descriptions of how and why the data gaps needs to be filled.

1.4 Previous assessments

Several previous benefit and risk assessments of fish and other seafood have been conducted in Norway and internationally. Generally, previous reports have concluded that fish consumption overall benefits health.

Short summaries of previous reports from VKM and EFSA can be found in Chapter 21, Appendix VIII (VKM, 2006; VKM, 2014; EFSA, 2014; EFSA, 2015; VKM, 2019). For a more comprehensive overview of other benefit and risk assessments of fish and other seafood published since 2000, we refer to the scoping review by Thomsen and colleagues published in 2021 (Thomsen et al., 2021).

1.5 References

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2 Recommendations for fish consumption and reference values for selected nutrients and undesirable substances

2.1 Recommendations for fish consumption

In 2011, the Norwegian National Council for Nutrition published the report “Dietary advice to promote public health and prevent chronic diseases in Norway” (Norwegian National Council for Nutrition, 2011). Based on this report, the Norwegian Directorate of Health published quantitative recommendations for fish intake, recommending fish as dinner meal 2-3 times per week for all age groups (Norwegian Directorate of Health, 2014). Fish as bread spread is also recommended. Translated into grams the recommendation represents 300-450 g prepared fish per week for adults, and less for children. For adults, at least 200 g is recommended as fatty fish. Six portions of bread spreads represent approximately one dinner portion.

To derive recommended fish intakes for children for this benefit and risk assessment, we have scaled down the recommended intake in adults using energy adjustment. Based on the reference energy requirement for men (11.7 MJ) and women (9.4 MJ) with average activity level (NNR, 2012), a reference energy intake was set to 10 MJ/day (100%) in adults and the recommended fish intakes of 300-450 g total fish and 200 g fatty fish per week, gave scaling factors of 30 g/MJ, 45 g/MJ and 20 g/MJ, respectively. Reference energy intakes in children were based on Nordic Nutrition Recommendations (NNR) (2012) using the average of girls and boys for an average physical activity level. The recommended intake in adults and estimated recommended fish intakes in children (Table 2.1-1) are used for comparison with current fish intake and the fish intake scenarios (in Chapter 9).

Table 2.1-1 Estimations of recommended intake of total fish and fatty fish for children and adolescents where no quantitative recommendations on fish are available (g/week). The estimations are based on recommendations for adults.

	Energy requirement MJ/day NNR	Proportion to estimate recommended fish intake for children and adolescents based on recommendations for adults	Estimated recommended intake of total fish g/week, based on adult recommendation of 300 g/week	Estimated recommended intake of total fish g/week, based on adult recommendation of 450 g/week	Estimated recommended intake of fatty fish g/week, based on adult recommendation of at least 200 g/week
Adults (ref)	10	100%	300	450	200
13-year-olds	9.6	96%	288	432	192

	Energy requirement MJ/day NNR	Proportion to estimate recommended fish intake for children and adolescents based on recommendations for adults	Estimated recommended intake of total fish g/week, based on adult recommendation of 300 g/week	Estimated recommended intake of total fish g/week, based on adult recommendation of 450 g/week	Estimated recommended intake of fatty fish g/week, based on adult recommendation of at least 200 g/week
9-year-olds	7.4	74%	222	333	148
4-year-olds	5.7	57%	171	257	114
2-year-olds	4.3	43%	129	194	86
1-year-olds	3.4	34%	102	153	68

In addition to the general recommended intake of 300-450 g fish per week, the national food safety authorities continuously issue regional advice to restrict consumption of fish from certain polluted lakes, fjords, and harbours as well as fish species or fish liver known to have high concentrations of pollutants. Several of such warnings are directed to pregnant and lactating women.

The basis for the quantitative recommendations in Norway is described in more detail in the report "Dietary advice to promote public health and prevent chronic diseases in Norway" (Norwegian National Council for Nutrition, 2011). In brief, recommendations are based on a summary of systematic literature reviews, meta-analyses, other reviews, statement papers and Cochrane reports published between 2000 and 2010.

A new edition of the Nordic Nutrition Recommendations (NNR) will be published in 2023. This edition will be the basis for new food-based dietary guidelines in Norway.

In July 2021, the US Food and Drug Administration (FDA) and the US Environmental Protection Agency (EPA) issued an updated advice for fish consumption encouraging women of childbearing age, pregnant women, and breastfeeding mothers to eat 2-3 servings of fish low in mercury per week and young children to eat 2 servings of fish low in mercury per week (EPA/FDA, 2021).

EFSA suggested that recommendations for fish consumption are issued on national or local levels because of great variations in fish consumption and in concentrations of contaminants in the various fish species among member states (EFSA, 2015).

2.2 Dietary reference values for comparison for selected nutrients in fish

The sections below give an overview of relevant dietary reference values established by various competent bodies for the selected nutrients included for this benefit and risk assessment.

For the purpose of comparison with the levels of nutrient exposures described in Chapter 8.3 for current exposures and Chapter 9.3 in the scenarios, the dietary reference value average

requirement (AR) is used in the semi-quantitative benefit assessment of the included nutrients. This is in line with a proposal for harmonised dietary reference values from WHO, FAO and National Academies of Science, Engineering and Medicine (NASEM), where AR and tolerable upper intake level (UL) are considered the core nutrient intake reference values for evaluating adequacy and safety for population groups (Allen et al., 2020). The US Institute of Medicine (IOM) has recently been renamed and incorporated into NASEM. Thus, all DRI reports through 2011 were published by IOM, while all subsequent reports are published by NASEM. The US term estimated average requirement (EAR) equals the average requirements (AR).

The AR/EAR is the primary reference value for evaluation of nutrient intakes, and the recommended intake (RI), lower intake (LI) and tolerable upper intake level (UL) can be used as complementary values (NNR Project Group, 2012).

AR/EAR is defined as an intake that is estimated to meet the requirement of approximately half the population of healthy individuals in a life stage and gender group (i.e., median requirement). RI (equal to PRI – population reference intake) is derived by adding two standard deviations to AR/EAR and is defined as the *average* long-term intake level of a nutrient that is estimated to meet the requirement of and maintain good nutritional status in almost all healthy individuals in a group.

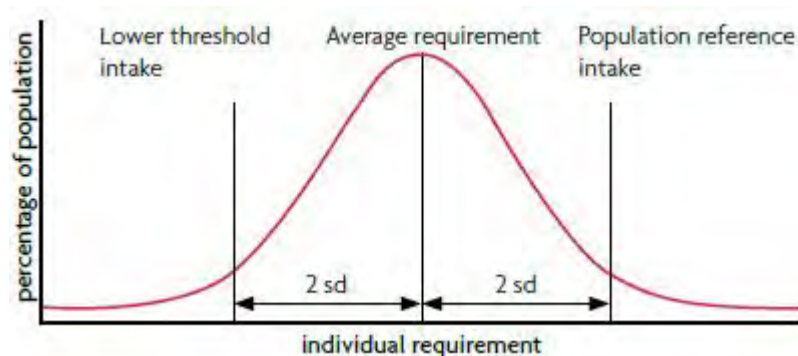


Figure 2.2-1 Population reference intake (PRI) and average requirements (AR), if the requirement has a normal distribution and the inter-individual variation is known (EFSA, 2010a).

Generally, UL is the maximum level of total chronic daily intake judged to be unlikely to pose a risk of adverse health effects (SCF, 2002). UL is considered relevant as reference value for comparison when evaluating fortifications of foods and drinks or food supplements but is not considered relevant for evaluating nutrients related to fish consumption or other foods that are not fortified with vitamins or minerals or do not contain extreme concentrations.

2.2.1 Inclusion and exclusion of nutrients

The project group has defined general inclusion/exclusion criteria for nutrients included in this benefit and risk assessment. These criteria are given in Table 2.2.1-1.

Table 2.2.1-1 Criteria for inclusion or exclusion of nutrients.

Criteria for inclusion	Criteria for exclusion
- Fish is an important source of the nutrient intake AND - Good and consistent evidence exists for a beneficial health effect of the nutrient	- Fish is not an important source of the nutrient intake - Lack of evidence for beneficial health effect and/or exposure

Fish as “important source” was defined by the project group as at least 20% contribution from fish to the total mean dietary intake of a specific nutrient (not including contribution from food supplements). According to intake calculations in adults for all nutrients fish contributed $\geq 20\%$ to the total intake of long chain n-3 fatty acids (LC n-3 FA), vitamin D, iodine, selenium, and vitamin B₁₂ from foods excluding supplements.

Table 2.2.1-2 gives an overview of contribution from fish and fish products to the total intake of nutrients contained in the Norwegian food and nutrient database and calculation system KBS (KBS-AE18) estimated for adults (mean of two registration days, Norkost 3).

Table 2.2.1-2 Overview of mean contributions in percent from fish to the total dietary intake of nutrients (Norkost 3), excluding food supplements.

Nutrient	Women, 18-70 years n= 925 % contribution from fish	Men, 18-70 years n= 862 % contribution from fish
EPA	39	42
DHA	38	42
DPA	23	23
Selenium	23	24
Iodine	22	24
Vitamin B ₁₂	22	24
Vitamin D	20	21
Vitamin E	7	8
Phosphorus	7	7
Potassium	5	6
Magnesium	4	5
Copper	4	4
Zink	3	4
Retinol	3	3
Folate	2	3
Iron	2	3
Calcium	2	2

In the sections below, we have given a brief summary of the derivation of AR for LC n-3 FA, vitamin D, iodine, selenium, and vitamin B₁₂ for adults and AR for vitamin D for children from the NNR (2012). AR for children for iodine, selenium, and vitamin B₁₂ are derived from the Institute of Medicine, USA, as NNR has only established ARs for children for vitamin D.

EFSA has established more recent adequate intakes (AI), but no ARs are available for our selected nutrients in the EFSA opinions. AI is the value estimated when a RI cannot be established because an average requirement cannot be determined. AI is the average observed daily level of intake by a population group that is assumed to be adequate (EFSA, 2017). The AIs for vitamin D, iodine, selenium, and vitamin B₁₂ from EFSA, are in the same orders of magnitude as the RIs from NNR given in Table 2.2.7-2 below.

We have, additionally, presented the recommended intakes (RI) for the selected nutrients. We have only presented recommended intakes for adults, and only the present Norwegian recommendations which are based on NNR (2012). These reference values are, however, not used in our benefit and risk characterisation.

2.2.2 LC n-3 fatty acid requirement

LC n-3 FAs are commonly known as the fatty acids, eicosapentanoic acid (EPA), docosapentanoic acid (DPA) and docosahexanoic acid (DHA). No AR or RI have been established for EPA, DHA or total LC n-3 FAs.

In NNR (2012), a recommendation is given that energy from n-3 fatty acid (including α -linolenic acid (ALA), as well as the marine LC-n-3 FAs) should be above 1% of the total energy intake, but no specific requirement or recommendation is given for the LC n-3 FAs.

In 2012, based on considerations of cardiovascular health, EFSA set an AI of 250 mg for EPA plus DHA for adults. For infants and young children (6-24 months) an AI for DHA was set at 100 mg (EFSA, 2010b).

For the total LC n-3 FAs (EPA, DPA, and DHA), no values for comparison with exposure estimates are presented in Chapter 9.3. VKM use the AI established for EPA plus DHA by EFSA (2010b) for comparison to exposures in adults presented in Chapter 9.3.

In addition, the inclusion of LC n-3 FAs in this benefit and risk assessment of fish is based on weight of evidence for specific health outcomes related to EPA, DHA or total LC n-3 FAs (see Chapter 5.2).

2.2.3 Vitamin D requirement and recommended intakes

Bone health is the selected indicator to form the basis for reference values for vitamin D intake in NNR (2012). The selection of bone health as indicator is based on a thorough evidence-based systematic reviews for all potential health endpoints for vitamin D (IOM, 2011, Lamberg-Allardt, 2013).

IOM (2011) considered calcium absorption together with bone mineral density, rickets, and osteomalacia to establish an optimal serum 25-hydroxyvitamin D (25OHD) concentration, the preferred marker of vitamin D-status reflecting both dietary intake and cutaneous production of vitamin D. IOM found congruence among these outcomes with a plateau of the effect between 30 and 40 nmol/L and no additional benefits of serum 25OHD concentrations higher than 50 nmol/L. IOM suggested that this level is consistent with an "recommended dietary

allowance-type" reference value in that this level appears to cover the needs of 97.5% of the population. A serum 25OHD concentration <30 nmol/L is regarded as indicating deficiency and between 30 nmol/L and 50 nmol/L is considered an insufficient vitamin D status (IOM, 2011). For the population, 40 nmol/L was consistent with the median requirement. In NNR (2012), a serum 25OHD concentration of 50 nmol/L is used as an indicator of sufficiency, a concentration of 30–50 nmol/L is considered to indicate insufficient status and a concentration of <30 nmol/L indicate vitamin D-deficiency.

NNR set AR for children and adults to be 7.5 µg/day. The identical ARs across age groups are notable and reflect the concordance of serum 25OHD levels with the integrated bone health outcomes as well as the lack of an age effect on the simulated dose-response (IOM, 2011).

In NNR (2012) some contribution of vitamin D from outdoor activities during the summer season is taken into account. This is in line with normal, everyday life and with recommendations on physical activity. It is however stated that a higher intake might be necessary in groups with limited sun exposure, limited access to outdoor activities, or skin pigmentation. In general, studies suggest that mean concentration of 25OHD is well above 50 nmol/L in the Norwegian population (Itkonen et al., 2021). However, there are seasonal variations, and a higher proportion of the population has concentration <50 nmol/L during winter. In addition, much lower concentrations have been reported in immigrants from Asia and Africa compared to the majority population.

For this benefit and risk assessment of fish consumption, VKM use the AR established by NNR. The ARs for vitamin D for adults and children that are used for comparison to exposures presented in Chapter 9.3 are given in Table 2.2.7-1. The Norwegian recommendations for intake of vitamin D is given in Table 2.2.7-2.

2.2.4 Iodine requirement and recommended intakes

In IOM (2001), the thyroid iodine accumulation and turnover were used to set the EAR. The normal thyroid gland takes up the amount of circulating iodine necessary to make the proper amount of thyroid hormone for the body's needs. Assuming iodine equilibrium, the mean daily thyroid iodine accumulation and release are similar. Thus, the average daily uptake and release (turnover) of iodine in the body can be used to estimate the average requirement of iodine, provided that the subjects tested have adequate iodine status and a normal thyroid gland function. Turnover studies are based on the intravenous administration of ¹³¹I and the calculation of thyroid iodine accumulation from measurements of thyroidal and renal radioiodine clearances, urinary iodine excretion and fractional thyroidal release rate. Over 90% of dietary iodine is excreted in the urine (Nath et al., 1992; Vought and London, 1967 in IOM 2001). IOM (2001) proposed the following equation to calculate daily iodine intake from the urinary iodine concentration (UIC):

$$\text{Daily iodine intake} = \text{UIC } (\mu\text{g/L}) \times 0.0235 \times \text{body weight (kg)}$$

The equation assumes that 92% of dietary iodine is absorbed. Although body weight is poorly correlated with urine volume in adults, the equation is a good approximation

considering an average 24-h urine volume of 1.5 L/day in adults. Alternatively, daily iodine intake can be estimated from UICs by estimating the daily urinary iodine excretion by means of the urinary creatinine concentration.

In a systematic literature review obtained prior to NNR (2012) aiming to summarise the scientific basis for the previous iodine recommendation in the Nordic countries (Gunnarsdottir and Dahl, 2012), the iodine requirement to prevent goiter was estimated to be 50-75 µg/day for adult women and men, and the AR was estimated to be 100 µg/day, an intake level at which the iodine concentration in the thyroid gland reaches a plateau. The NNR expert group also evaluated the scientific rationale for the recommended increased iodine intake during pregnancy and lactation from WHO (Gunnarsdottir and Dahl, 2012). In pregnancy, a higher iodine intake is recommended to cover for the higher thyroid hormone production and simultaneously increased excretion in the urine (Andersen and Laurberg, 2016). A higher iodine intake is also recommended during lactation to ensure sufficient iodine in the breast milk.

The AR/EARs for iodine for adults and children that are used for comparison to exposures presented in Chapter 9.3 are given in Table 2.2.7-1. The Norwegian recommendations for intake of iodine is given in Table 2.2.7-2.

2.2.5 Selenium requirement and recommended intakes

Keshan disease is a cardiomyopathy that occurs in children, and it is the only human disease that is firmly linked to selenium deficiency (IOM, 2000). The disease occurs with varying frequency in areas of China where the population is severely selenium-deficient (intake <20 µg/day) and selenium supplementation was able to prevent the condition (Ge et al., 1983). Twenty-five genes code for selenoproteins in which selenium is found as selenocysteine. Many selenoproteins are enzymes with important functions catalysing red/ox reactions. These selenoproteins require selenium for their synthesis and for maintenance of their activities in tissues. Several studies indicate that a higher intake than that preventing Keshan disease is beneficial for health, however, there have not been studies that can be used as a basis to determine selenium requirement in humans.

In the absence of a health indicator for determination of the selenium requirement, two plasma selenoproteins (glutathione peroxidase3 and selenoprotein P) can serve as indicators of selenium status. These plasma selenoproteins have been measured in individuals consuming varying amounts of selenium and have been used as basis for determination of a required intake of selenium. The SELENOP concentration was optimized by a daily intake of 49 µg and GSHPX3 activity was optimized by a daily intake of 35 µg (NNR, 2012). Translating the results of the Chinese intervention study to Nordic conditions and correcting for average body size, the recommended intake in the Nordic countries should be 60 µg/d for men and 50 µg/day for women (NNR, 2012). NNR (2012) set an AR of 30 and 35 µg/day for women and men, and it appears that the NNR (2012) without any further explanation based this on the optimization of GSHPX3 as NNR did in 2004. IOM (2000) established EAR for adult men and women (19-50 years) at 45 µg/day.

No data were found on which to base EARs for selenium for children or adolescents. EARs for children and adolescents from IOM were extrapolated downward using an adjustment for metabolic body size and growth. The formula for the extrapolation is given:

$$EAR_{\text{child}} = EAR_{\text{adult}} \times (\text{weight}_{\text{child}}/\text{weight}_{\text{adult}})^{0.75} \times (1+\text{growth factor})$$

NNR (2012) set AR to be 30 µg/day for women, and 35 µg/day for men. NNR and IOM have concluded differently regarding AR/EAR for selenium. There is no explanation given for this difference. For this benefit and risk assessment of fish consumption, VKM will use the AR established by NNR for adults, and the EAR established by IOM for children and adolescents.

The AR/EARs for selenium for adults and children that are used for comparison to exposures presented in Chapter 9.3 are given in Table 2.2.7-1. The Norwegian recommendations for intake of selenium is given in Table 2.2.7-2.

2.2.6 Vitamin B₁₂ requirement and recommended intakes

An AR is derived for vitamin B₁₂ (cobalamin) by IOM (1998) and NNR (2012).

In IOM (1998), no single indicator was judged to be a sufficient basis for deriving an EAR for vitamin B₁₂ for adults. To determine the amount of vitamin B₁₂ needed to maintain adequate hematological status (stable hemoglobin value, normal MCV and normal reticulocyte response), serum B₁₂ values in persons with pernicious anemia or with known intakes that were very low in dietary vitamin B₁₂, were used for deriving an EAR. It was the only approach for which there were sufficient and reliable data. Data on men and women were examined together because of small numbers.

The major cause of vitamin B₁₂ deficiency is pernicious anemia, and the hematological effects of vitamin B₁₂ deficiency are indistinguishable from those of folate deficiency. Neurological complications are present in 75-90% of individuals with clinically observable vitamin B₁₂ deficiency and may be the only clinical manifestation of vitamin B₁₂ deficiency. Vitamin B₁₂ deficiency is also frequently associated with various gastrointestinal discomforts, including sore tongue, appetite loss, flatulence and constipation (IOM, 1998).

In IOM (1998), the EAR for vitamin B₁₂ for adults was set to be 2 µg/day for both men and women. Data to set EAR for children were considered to be insufficient, and EAR for children and adolescents were extrapolated down from adult values and rounded up. The formula for the extrapolation is given:

$$EAR_{\text{child}} = EAR_{\text{adult}} \times (\text{weight}_{\text{child}}/\text{weight}_{\text{adult}})^{0.75} \times (1+\text{growth factor})$$

In NNR (2012), the AR for vitamin B₁₂ for adults was set to be 1.4 µg/day. NNR and IOM have concluded differently regarding AR/EAR for vitamin B₁₂. There is no given explanation for this difference. For this benefit and risk assessment of fish consumption, VKM will use the AR established by NNR for adults, and the EAR established by IOM for children and adolescents.

The AR/EARs for vitamin B₁₂ for adults and children that are used for comparison to exposures presented in Chapter 9.3 are given in Table 2.2.7-1. The Norwegian recommendations for intake of vitamin B₁₂ is given in Table 2.2.7-2.

2.2.7 Summary of reference values for comparison for the selected nutrients

Fish is an important source in the diet for LC n-3 FA (EPA, DHA and DPA), vitamin D, iodine, selenium, and vitamin B₁₂, and more than 20% of the total intake of these nutrients are derived from fish.

There are various dietary reference values for the micronutrients, and AR/EAR are considered the core nutrient intake reference values for evaluating population intakes for vitamins and minerals (Allen et al., 2020). Summary of average requirements for vitamin D, iodine, selenium, and vitamin B₁₂ from NNR (2012) and IOM (2001, 2000 and 1998) used for comparison with exposure estimates presented in Chapter 9.3 are given in Table 2.2.7-1. No ARs have been derived for either EPA, DHA or total LC n-3 FAs.

Table 2.2.7-1 Average requirements for vitamin D, iodine, selenium, and vitamin B₁₂ used for comparisons with exposure estimates presented in Chapter 9.3. All the values for adults and the value for vitamin D for children and adolescents are from NNR (2012). The other values for children and adolescents are from IOM (2001, 2000 and 1998).

Population group	Vitamin D µg/day	Iodine µg/day	Selenium µg/day	Vitamin B ₁₂ µg/day
Men, 19-<70y	7.5	100	35	1.4
Women, 19-<70y	7.5	100	30	1.4
Pregnancy	7.5	160	49	2.2
Lactation	7.5	209	59	2.4
Boys, 14-18y	7.5	95	45	2.0
Girls, 14-18y	7.5	95	45	2.0
Boys, 9-13y	7.5	73	35	1.5
Girls, 9-13y	7.5	73	35	1.5
Children, 4-8y	7.5	65	23	1.0
Children, 1-3y	7.5	65	17	0.7

The recommended intakes (RI) for vitamin D, iodine, selenium, and vitamin B₁₂ based on NNR (2012) are given in table 2.2.7-2. No recommended intakes have been established for either EPA, DHA or total LC n-3 FAs in Norway, but EFSA has established an AI for EPA plus DHA (EFSA, 2010b) at 250 mg/day.

Table 2.2.7-2 Norwegian recommendations for intake of vitamin D, iodine, selenium, and vitamin B₁₂ based on NNR (2012) and AI for LC n-3 FA from EFSA (2010b).

Population group	Vitamin D µg/day	Iodine µg/day	Selenium µg/day	Vitamin B ₁₂ µg/day	EPA+DHA, mg/day
≥75y	20	-	-	-	-
Men, 18-<70y	10	150	60	2	250

Population group	Vitamin D µg/day	Iodine µg/day	Selenium µg/day	Vitamin B ₁₂ µg/day	EPA+DHA, mg/day
Women, 18-<70y	10	150	50	2	250
Children, 10-13y	10	150	40	2	-
Children, 6-9y	10	120	30	1.3	-
Children, 2-5y	10	90	25	0.8	-

2.3 Established tolerable intakes for contaminants in fish

2.3.1 Inclusion and exclusion of contaminants

The present subchapter explains the considerations around selection of contaminants to include or exclude from the present benefit and risk assessment of fish consumption in Norway. The contaminant groups dioxins and dl-PCBs, and PFASs, were listed in the mandate from the NFSA and are therefore to be included in the present benefit and risk assessment. According to the terms of reference, it was up to VKM to decide which additional substances to include in the assessment.

As a starting point, a long list covering a wide range of possibly relevant substances was suggested for consideration by members of the project group and members of the Scientific steering committee (Chapter 18 Annex III). These suggestions were made based on general knowledge on toxic substances possibly present in fish. VKM made a selection following a decision process as outlined in the text below and in Figure 2.3.1-1. For completeness, also the substance groups already outlined in the mandate were included in the selection process.

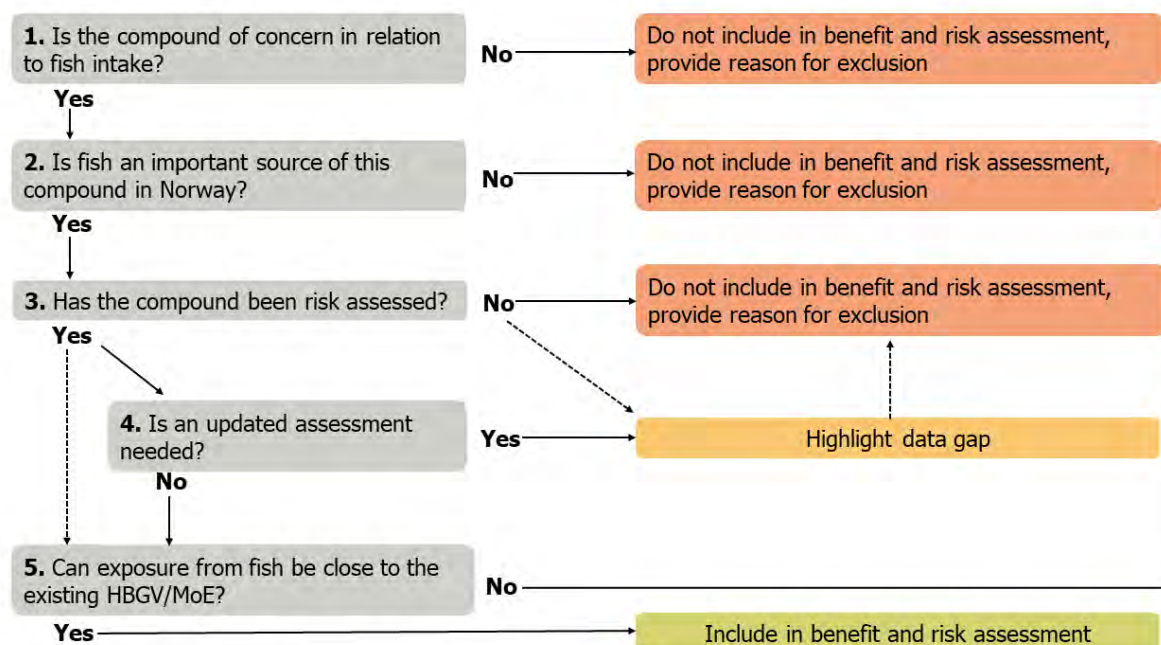


Figure 2.3.1-1 Flow chart describing the decision process for inclusion or exclusion of candidate contaminants for the benefit and risk assessment of fish in the Norwegian diet. Grey boxes are questions that require an evaluation, yellow boxes are steps that require

further action, and the green and red boxes are the final steps. HBGV: health-based guidance value, MoE: margin of exposure.

The first question that was asked for each compound or group is whether the compound is of concern in relation to fish intake, i.e., whether the compound is normally found in fish species that are consumed as food in Norway, and further, if fish is an important source for this compound in Norway. If the answer to one or both of these questions were 'no', VKM considered this as reasons not to include the compound in the benefit and risk assessment. If the answer to both questions were 'yes' we moved to the next question in the flowchart.

The third was whether the compound has been assessed previously, i.e., if there is an established Health Based Guidance Value (HBGV, e.g., tolerable daily intake; TDI, tolerable weekly intake; TWI) or a safe Margin of Exposure (MoE) for the compound. If the answer was 'yes', we moved to the fourth question. If the answer was 'no', the next step would be to highlight the need for a risk assessment as a data gap in the assessment.

Question four was whether an updated assessment is needed, i.e., if there is new knowledge/data available that could justify a new HBGV/MoE. If the answer to this question was 'yes', the next step would again be to highlight the need for a new risk assessment as data gap. If the answer was 'no', we moved to the next question.

Question five was to consider if exposure to the compound (from fish intake) can be close to the HBGV or MoE. If the answer to Q5 was 'yes', then VKM concluded that the compound should be included in the benefit and risk assessment. If the answer to the question was 'no', VKM considered this as a reason not to include the compound in the benefit and risk assessment.

Description of each substance or substance group and considerations made when deciding inclusion or exclusion is detailed in Chapter 17, Appendix IV.

Methyl mercury (MeHg) was the only compound that was included based on this decision process. As shown in Appendix IV PCDD/Fs and DL-PCBs and PFASs, the compounds given in the mandate, would also have been included based on these inclusion criteria.

To investigate whether the risk assessment of mercury conducted by EFSA in 2012 was still valid (Q4), a literature search on systematic review papers was conducted and a search for original publications addressing mercury exposure and health outcomes in the Norwegian population were conducted (described in Appendix IV). The literature review did not provide strong indications that the risk assessment by EFSA of methylmercury from 2012 needs revision. However, VKM notes that there might be new primary studies and outcomes that have not been captured by the available reviews.

As concluding remarks from the selection process, fish may contain a wide range of contaminants, and as illustrated in the text in Appendix IV, there are various reasons why not all of them are considered separately in the present benefit and risk assessment.

However, it needs to be kept in mind that all these substances, as well as the nutrients, are present in the fish consumed in the epidemiological studies on associations between fish intake and health outcomes that are described in detail in Chapter 4.

2.3.2 PCDD/F and DL-PCB

The group of persistent organic pollutants (POPs) often referred to as “dioxins and dioxin-like PCBs” is a group of environmental contaminants that are assessed together based on their similar toxicity. The group refers to 29 individual substances belonging to polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (DL-PCBs). Substances in each group with similar backbone and different numbers and positions of chlorines are called chemical congeners. The term used in the present opinion for the sum of the 29 congeners is PCDD/Fs and DL-PCBs.

PCDD/Fs and DL-PCBs are fat-soluble and persistent to degradation, they bioaccumulate and are biomagnified in the environment. They are found in the highest concentrations in organisms located high up in the food chain. Fat of animal origin, and in particular marine fat, is the major dietary exposure source.

Based on the dioxin toxic equivalency (TEQ) scheme (see Chapter 2.3.2.1), human health risk assessments have been conducted for the total exposure to PCDDs, PCDFs and DL-PCBs. In 2018, the European Food Safety Authority (EFSA) reassessed the hazards of PCDDs, PCDFs and DL-PCBs and established a new and lower Tolerable Weekly Intake (TWI) at 2 pg TEQ/kg bw/week (EFSA, 2018b). The new TWI is 1/7 of the previous TWI of 14 pg TEQ/kg bw/week established in 2001 (SCF, 2001). The TWI was reduced based on new epidemiological and experimental animal data on the toxicity of these substances, and more refined methods for predicting the concentrations of the substances in the human body over time. The epidemiological studies have been conducted in subjects/cohorts exposed to PCDD/Fs and DL-PCBs at different life stages under different exposure conditions, e.g. from industrial accidents or contamination incidents, from occupational exposure or from background levels mainly via the diet in the general population. In the present assessment the new TWI is used for assessing the risk connected to dietary intake of hazardous PCDD/Fs and DL-PCBs in Norway (see also VKM, 2022: Risk assessment of dioxins, furans and dioxin-like PCBs in food in Norway).

The TWI is based on an association between serum levels of the sum of PCDD/Fs and the decreased sperm concentrations as the critical effect. The evidence suggests a postnatal period of sensitivity that might expand into puberty.

2.1.1.1 *PCDD/F and DL-PCB Toxic equivalent factors (TEFs)*

Both these compound groups have various detrimental health effects most of which are mediated through the aryl hydrocarbon receptor (AhR). Toxic equivalency factors (TEFs) have been set based on experimental evidence for 17 PCDD/F congeners and 12 dioxin-like PCB congeners with respect to their potency to induce toxic or biological effects through the AhR. The TEF value of the most toxic congener, 2,3,7,8-tetrachlorodibenzodioxin (TCDD), has been set to 1, and the TEF values of other congeners to 0.00003–1. Multiplication of the

measured amount of each congener by its respective TEF value gives the amount that is toxicologically equivalent with TCDD. Summing up the TEF-weighted amounts of all congeners in a mixture gives the approximate amount that is equivalent to TCDD in toxicity (toxic equivalent sum, TEQ) (Van den Berg et al., 2006). The toxicity equivalency factors proposed by the World Health Organization in 2005 (WHO₂₀₀₅-TEFs) are used in this assessment unless otherwise stated (Chapter 20, Appendix VII).

It needs to be noted that *"TEFs are internationally agreed weighted values that are based on animal studies and supported by in vitro studies. TEFs are used to enable expressing the toxicities of PCDD/Fs and DL-PCBs on a common scale, relative to TCDD. When setting TEFs, the underlying relative effect potencies that are determined for each congener show a large range of values, due to factors like animal species/strain, measured endpoint and duration of exposure."* The most recent TEFs (WHO-TEF₂₀₀₅) are rounded based on a log scale, and each value as such presents an order of magnitude in different potencies (see values in Appendix VII, Chapter 20). TEFs are thus not a precise estimate of the toxic potency of a congener, and this may affect the interpretation of both human and animal studies. In particular, the TEF of PCB-126 was discussed in the EFSA opinion in 2018, and EFSA referred to studies indicating lower potencies in humans than in rodents, which are presently the major basis for the PCB-126 TEF. EFSA 2018 stated that:

"The CONTAM Panel noted that in the Russian Children's Study, no association was observed for DL-PCB TEQ or the sum-TEQ of PCDD/Fs and DL-PCBs. This might be explained by observations from in vitro studies with human cells, showing that PCB-126 is much less potent in humans than suggested by the WHO₂₀₀₅-TEF of 0.1. PCB-126 is the DL-PCB contributing most to the current intake of PCDD/Fs and DL-PCBs, but also in the serum of boys from the Russian Children's Study."

Since the TEF of PCB-126 is relatively high compared to the TEFs for the other DL-PCBs, it has high impact on the total TEQ concentration in food or in blood. Of note, the TEFs are updated at irregular intervals based on new scientific information. The TEFs set by WHO in 2005 as published in Van den Berg et al (2006) are currently under revision by WHO and scheduled to be finalized by the end of 2022 (FAO, 2021).

As the hazard characterization done by EFSA is based on serum concentrations of the sum of PCDD/Fs in the critical study, and extended to include DL-PCBs, a change in TEFs for DL-PCBs will not affect the TWI set by EFSA in 2018. However, a change in TEFs for DL-PCBs will make it necessary to update the exposure assessment to the sum of PCDD/Fs and DL-PCBs based on new TEFs. If the revision of TEFs by WHO will also result in changes in TEFs for PCDD or PCDFs that are major contributors to serum levels, also a revision of the TWI might become necessary.

2.3.3 PFAS

Poly- and perfluoroalkyl substances (PFASs) is the collective name for a vast group of fluorinated substances containing more than 7000 compounds. PFASs are synthetic chemicals that are very persistent to environmental degradation, several of them bioaccumulate and are biomagnified in the environment and in humans. Earlier risk

assessments have focused on the most prevailing PFASs perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS), but an assessment from EFSA published in 2020 also included perfluorononanoic acid (PFNA) and perfluorohexane sulfonic acid (PFHxS) in a TWI (EFSA, 2020). The new TWI for the sum of the four PFASs PFOA, PFNA, PFHxS and PFOS is 4.4 ng/kg bw/week (EFSA, 2020). The new and lower TWI replaces the previous temporary TWIs set for PFOS and PFOA as individual substances (EFSA, 2018a).

The TWI is based on effects on the immune system (decrease in antibody response after vaccination of children), which were considered the most critical for the risk assessment based on available studies in animals and humans.

The present benefit-risk assessment is restricted to the four PFASs PFOA, PFNA, PFHxS and PFOS. The basis for focusing on these substances can be found in Appendix IX, Chapter 22.

2.3.4 Methyl mercury

Mercury is released to the environment from both natural and anthropogenic sources. Mercury is methylated to methylmercury by microorganisms both in water and in sediments. Methylmercury is readily bioavailable and bioaccumulates in aquatic food chains, leading to elevated mercury concentrations in predatory fish. Human exposure to methylmercury is mainly dietary, and fish and other seafood is the main dietary source. VKM apply a conservative approach, based on the assumption that all mercury found in fish and other seafood is methylmercury. EFSA has established a TWI for methylmercury of 1.3 µg/kg bw/week (expressed as mercury) based on human neurodevelopmental outcomes after prenatal exposure (EFSA, 2012). Pregnant women and their foetuses are therefore the population group most vulnerable to dietary methylmercury exposure.

2.3.5 Summary of tolerable weekly intakes for the selected contaminants

VKM based the risk characterization of contaminants on tolerable intakes set by EFSA for the three contaminant groups included based on the inclusion and exclusion process as outlined in Chapter 6 and Appendix IV, Chapter 17. The tolerable intakes are summarized in table 2.3.5-1.

Table 2.3.5-1 Overview of tolerable intakes of contaminants considered specifically.

Contaminant	Tolerable intake	Reference
PCDD/Fs and DL-PCBs	2 pg TEQ/kg bw/week	EFSA, 2018
PFASs (sum of PFOA, PFNA, PFHxS and PFOS)	4.4 ng/kg bw/week	EFSA, 2020
Methyl mercury	1.3 µg/kg bw/week	EFSA, 2012

2.4 References

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3 Systematic literature review and weight of evidence methods

Three systematic literature reviews (SLRs) were conducted for this benefit and risk assessment. The objective of the first SLR was to evaluate the epidemiological evidence for associations between fish consumption and selected health outcomes (summarized in Chapter 4). The first review on fish covered both primary studies and previous SLRs including meta-analyses. The objective of the second SLR was to evaluate the epidemiological evidence for associations between specific nutrients for which fish is an important source (LC n-3 FA, vitamin D, iodine, selenium, and vitamin B₁₂) and health outcomes (summarized in Chapter 5). The review on nutrients was limited to previous SLRs and meta-analysis and included nutrient intake from diet and/or supplements. For the third review on evaluation of risks related to contaminants in fish, we have based our work on existing EFSA opinions for PCDD/Fs and DL-PCBs, PFASs and methyl mercury. Because the most recent EFSA opinion for methyl mercury was from 2012, a third SLR was conducted to search for more recent studies that could imply the need for an update of the TWI for methyl mercury set by EFSA in 2012 (summarized in Appendix IV, Chapter 17).

The literature search methods are described in Chapter 3, sections 3.1 (fish), 3.2 (nutrients) and 3.3 (methyl mercury). The description includes the databases searched, inclusion and exclusion criteria, quality assessment of eligible studies, and data extraction.

The quality assessment tools used for primary studies (the fish SLR only) and systematic reviews and meta-analyses (the fish and nutrient SLRs) are described in Chapter 3.1.3.

The criteria used for weight of evidence for associations between fish consumption and various health outcomes, and for associations between the specific nutrients in fish (LC n-3 FA, vitamin D, iodine, selenium, and vitamin B₁₂) and various health outcomes are described in Chapter 3.1.6.

It should be noted that the evidence for associations between adverse health outcomes related to the included contaminants are evaluated by EFSA, not by VKM, and hence were not derived using the same quality assessment tools or weight of evidence criteria as the evidence for beneficial and adverse outcomes related to fish consumption or the nutrients in fish as performed by VKM. The level of evidence for associations is therefore not directly comparable.

3.1 Methods for the systematic literature review of fish consumption and health outcomes

The objective of the systematic literature review was to identify beneficial or adverse health effects associated with consumption of fish as such. This was conducted through a systematic literature search, quality assessment of the identified literature, and a weight of evidence (WoE) approach. The systematic literature review process generally followed

commonly accepted guidelines (e.g., PRISMA or JBI) for searching, selecting and reporting the literature, but data extraction was mainly performed by only one person. Samples of extracted data were however controlled by a second reviewer, and the parameters to be extracted were thoroughly discussed and agreed upon by the project group. When possible, the dose-response relationship for beneficial or adverse associations are described.

The systematic literature review on fish had two parts: one search for original (primary) studies and one for previous systematic reviews and meta-analyses (for more details see section 3.1.1 below).

Chapter 3.1 is divided into the following sections: (3.1.1) a presentation of the literature searches, (3.1.2) selection of studies for inclusion, (3.1.3) quality assessment, (3.1.4) data extraction from primary studies, (3.1.5) data extraction from meta-analyses and systematic reviews, (3.1.6) a presentation of the guidelines for grading evidence, and (3.1.7) pooled analysis estimates.

The searches and methods for selection and evaluation of eligible studies described in this section correspond to the results presented in Chapter 4 Fish intake and health outcomes.

3.1.1 Search strategies for fish and health outcomes

Literature searches were conducted to retrieve the best available evidence on health effects of fish consumption to respond to the terms of reference questions:

Which health consequences will occur for the Norwegian population if the population:

- Continues with the same consumption levels of fish as of today
- Increases the consumption of fish to match the recommendations given by the Norwegian Directorate of Health

The general search strategy was guided by PICO as shown below.

Table 3.1.1-1 The research question and PICO diagram used for our literature search.

PICO-question	Population	Intervention	Comparison	Health outcomes
What could be the potential health consequences if the Norwegian population maintains, increases, or reduces their consumption of fish	General population	Fish intake	High-low	CVD-outcomes Mortality Neurodevelopmental outcomes Birth outcomes Type 2 diabetes Bone health Dental enamel changes Overweight and obesity Immunological diseases Male fertility

We selected the health outcomes based on:

- 1) established knowledge about fish consumption and health outcomes
- 2) relevance for fish consumption and common non-communicable diseases
- 3) health outcomes relevant for the included nutrients and contaminants

Health outcomes with a well-established association to fish were identified from published systematic reviews and meta-analyses, NNR (2012), IOM (2001 and 2011), as well as previously published benefit and risk assessments of fish from VKM and EFSA. To identify these systematic reviews and meta-analyses VKM performed a non-systematic, preparatory search in Google Scholar and MEDLINE. This preparatory search was not a part of the systematic literature review. The search is described in Appendix II, Chapter 15.1.

VKM then used the abstracts and method sections in these publications to identify health outcomes, search terms and inclusion and exclusion criteria. To identify search terms and text words for the relevant health outcomes, VKM also used the project group's expertise, and when needed, consulted other experts.

VKM also identified health outcomes for the included nutrients (LC n-3 FA, vitamin D, iodine, selenium, vitamin B₁₂) and contaminants (PCDD/F and DL-PCBs, PFAS, methyl mercury) and included them in the search strategy for fish and health outcomes.

With exception from semen quality parameters (biomarker for male fertility), intermediate endpoints such as biomarkers for disease – e.g., cholesterol, blood pressure or other intermediate markers were not included in the search.

Cancer was not included in the search as our conclusions regarding cancer will be based on World Cancer Research Fund's thorough reviews of evidence for different food groups/compounds and risk of cancers (WCRF, 2018).

3.1.1.1 Literature search for primary studies

The health outcomes that were included in the literature search for fish consumption and primary studies can be divided into the following main categories: coronary heart diseases and cardiovascular diseases, mortality, neurodevelopment and cognitive functioning, bone health, dental enamel changes, immunology and allergy, male fertility, overweight and obesity, birth outcomes, diabetes, and goitre.

Two research librarians at the Norwegian Institute of Public Health drafted the search strategy for human primary studies on fish consumption and health outcomes. This strategy was further refined based on discussions among members of the project group. We searched the databases Ovid MEDLINE®, Embase and PsycINFO from inception to the 25th of November 2019. An updated search was performed the 8th of October 2021. The search strategy and search terms are available in Appendix II, Chapter 15).

We imported the identified records into EndNote (Thomson Reuters, version X9), removed duplicates, and imported the records into Rayyan (Ouzzani et al., 2016) for screening. Before duplicate check by the librarians in Endnote, this search encountered 30 558 hits, and 21 857 hits were left after duplicate check. However, the members in the project group found several duplicates in the further exclusion process in Rayyan. These may later have been registered as excluded and we may therefore not have the exact number of original hits. The updated literature search resulted in 5744 hits before duplicate check, and 4527 after duplicate check. The numbers shown in the flow chart in Figure 3.1.3.1-1 includes both original and updated searches.

3.1.1.2 Literature search for systematic reviews and meta-analyses

In addition to the search for human primary studies, the librarians also conducted a search with identical search terms, filtered for systematic reviews and meta-analyses, to check for systematic reviews or meta-analyses that had weighted the evidence for fish intake and any of the included health outcomes. This search was limited back in time to 2016 and performed in Ovid MEDLINE® and Embase. This search was originally performed the 15th of December, 2020. An updated search was performed the 5th of October, 2021. The search strategies and search terms for both the original and the updated searches are available in Appendix II, Chapter 15.3.

We imported the identified records into EndNote (Thomson Reuters, version X9), removed duplicates, and imported the records into Rayyan (Ouzzani et al., 2016) for screening. After duplicate check by the librarians in Endnote, this search encountered 488 hits. However, the members in the project group found some duplicates in the further exclusion process in Rayyan. These may later have been registered as excluded and we may therefore not have the exact number of original hits. The updated literature search resulted in 310 hits after duplicate check. The numbers shown in the flow chart in Figure 3.1.3.1-2 includes both original and updated searches.

3.1.2 Selection of studies

A systematic approach was used for the selection of papers/studies from both literature searches. Screening of titles and abstracts was performed in a pairwise blinded manner using Rayyan, a web application for systematic reviews (Ouzzani et al., 2016). The screening was performed against pre-defined inclusion/exclusion criteria. These criteria are detailed in Tables 3.1.2-1 and 3.1.2-2 below, including study design, population groups and fish consumption criteria. Cross-sectional studies were not filtered out in the setup of the literature search but were excluded in the selection process in Rayyan.

After the first round of screening, the blinding was removed, and the reviewers discussed conflicting decisions. If the two reviewers were unable to reach an agreement, the paper in question was included. If two articles were published from the same cohort data, but in different follow-up durations, the article with the longest follow-up study was chosen.

The potentially relevant papers selected via the screening procedure based on title and abstract was then reviewed in full text. For the primary studies, this was done in a pairwise

blinded manner, using Rayyan, and based on the same inclusion and exclusion criteria. The two rounds of screening resulted in 346 full text papers (primary studies). These were quality assessed as described in the Chapter 3.1.3.1 below.

All assumed relevant meta-analyses and systematic reviews that fulfilled the inclusion criteria were evaluated in full text (84 in original search, 18 in updated search) included papers after screening of titles and abstracts). However, some reviews were excluded after full text reading as they turned out not to be systematic (e.g., a reproducible methodology with search strategy and eligibility criteria for studies were not described), or not relevant for fish (i.e., dietary pattern reviews not including specific intake data for fish). All included systematic reviews and meta-studies were quality assessed using AMSTAR (see 3.1.3.2 below).

Table 3.1.2-1 Inclusion/exclusion criteria for literature from the systematic searches for primary studies related to fish intake and health outcomes.

Criteria for inclusion	• Studies investigating fish intake in relation to one or more health outcomes included in the systematic search • Study designs: - o Longitudinal observational studies, such as: Cohort studies, Case-cohort studies, Nested case-control studies, Case-control studies - o Experimental studies, such as: Randomised Controlled Trials (RCTs), Controlled Clinical Trials, Controlled Before-and-After studies • Population: general population, all age groups. Persons with the following conditions are considered part of the general population and will be included: - o Diabetes type 2 - o Obesity - o Musculoskeletal disorders • Publication type: original papers • Other: fish intake needs to be measured at the individual level, effect estimates must be given. Studies on secondary prevention should be included
Criteria for exclusion	• Studies investigating fish intake without any relation to the specific health outcomes included in the search. • Studies investigating exposure to supplements only (n-3/fish oil/vitamin D). • Dietary pattern studies • Publication types: - o reviews - o case histories - o letters to editors - o book chapters - o posters - o abstracts • Population: specific patient groups (see inclusion criteria for exceptions) • Study designs: - o Cross-sectional studies - o Animal studies - o <i>In vitro</i> studies

Table 3.1.2-2 Inclusion/exclusion criteria for literature from the systematic searches for systematic reviews and meta-analyses related to fish intake and health outcomes.

Criteria for inclusion	• Studies investigating fish intake in relation to one or more health outcomes that were included in the systematic search • Study designs: ◦ Systematic reviews and meta-analyses of observational studies, such as: Cohort studies, Case-cohort studies, Nested case-control studies, Case-control studies, AND/OR of experimental studies, such as: Randomised Controlled Trials (RCTs), Controlled Clinical Trials, Controlled Before-and-After studies • Population: general population, all age groups. Persons with the following conditions are considered part of the general population and will be included: ◦ Diabetes type 2 ◦ Obesity ◦ Musculoskeletal disorders • Publication type: Systematic reviews and meta-analyses • Other: fish intake needs to be measured at the individual level, effect estimates must be given. Studies on secondary prevention should be included
Criteria for exclusion	• Systematic reviews or meta-analyses investigating fish intake without any relation to the specific health outcomes included in the search. • Systematic reviews or meta-analyses investigating exposure to supplements only (n-3/fish oil/vitamin D). • Dietary pattern reviews or meta-analyses without specified estimates for fish intake • Systematic reviews and meta-analyses with estimates only from: ◦ Cross-sectional studies ◦ Animal studies ◦ <i>In vitro</i> studies • Population: specific patient groups (see inclusion criteria for exceptions)

3.1.3 Quality assessment

3.1.3.1 *Quality assessment of primary studies*

All the included full text papers were graded in a three-category (A, B or C) rating system considering internal validity. The quality assessment tool tables have been developed for Nordic Nutrition Recommendations (NNR) (Nordiska ministerrådet, 2014). There are quality assessment tools especially designed for the different study designs. We used the tools for randomised controlled trials, prospective cohort studies, case-control studies and nested case-control studies. The quality assessment tools were modified to fit our purpose, and for prospective cohort studies the scoring was also slightly adapted for our purpose. The review of the full text papers and the quality assessment was conducted independently by two reviewers. Disagreement on the final rating of a paper was resolved by consensus. When necessary, a third reviewer was included for decision. Only papers graded as A or B category in the quality assessment have been included in the further process. Very few papers were graded A, and a distinction between A and B has not been pointed out as it had no impact on any conclusions. Papers graded as C were excluded from this benefit and risk assessment. They are listed with reason for category C in [Supplement A](#). The quality

assessment tool templates and an overview of the modifications made, are given in Appendix III, Chapter 16.1.

As previously noted, VKM did not evaluate the evidence for associations between adverse health outcomes related to the included contaminants. These were evaluated by EFSA. In the EFSA Opinion on PCDD/F and DL-PCBs, EFSA used the OHAT Risk of Bias Tool for assessment of the reliability of primary studies (Rooney et al., 2014). In the opinions on PFASs and methyl mercury, no standardized tools for quality or risk of bias assessment was used, and the quality of the included studies in the EFSA opinions was assessed by expert judgement.

Adaptation of the scoring in the quality assessment tool for our purpose

In the quality assessment tool for prospective cohort studies, question 2 b.) "Response rate reported and acceptable?"; this question was originally a question that if not answered Yes, would lead to a category C classification. However, this turned out to be too strict. Most often the response rate was not reported or given in supplementary material or other references. Due to the high number of publications included in the assessment at this stage, checking all supplementary material was not feasible. Therefore, in the present assessment Question 2b) did not need to achieve the answer 'YES' to obtain the B category.

Several of the members in the project group participated in the process of selecting the studies and assessing their quality. The reviewers held repeated calibration meetings along the way to minimize different interpretations and handling among the reviewers.

Box 1 includes the criteria for assessing the methodological quality of the studies for A, B and C categories. These criteria were developed for the Nordic Nutrition Recommendations (NNR) (Nordiska ministerrådet, 2014).

Box 1. Criteria for assessing the methodological quality of the studies: The three category quality grading system. The studies should be evaluated and graded within their own design strata.

A. The results from studies that have an acceptably low level of bias are considered valid. These studies adhere mostly to the commonly held concepts of high quality including the following: a comprehensive study design; clear description of the participants, setting, interventions, and control group(s); appropriate measurement of outcomes; appropriate statistical and analytical methods and reporting; less than 30% dropout (depending on the length of the study see the QAT for clinical studies) or over 50% participation rate for prospective cohort studies; clear reporting of dropouts; and no obvious bias. Where appropriate, studies must provide a valid estimation of food intake/nutrient exposure, from dietary assessments and/or biomarkers with a reasonable range of measurement error, and justification for approaches to control for confounding in the design and analyses.

B. Studies may have some bias, but not sufficient to invalidate the results. They do not meet all the criteria in category "A", they have some deficiencies but none likely to cause major bias. The study may be missing information, making it difficult to assess limitations and potential problems.

C. Studies have significant bias that may invalidate the results. These studies have serious errors in design, analysis, or reporting; there are large amounts of missing information, or discrepancies in reporting.

A flow chart for the complete process of including and excluding papers from the search for primary literature on health outcomes related to fish consumption as such is shown in Figures 3.1.3.1-1 and 3.1.3.1-2.

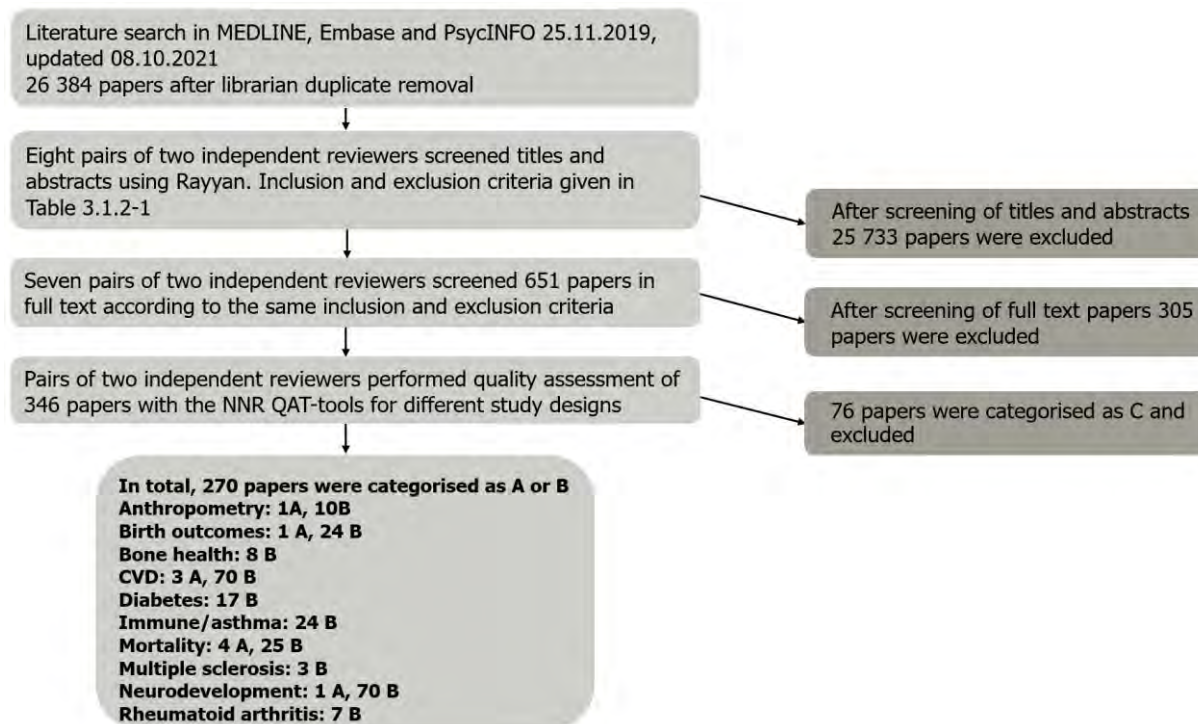


Figure 3.1.3.1-1 Flow chart for the complete process of including and excluding primary studies for health outcomes related to fish consumption. The numbers include both original and updated searches.

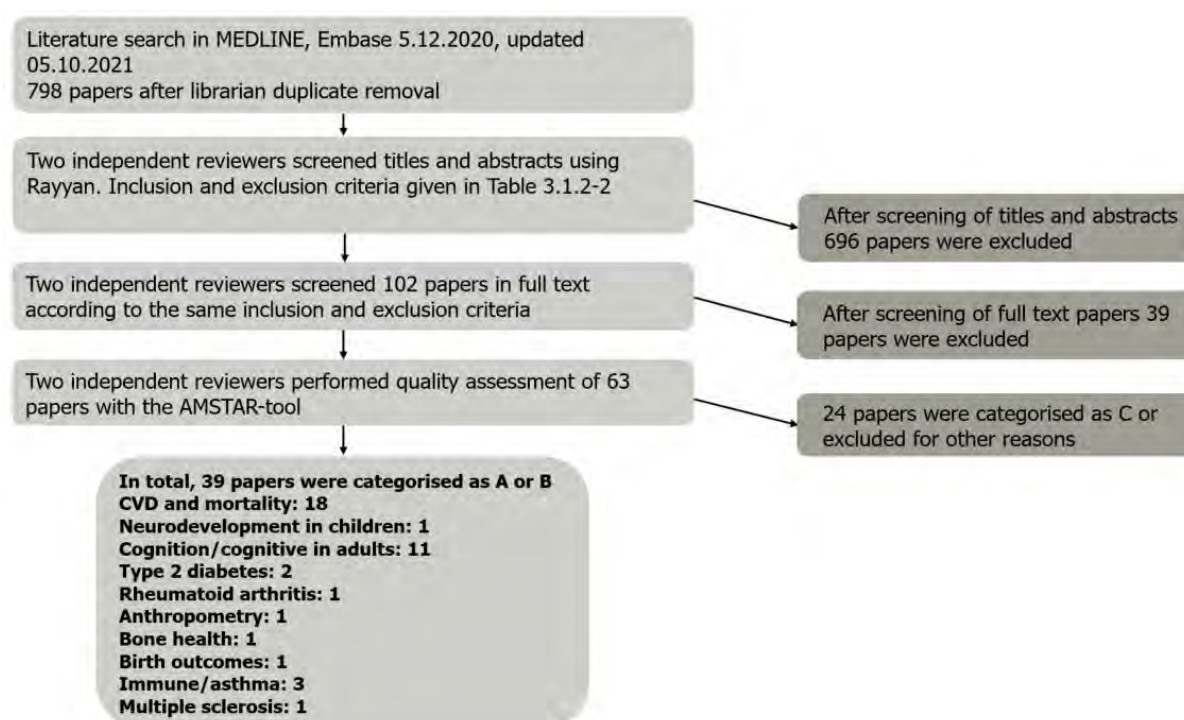


Figure 3.1.3.1-2 Flow chart for the complete process of including and excluding systematic reviews and meta-analyses for health outcomes related to fish consumption. The numbers include both original and updated searches.

3.1.3.2 *Quality assessment of systematic reviews and meta-analyses (AMSTAR)*

For assessment of the methodological quality of the included systematic reviews and meta-analyses the AMSTAR tool (version 1; Shea et al., 2007) was used. However, AMSTAR version 1 was originally created for systematic reviews of RCTs, while AMSTAR version 2 has been developed to appraise systematic reviews of both randomised and non-randomized studies. Therefore, when in doubt, we checked AMSTAR version 2 (Shea et al., 2017) for interpretation. After separate quality assessment of the papers by two independent reviewers, they discussed and agreed on category A, B or C for each paper. If in doubt, a third reviewer was contacted. Minor adaptations were made to fit our purpose, these are described below. The AMSTAR tool questions, and grading template can be found in Appendix III, Chapter 16.2. Papers graded A or B were kept, while papers graded C were excluded.

Box 1 (in Chapter 3.1.3.1) includes the criteria for assessing the methodological quality of the studies for A, B and C categories.

Adaption of the grading in AMSTAR quality assessment tool for our purpose

In the AMSTAR quality assessment tool (AMSTAR version 1), 'Yes' to question 5 "Was a list of studies (included and excluded) provided"; was originally a category B requirement.

However, this turned out to be too strict, as very few publications included a proper list of excluded studies, and the project group realised that this would cause too many highly relevant papers of otherwise good quality to be excluded. The criteria were therefore changed, and 'No' to question 5 was allowed for papers graded as category B.

3.1.4 Data extraction from primary studies

Data were extracted from all category A and B papers by one reviewer. Samples of extracted data were controlled by a second reviewer. The parameters to be extracted were thoroughly discussed and agreed upon by the project group.

Data were extracted using a template with two parts. The first part included data related to the study overall: the first author's last name, publication year, country or countries where the study was conducted, study name and design, study aim, study period and follow-up time (prospective studies), study population (gender, age, other characteristics), sample size (after exclusions), exclusion criteria, dietary assessment method (type, reference period for intake, whether it was validated), data collected on fish intake in study (items assessed and whether fish overall included shellfish or seafood items) or in the case of the intervention studies, the type of intervention was extracted. Study funding sources and conflict of interest statements were reviewed for the different studies during extraction.

The second part of the template included details on each specific study result extracted: outcome and outcome definition, gender (men, women, or combined), number of cases, fish exposure (type of fish such as total fish, fatty- or lean fish, dark- or white fish, fried- or non-fried fish, saltwater- or fresh water fish, species of fish; intake unit, intake range, quantity in grams when available), effect estimate with confidence interval for the highest versus lowest intake (or continuous intake), and a description of the overall result (null finding, or direction of association, test for trend if given), variables adjusted for, and information regarding sensitivity analyses and/or effect modification (also referred to as interaction effects). For studies that presented major results as figures only, WebPlot Digitizer was used in a few cases to extract the high-low risk estimate. Studies that investigated potential non-linearity of associations were commented on in the text, but data was not extracted from curvilinear figures.

Most studies contributed results on multiple fish-exposures and/or outcomes. Data were extracted for every relevant exposure-outcome combination within each study. Estimates were extracted from the maximally adjusted risk model to account for the largest number of potential confounding factors, including lifestyle factors, except when the purpose of the model was to study mediation. Most studies analyzed and presented categories or quantiles of fish intake and the effect estimate with confidence interval was recorded for the highest versus lowest intake category for comparison with previous high-low meta-analyses.

The completed extraction templates were combined into databases used for generating tables and synthesizing evidence as presented in Chapter 4.

3.1.5 Data extraction from systematic reviews and meta-analyses of fish intake

Data were extracted from systematic reviews graded A or B that also included a quantitative meta-analysis. The following data were extracted: first author's last name, publication year, health outcome, general population and/or patient population, measure of disease (incidence or mortality) if relevant, type of fish included (e.g. all seafood, all fish, fatty fish only), type of study design(s) included, total number of studies, total number of cases, comparison (high-low effect estimate, and/or continuous effect estimate), summary relative risk (RR) with 95% confidence interval, measures of heterogeneity (I^2 and/or P-value for test of heterogeneity), results from linear and/or non-linear meta-dose response analyses (including P-value for test for non-linearity and description of dose response relationship), overall conclusion for each relevant analysis, tool used for grading the quality of primary studies (e.g. Newcastle Ottawa Scale (Wells et al.), ROBINS-E tool for Risk Of Bias In Non-randomized Studies of Exposures (Wang et al., 2022), Data S2 (Zara et al., 2000), or other), summary of quality scores, and the overall grading of the meta-evidence if reported (e.g. NutriGrade score). Of note, figures in meta-analyses (forest plots and dose-response curves) were evaluated but are not presented in this report.

Although evidence based on cross-sectional studies was not considered by VKM, some meta-analyses with cross-sectional studies were included if results were stratified by study design. In this case, results from sub-group analyses of prospective studies and case-control studies were extracted and used as the main result.

3.1.6 Guidelines for grading of the evidence

After the quality assessment and extraction of data from each study, an overall assessment of the weight of evidence for the associations between fish intake and health effects was performed. In the weighting of evidence, the results from the included systematic reviews and meta-studies were compared to and considered together with the results from the systematic review of the primary studies, including the summary relative risks for the outcomes where this was calculated (see Chapter 3.1.7).

The weighing of the evidence followed the guidelines described by the World Cancer Research Fund (WCRF, 2018).

According to the guidelines, evidence is classified as "convincing" (strong evidence), "probable" (strong evidence), "limited, suggestive", "limited, no conclusion" and "substantial effect on risk unlikely" (strong evidence). Box 2 below shows the list of criteria for grading used in the present assessment and explains the meaning of the evidence grading (from WCRF, 2018). The evidence should be robust enough to be unlikely to be modified in the near future as new evidence accumulates. The WCRF grading system also have special upgrading factors that may upgrade the reached judgement of the evidence.

In this assessment, we have based our WoE on the following steps:

3.1.6.1 *Evidence on the relationship of interest based on published studies*

We compiled the knowledge on the relationship of exposure and outcome of interest based on systematic reviews or meta-analyses published the last 5 years and/or the primary studies from the systematic literature review conducted by VKM. The systematic reviews and meta-analyses included were quality assessed by AMSTAR version 1, moreover the studies included in the systematic literature review conducted by VKM were quality assessed as described in Chapter 3.1.3. In that way, we only include good quality studies, and exclude or minimize the risk that the observed association results from random or systematic error, confounding, measurement error and selection bias.

3.1.6.2 *Heterogeneity*

It is important to consider to what extent the results of studies are consistent and if results vary more than that expected by chance (heterogeneity) or sampling variation. Heterogeneity can be statistically quantified using the I^2 index that describes the proportion of total variation in study estimates that is due to heterogeneity (range 0-100%). Guiding thresholds have been proposed for the interpretation of I^2 . The Cochrane Handbook for Systematic Reviews of Interventions (V6.2) present the following values as a rough guide to interpretation of I^2 :

- 0% to 40%: might not be important
- 30% to 60%: may represent moderate heterogeneity
- 50% to 90%: may represent substantial heterogeneity
- 75% to 100%: considerable heterogeneity

The WCRF considers heterogeneity to be low when I^2 is below 30% and high when substantially higher than 50%.

I^2 should be used with caution as the importance of inconsistency depends on several factors, including the magnitude and direction of effects, as well as the strength of evidence for heterogeneity. Inconsistencies in the direction of association or effect are of more concern than differences in magnitude. In particular in high-low meta-analyses, some heterogeneity in the magnitude of associations is expected because the highest and lowest exposure levels often vary between studies and populations in observational studies.

A commonly reported test for heterogeneity (null hypothesis that all studies are evaluating the same effect) is Cochran's Q. The statistic is computed as the weighted sum of squared differences by summing the squared deviations of each study's estimate from the overall meta-analytic estimate (weights are those used in the pooling method). The P -value is then obtained by comparing the statistic with a χ^2 distribution with $k-1$ degrees of freedom (where k is the number of studies). The test is affected by the number of studies included in the meta-analysis and may have low power in meta-analyses based on few studies, and too much power if the number of studies is large.

If there are relevant and high-quality meta-analyses of the relationship of interest, we will use them in the evaluation of heterogeneity based on I^2 and the Cochran's Q test. VKM also

evaluated heterogeneity between primary studies included in summary RRs (Chapter 3.1.7) using an equivalent of Cochran's Q test (significance level at 5%) or qualitatively by comparing estimates from primary studies. According to the WCRF criteria, strong evidence of association or effect require that there should be "no substantial unexplained heterogeneity between or within study types in the presence or absence of an association, or direction of effect" (see Box 2).

3.1.6.3 Biological gradient (dose-response)

If there are dose-response curves from relevant and high-quality meta-analysis of the relationship of interest, we use them in the evaluation of a biological gradient. Dose-response curves from large, high quality, single studies may also be used. A dose-response do not need to be linear or even in the same direction across the different levels of exposure, so long as this can be explained plausibly. We use the criteria from WCRF to guide the weighing of biological gradient (see Box 2). When available, we have evaluated dose-response relationship as potential upgrading factor.

3.1.6.4 Mechanisms (experimental evidence)

We describe the plausible mechanism(s) behind the relationship between the exposure and outcome of interest. The mechanisms can be based on both human and animal studies, with a preference for human studies whenever possible. We only cover the primary hypothesis that are current prevailing and do not do a systematic or exhaustive search of the literature.

3.1.6.5 Weight of evidence

Based on the four criteria described above and the grading system by WCRF presented in Box 2, we rate the evidence as "convincing", "probable", "limited, suggestive", or "limited, no conclusion" or "substantial effect on risk unlikely". Only effects for which the total body of evidence (across all types of studies) is rated as strong ("convincing" or "probable") according to the WCRF grading is included in the quantitative benefit and risk assessment.

Box 2. List of criteria for grading used in the present assessment, based on WCRF cancer report (2018)

Convincing (strong evidence)

Evidence strong enough to support a judgement of a convincing causal (or protective) relationship, which justifies making recommendations designed to reduce risk of an outcome. The evidence is robust enough to be unlikely to be modified in the foreseeable future as new evidence accumulates. All of the following are generally required:

- Evidence from more than one study type.
- Evidence from at least two independent cohort studies.
- No substantial unexplained heterogeneity within or between study types or in different populations relating to the presence or absence of an association, or direction of effect.
- Good quality studies to exclude with confidence the possibility that the observed association results from random or systematic error, including confounding, measurement error, and selection bias.
- Presence of a plausible biological gradient ('dose-response') in the association. Such a gradient need not be linear or even in the same direction across the different levels of exposure, so long as this can be explained plausibly.
- Strong and plausible experimental evidence, either from human studies or relevant animal models, that typical human exposures can lead to relevant outcomes.

Probable (strong evidence)

Evidence strong enough to support a judgement of a probable causal (or protective) relationship, which generally justifies recommendations designed to reduce the risk of an outcome. All the following criteria are generally required:

- Evidence from at least two independent cohort studies, or at least five case-control studies.
- No substantial unexplained heterogeneity between or within study types in the presence or absence of an association, or direction of effect.
- Good quality studies to exclude with confidence the possibility that the observed association results from random or systematic error, including confounding, measurement error and selection bias.
- Evidence for biological plausibility (see below).

Limited, suggestive

Evidence that is too limited to permit a probable or convincing causal judgement but is suggestive of a direction of effect. The evidence may be limited in amount or by methodological flaws, but shows a generally consistent direction of effect. This judgement is broad, and includes associations where the evidence falls only slightly below that required to infer a probably causal association through those where the evidence is only marginally strong enough to identify a direction of effect. This judgement is very rarely sufficient to justify recommendations designed to reduce the risk of an outcome; any exceptions to this require special, explicit justification. All the following criteria are generally required:

- Evidence from at least two independent cohort studies or at least five case-control studies.
- The direction of effect is generally consistent though some unexplained heterogeneity may be present.
- Evidence for biological plausibility.

Limited, no conclusion

Evidence is so limited that no firm conclusion can be made. This judgement represents an entry level and is intended to allow any exposure for which there are sufficient data to warrant Panel consideration, but where insufficient evidence exists to permit a more definitive grading. This does not necessarily mean a limited quantity of evidence. A body of evidence for a particular exposure might be graded 'limited — no conclusion' for a number of reasons. The evidence may be limited by the amount of evidence in terms of the number of studies available, by inconsistency of direction of effect, by methodological flaws (for example, lack of adjustment for known confounders), or by any combination of these factors.

When an exposure is graded 'limited — no conclusion', this does not necessarily indicate that the Panel has judged that there is evidence of no relationship. With further good-quality research, any exposure graded in this way might in the future be shown to increase or decrease the risk of an outcome. Where there is sufficient evidence to give confidence that an exposure is unlikely to have an effect on risk, this exposure will be judged 'substantial effect of risk unlikely'.

Substantial effect on risk unlikely (strong evidence)

Evidence is strong enough to support a judgement that fish is unlikely to have a substantial causal relation to an outcome. The evidence should be robust enough to be unlikely to be modified in the foreseeable future as new evidence accumulates. All the following criteria are generally required:

- Evidence from more than one study type
- Evidence from at least two independent cohort studies.
- Summary estimate of effect close to 1.0 for comparison of high versus low exposure categories.
- No substantial unexplained heterogeneity within or between study types or in different populations.
- Good-quality studies to exclude, with confidence, the possibility that the absence of an observed association results from random or systematic error, including inadequate power, imprecision or error in exposure measurement, inadequate range of exposure, confounding and selection bias.
- Absence of a demonstrable biological gradient ('dose-response').
- Absence of strong and plausible experimental evidence, from either human studies or relevant animal models, that typical human exposure levels lead to relevant outcomes.

Special upgrading factors

These are factors that form part of the assessment of the evidence that, when present, can upgrade the judgement reached. An exposure that might be deemed a 'limited-suggestive' causal factor in the absence, for example, of a biological gradient, might be upgraded to 'probable' if one were present. The application of these factors (listed below) requires judgement, and the way in which these judgements affect the final conclusion in the matrix are stated. Factors may include the following:

- Presence of a plausible biological gradient ('dose-response') in the association. Such a gradient need not be linear or even in the same direction across the different levels of exposure, so long as this can be explained plausibly.
- A particularly large summary effect size (an odds ratio or relative risk of 2.0 or more, depending on the unit of exposure) after appropriate control for confounders.
- Evidence from randomized trials in humans.
- Evidence from appropriately controlled experiments demonstrating one or more plausible and specific mechanism actually operating in humans.
- Robust and reproducible evidence from experimental studies in appropriate animal models showing that typical human exposures can lead to relevant outcomes.

3.1.7 Calculation of summary relative risk estimates

VKM calculated summary relative risks (SRRs) with 95% confidence intervals (CIs) for binary disease outcomes in relation to the highest versus lowest fish intake. The SRRs were used to conclude overall on the direction of association (or lack of association) and for comparison with previous meta-analyses. The inclusion of studies for each SRR was based on an evaluation of similarity in outcome, fish exposure, study design, and statistical measure of association. A SRR could not be calculated for all outcomes.

For some outcomes, prospective studies reported relative risks from different regression models (logistic, log binomial, Cox, or Poisson). Under the rare disease assumption (prevalence of disease <10%) estimates will be approximately similar. For outcomes with a higher prevalence, the magnitude of the relative risks (ratio of risks, incidences, or odds) may differ and contribute to heterogeneity between studies, but the direction of association will be consistent. The number of studies with a retrospective design (case-control studies and retrospective cohort studies) was generally low. These studies were summarized separately or left out of the main SRR because dietary intake has been assessed after disease with potential for recall bias.

The SRRs were calculated using a random-effects model where the relative risk from each study were weighted by the DerSimonian and Laird method (DerSimonian et al., 1986). Standard errors of estimates were derived from the log transformed relative risk with 95% confidence intervals reported in each study. The analyses were performed in Episheet for Excel (version of the 29th of October 2015) which is freely available (<https://www.rtihs.org/episheet>). Episheet provides a test for the assumption of homogeneity (P -homogeneity), i.e., a common effect size underlying all studies. This test is similar to the more commonly reported Cochran's Q test for heterogeneity (P -heterogeneity). For simplicity, the P for homogeneity in Episheet is referred to as P for heterogeneity as a low p-value ($P < 0.05$) for either test provides evidence of significant heterogeneity. Episheet does not give a value for the I^2 statistic frequently reported in meta-analysis as a measure of heterogeneity, i.e., the amount of total variation that is explained by between-study variation rather than chance alone (homogeneity). The methods implemented in Episheet are described in more detail in Fleiss et al. (1993).

For studies that reported results separately for men and women, but not combined, we pooled the results in advance using a fixed-effects model to obtain the overall estimate before combining with the rest of the studies using random effects. In a fixed effects model the included studies are the only studies of interest whereas in a random effects model, the included studies are regarded as a sample from a larger population of possible studies. For studies that did not use the lowest intake category as the reference, relative risks were recalculated for the highest versus lowest category before combining with the other studies using the Excel macro RRest9 that implements the method described in Hamling et al. (2008).

VKM performed simple influence-analysis in some cases to assess the influence of specific studies on the summary RR and P -value for the test of heterogeneity. VKM did not perform

other aspects of meta-analyses (dose-response analysis, sub-group analyses of heterogeneity, or analysis of small-study effects/publication bias) but relied on the results from other quality assured meta-analyses identified in the systematic literature review.

3.2 Methods for the systematic literature review of nutrients in fish and health outcomes

The nutrients included for this benefit and risk assessment of fish consumption are long chain n-3 fatty acids (LC n-3 FA), vitamin D, iodine, selenium, and vitamin B₁₂, see Chapter 2 for inclusion criteria and evaluation. The description of the literature searches and methods for selection and evaluation of eligible studies in this chapter section corresponds to the results presented in Chapter 5.

The aim of the systematic literature review for nutrients and health outcomes was to identify relevant beneficial (and potential adverse) health effects associated with nutrients in fish, i.e., to:

- a) Evaluate the scientific evidence for the associations between nutrients and health outcomes through a systematic literature search, quality assessment of the identified literature, and a weight of evidence approach
- b) Characterise the beneficial (and potential adverse) health outcomes

As this report primarily is a benefit and risk assessment of fish, and not of the nutrients, we have based our weight of evidence analyses for nutrients in fish on results from systematic reviews and meta-analyses. All systematic literature searches for nutrients in fish were conducted by a librarian at the Norwegian Public Health Institute, in the databases MEDLINE, Embase, Cochrane and Epistemonikos for systematic reviews and meta-analyses. The identification of need for updated searches is described in section 3.2.1.1 below. Altogether, four systematic searches were conducted:

1. A search including all the selected nutrients in fish (LC n-3 FA, vitamin D, iodine, selenium, and vitamin B₁₂) and semen quality/male fertility, conducted the 25th of October 2021. The search was performed without any limitations in time.
2. A search for CVD/mortality outcomes and LC n-3 FA, conducted the 23rd of June 2021. This search was limited back in time to 2016 (5 years).
3. A search for neurodevelopment in children/cognition and cognitive decline in adults, mental health in adults, birth outcomes, type 2 diabetes, rheumatoid arthritis and multiple sclerosis and LC n-3 FA, conducted the 14th of May 2020. This search was limited back in time to 2015 (5 years).
4. A search for birth weight and respiratory tract infection and vitamin D, conducted the 29th of April 2020. This search was limited back in time to 2015 (5 years).

The processes of selecting the relevant health outcomes associated with the included nutrients are described below (Chapter 3.2.1). The search strategy and search terms are available in Appendix II, Chapter 15.1.

Inclusion and exclusion criteria for the systematic reviews and meta-analyses included for nutrients in fish are presented in Table 3.2-1.

Table 3.2-1 Inclusion and exclusion criteria for systematic reviews and meta-analyses included for evaluation of evidence for associations between nutrients in fish and various health outcomes.

Criteria for inclusion	• Systematic reviews and meta-analyses investigating intake of specified nutrients (either LC n-3 FA, vitamin D, iodine, selenium or vitamin B₁₂) in relation to one or more health outcomes that was included in the systematic search. • Study designs: - o Systematic reviews and meta-analyses including: observational studies, such as: Cohort studies, Case-cohort studies, Nested case-control studies, Case-control studies - o Systematic reviews and meta-analyses including experimental studies, such as randomised controlled trials (RCTs), controlled clinical trials, controlled before-and-after studies • Population: general population, all age groups. Persons with the following conditions are considered part of the general population and will be included: - o Type 2 diabetes - o Obesity - o Musculoskeletal disorders • Publication type: Systematic reviews and meta-analyses. • Other: Nutrient intake needs to be measured at the individual level, effect estimates must be given. Studies on secondary prevention should be included
Criteria for exclusion	• Systematic reviews and meta-analyses investigating nutrient intake without any relation to the specific health outcomes included in the search. • Population: specific patient groups (see inclusion criteria for exceptions) • Study designs: - o Umbrella reviews - o Systematic reviews and meta-analyses with estimates only for cross-sectional studies, animal studies, <i>in vitro</i> studies

A systematic approach was used for the selection of papers/studies from all the four literature searches for nutrients. Screening of titles and abstracts were performed in a pairwise blinded manner using Rayyan, a web application for systematic reviews (Ouzzani et al., 2016). The screening was performed against the pre-defined inclusion/exclusion criteria detailed in Table 3.2-1 above.

After the first round of screening, the blinding was removed, and the reviewers discussed conflicting decisions. If the two reviewers could not reach an agreement, a third reviewer was consulted. In case of systematic reviews/meta-analyses from the same research groups updating previous meta-analyses, only the last update was kept.

The potentially relevant papers selected via the screening procedure based on title and abstract was then reviewed in full text. Full-text systematic reviews and meta-analyses that fulfilled the inclusion criteria were quality assessed using the AMSTAR tool (version 1, slightly adapted), see Chapter 3.1.3.2, and categorized as A, B or C-papers. Only systematic reviews and meta-analyses categorized as A or B were data extracted and included for the weight of evidence.

A systematic data extraction was made for all category A and B papers by one reviewer. The parameters to be extracted was thoroughly discussed and agreed upon by the project group. Extracted data were study design, inclusion year(s), end of inclusion, study size of included studies, population groups, dietary assessment methods, quality assessment/risk of bias in included studies and exposure characteristics.

After the quality assessment and extraction of data of each systematic review and meta-analysis, an overall assessment of the weight of evidence for the associations between nutrient intake and health outcome was performed. The weighing of the evidence followed the guidelines described by the World Cancer Research Fund (WCRF) (see Chapter 3.1.6), but applied to evaluation of systematic reviews and meta-analyses.

Chapter 3.2 is divided into the following sections; (3.2.1) inclusion and exclusion of health outcomes for nutrients; (3.2.2) all nutrients and semen quality/male fertility; (3.2.3) LC n-3 FA and CVD and mortality; (3.2.4) LC n-3 FA and neurodevelopment/CNS/cognitive functioning, birth outcomes, type 2 diabetes and rheumatoid arthritis/multiple sclerosis; and (3.2.5) vitamin D and birth weight and respiratory tract infection.

3.2.1 Inclusion and exclusion of health outcomes for nutrients

This chapter section elaborates on which health outcomes that have been evaluated and included for the selected nutrients.

Table 3.2.1-1 shows criteria for inclusion/exclusion of health outcome related to the selected nutrients.

Table 3.2.1-1 Criteria for inclusion or exclusion of health outcome for the nutrients.

Criteria for inclusion of health outcome	Criteria for exclusion of health outcome
• Evidence for an association between nutrient and health outcome is good and consistent. • Source of evidence: Systematic reviews or meta-analyses published in one of the following (or equivalent): Cochrane Database, NNR, IOM/NASEM; or assessment published by EFSA or VKM. • If previous systematic reviews from these competent bodies are inconclusive regarding the evidence and new literature has emerged, we will include other published systematic reviews and meta-analyses.	• Evidence for an association is limited or inconsistent

Due to a large number of publications, it was not possible to conduct open searches for each included nutrient. To be able to manage the results from the searches within the available timeline, we therefore had to identify which health outcomes to be included for each nutrient, and thereafter decide which of these health outcomes that needed an updated systematic literature review to conclude.

We identified all health outcomes that have been investigated for the nutrients LC n-3 FA, vitamin D, iodine, selenium, and vitamin B₁₂ in systematic reviews by competent bodies such as NNR, IOM/NASEM, VKM or EFSA.

NNR conducted systematic literature reviews for LC n-3 FA (Schwab et al., 2014) and vitamin D (Lamberg-Allardt et al., 2013). The systematic literature review by Lamberg-Allardt et al. (2013) was based upon a systematic literature review by IOM (2011) setting dietary reference intakes for calcium and vitamin D. VKM conducted a systematic literature review for health outcomes related to mild to moderate iodine deficiency in 2020 (VKM, 2020). No systematic literature reviews by competent bodies were found for selenium, and vitamin B₁₂.

Additionally, we searched for Cochrane reviews for the nutrients LC n-3 FA, vitamin D, selenium, and vitamin B₁₂ and all potentially relevant health outcomes mentioned in NNR, IOM or EFSA opinions for the specific nutrients. This was not considered to be necessary for iodine since relevant health outcomes for this nutrient already were included in the recent systematic review by VKM (2020).

The following health outcomes were considered to be relevant for inclusion: CVD and mortality, neurodevelopment in children and cognition, cognitive decline and mental health in adults, birth outcomes, type 2 diabetes, overweight/obesity, immunological outcomes (such as e.g., asthma in children and rheumatoid arthritis), respiratory tract infection and multiple sclerosis.

Additionally, we included semen quality/male fertility as a relevant outcome for nutrients as this is the critical health outcome for setting the tolerable weekly intake for dioxins and DL-PCBs.

All these outcomes mirrored the health outcomes included in the systematic literature search for fish consumption.

3.2.1.1 Identification of need for updated systematic literature review for the included health outcomes for nutrients

After having identified all relevant health outcomes for LC n-3 FA, vitamin D, iodine, selenium, and vitamin B₁₂, we started the process to identify which of the nutrient and health outcome associations to prioritize for the systematic review and weight of evidence evaluation.

Based on the inclusion criteria in Table 3.2.1-1, we identified associations that were judged to be good and consistent in previous reports/systematic reviews by competent bodies. Such associations were identified for vitamin D in IOM (2011) and Lamberg-Allardt et al. (2013) for bone health, including fall, and mortality. For these associations we merely conducted brief literature searches to check if results from more recent high quality systematic reviews/umbrellas conclusions were still valid. For LC n-3 FA, iodine, selenium, and vitamin B₁₂, no good and consistent associations between the nutrients and the selected outcomes were identified in previous reports by competent bodies (NNR, IOM/NASEM, VKM or EFSA).

After the identification of these previous reports/systematic reviews, we conducted brief searches and checked if conclusions from Cochrane reviews indicated any good and consistent associations not covered in the previous reports/ systematic reviews by competent bodies. We have not listed all health outcomes that have been evaluated for all the included nutrients in this process, but it should be mentioned that several outcomes were evaluated for selenium (CVD, mortality, diabetes) and vitamin B₁₂ (cognition, cognitive decline, CVD), but the initial search did not encourage further investigations as the evidence was not likely to be good and consistent for these associations (intermediate markers of health not included for this benefit and risk assessment). We therefore concluded that it was not necessary to make updated systematic literature searches for selenium, and vitamin B₁₂ for any health outcomes.

The next step was to identify inconclusive evidence in previous reports/systematic reviews by competent bodies for which new literature have emerged. None of the associations investigated for LC n-3 FA in Schwab et al. (2014) was concluded as “probable” or “convincing” (good and consistent). In the last decade, several large RCTs investigating a broad range of the included health outcomes (CVD, mortality, neurodevelopment in children, cognition, cognitive decline and mental health in adults, type 2 diabetes, birth outcomes, asthma in children, rheumatoid arthritis and multiple sclerosis) have been published.

We therefore decided to include all these outcomes for the systematic literature review and weight of evidence analysis for LC n-3 FA for this benefit and risk assessment. However, asthma in children was not included as this will be conducted as a de novo literature review in the NNR 2023.

The review for vitamin D by Lamberg-Allardt et al. (2013) covered the following health outcomes: pregnancy outcomes and growth, bone health (all fractures, hip fractures, vertebral fractures, bone mineral density/osteoporosis, bone mass, bone quality, rickets, osteomalacia, dental health), muscle strength, falls; all cancers, breast cancer, colorectal cancer, prostate cancer, type 1 and type 2 diabetes, multiple sclerosis, obesity, total mortality, hypertension/blood pressure, cardiovascular disease (CVD) clinical outcomes, and infections. As mentioned above the associations for bone health, including fall, and mortality were evaluated as good and consistent. Based on brief searches we evaluated that for the other outcomes, birth weight and respiratory tract infections would be the outcomes in which new literature have emerged. We therefore decided to include birth weight and respiratory tract infections in the systematic literature review and weight of evidence analysis for vitamin D in this benefit and risk assessment.

To summarize, for LC n-3 FA, systematic literature reviews of systematic reviews and meta-analyses were conducted for the health outcomes CVD and mortality, neurodevelopment in children, cognition, cognitive decline and mental health in adults, type 2 diabetes, birth outcomes, rheumatoid arthritis, and multiple sclerosis. For vitamin D, systematic literature reviews of systematic reviews and meta-analyses were conducted for the health outcomes birth weight and respiratory tract infections. For iodine, selenium, and vitamin B₁₂ no updated searches were necessary.

It should be noted that EFSA also has published several opinions on “health claims” related to many of these nutrients. These opinions were not based on systematic literature reviews, and were published prior to 2015, and have therefore not been included in our work.

3.2.1.2 Overview of the process of selecting nutrients and associated health outcomes, and the need for updated literature searches

The process for the updated searches is described in the Chapters 3.2.2 to 3.2.5 below.

The processes of selecting the nutrients and relevant health outcomes associated with the included nutrients, and identification of the nutrient-outcome combinations that required updated literature searches are shown in Figure 3.2.1.2-1.

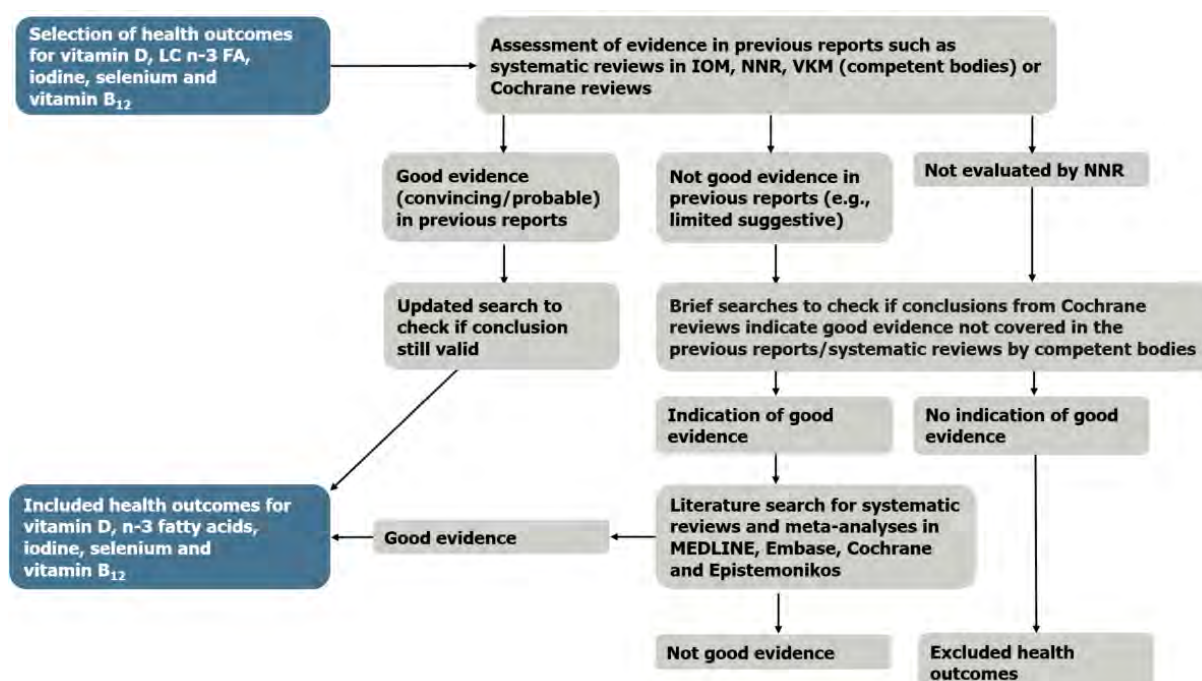


Figure 3.2.1.2-1 A process diagram for the selection of nutrients and health outcomes related to the nutrients in this benefit and risk assessment, and the identification of nutrients and outcomes where an updated literature search was needed.

3.2.2 LC n-3 FA, vitamin D, iodine, selenium, and vitamin B₁₂ and semen quality/male fertility

Because the critical endpoint for PCDD/Fs and DL-PCBs is semen quality, we examined the potential associations between the included nutrients and semen quality/male fertility. A search for all included nutrients and semen quality/male fertility without time restrictions was conducted by a librarian at the Norwegian Public Health Institute 25.10.21. We identified 140 systematic reviews and meta-analyses. After blinded screening in Rayyan by two independent reviewers, 19 of these papers were evaluated in full text. Fifteen papers were excluded because they did not fulfil the inclusion criteria (commentaries, not systematic

reviews, no estimates for exposure to LC n-3 FA, vitamin D, iodine, selenium, or vitamin B₁₂, or only related to female fertility). A quality assessment with slightly adapted AMSTAR for systematic reviews resulted in 2 B graded papers and 1 C graded paper. Additionally, 1 paper was excluded, and reasons for exclusion are given in tables in Chapters 5.2.17 and 5.5.1. Only papers investigating semen quality/male fertility in relation to LC n-3 FA or selenium were identified and included.

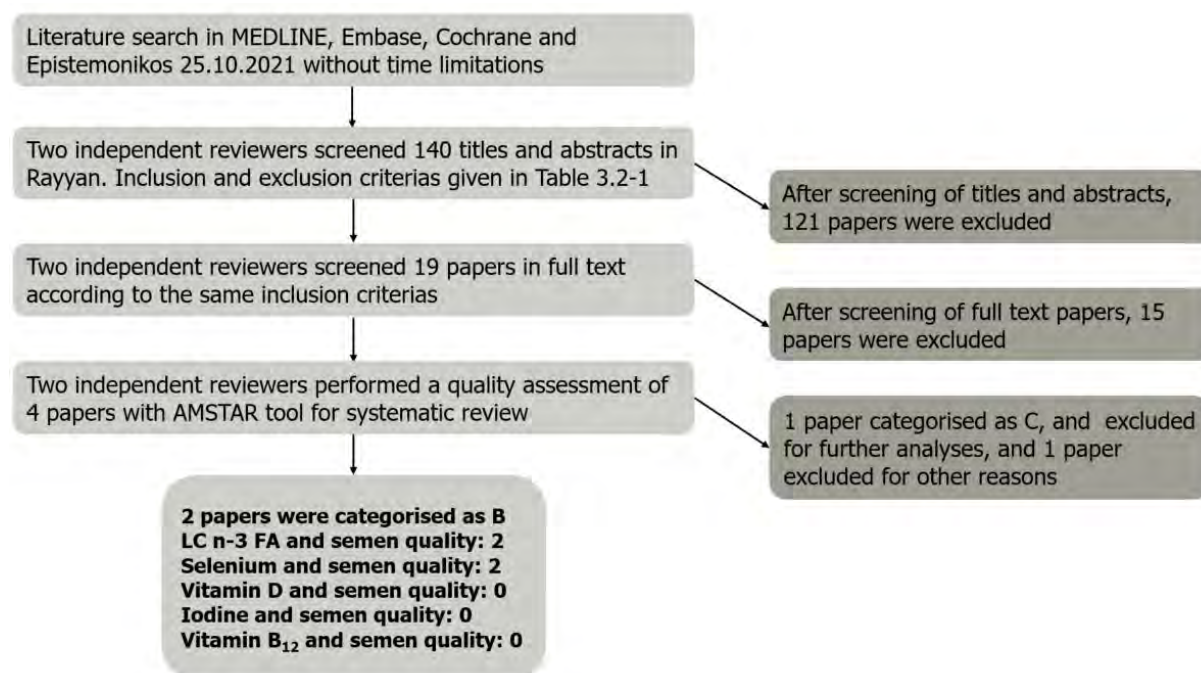


Figure 3.2.2-1 Flow chart for selection of systematic reviews and meta-analyses for LC n-3 FA, vitamin D, iodine, selenium, and vitamin B₁₂ and semen quality.

The results from the data extraction and weight of evidence from this literature search are described in Chapters 5.2.17 (LC n-3 FA) and 5.5.1 (selenium).

3.2.3 LC n-3 FA and CVD/mortality

The search for LC n-3 FA and CVD/mortality was conducted by a librarian at the Norwegian Public Health Institute 23.06.21. We identified 564 systematic reviews and meta-analyses from 2016 to the date of search. After blinded screening in Rayyan by two independent reviewers, 39 papers were evaluated in full text. Seventeen papers were excluded because they did not fulfil the inclusion criteria (umbrellas, not systematic reviews, no estimates for exposure to LC n-3 FA). A quality assessment with slightly adapted AMSTAR (see 3.1.3.2) for systematic reviews of 22 papers, resulted in 12 B graded papers and 7 C graded papers. Additionally, 3 papers were excluded, and reasons for exclusion are given in a table in Chapter 5.2.1.

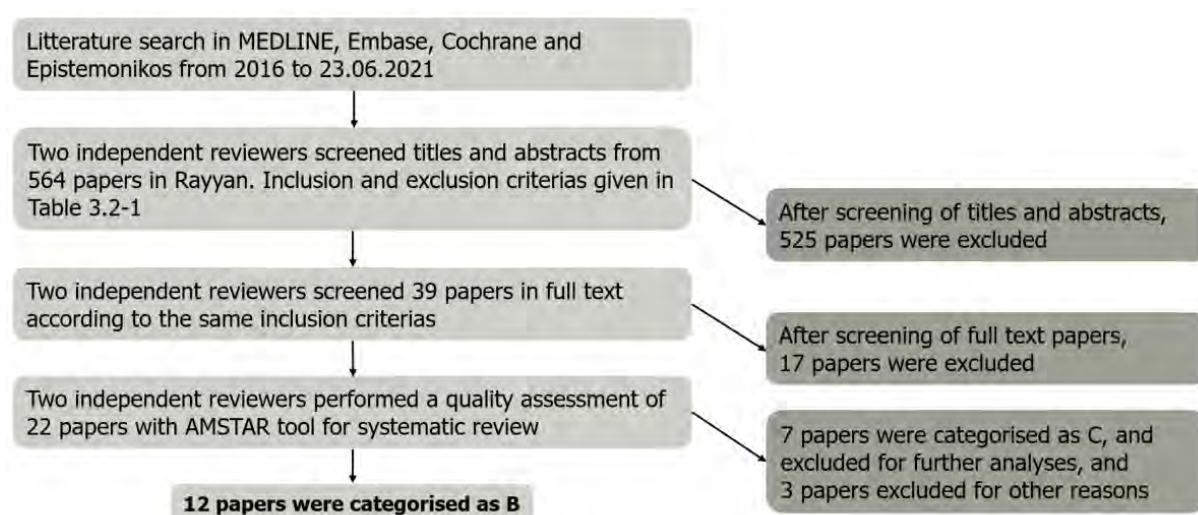


Figure 3.2.3-1 Flow chart for selection of systematic reviews and meta-analyses for LC n-3 FA and CVD and mortality.

The results from the data extraction and weight of evidence from this literature search are described in Chapters 5.2.1 to 5.2.9.

3.2.4 LC n-3 FA and neurodevelopment in children, cognition and cognitive decline in adults, mental health in adults, birth outcomes, type 2 diabetes, rheumatoid arthritis, and multiple sclerosis

The search for neurodevelopment in children, cognition and cognitive decline in adults, mental health in adults, birth outcomes, type 2 diabetes, rheumatoid arthritis, and multiple sclerosis was conducted by a librarian at the Norwegian Public Health Institute 14.05.20. The search was originally limited in time back to year 2010. We identified 1352 systematic reviews and meta-analyses. However, only articles from 2015 to the date of search were included, altogether 928 articles. After blinded screening of these in Rayyan by two independent reviewers, 62 papers were evaluated in full text. Twenty-two papers did not fulfil the inclusion criteria (umbrellas, not systematic reviews, no estimates for exposure to LC n-3 FA, included only patient groups). A quality assessment with slightly adapted AMSTAR for systematic reviews of 40 papers, resulted in 16 B graded papers and 17 C graded papers. Additionally, 7 papers were excluded, and reasons for exclusion are given in tables in Chapters 5.2.10-5.2.16.

A flow chart for selection of systematic reviews and meta-analyses on LC n-3 FA and the outcome measures neurodevelopment (including cognitive functioning), preterm birth and birth weight, type 2 diabetes, rheumatoid arthritis, and multiple sclerosis is given in Figure 3.2.4-1.

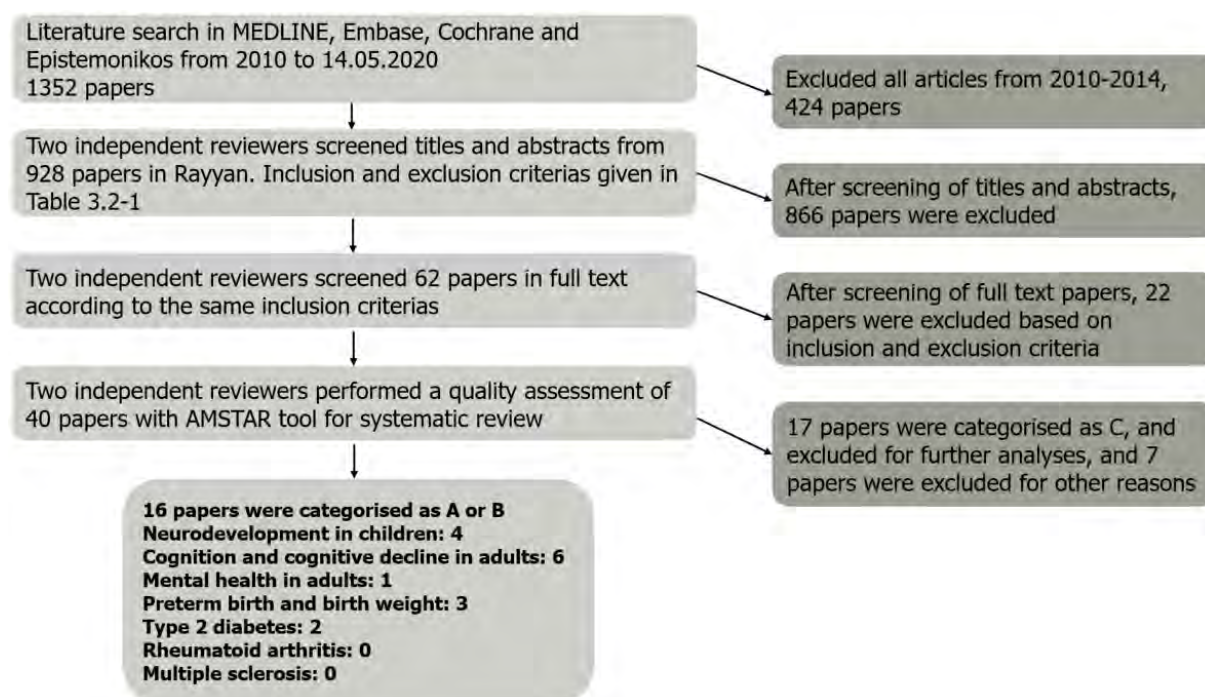


Figure 3.2.4-1: Flow chart for selection of systematic reviews and meta-analyses on LC n-3 FA and selected health outcomes.

3.2.5 Vitamin D and birth weight, and respiratory tract infection

The search for vitamin D and birth weight, and respiratory tract infections was conducted by a librarian at the Norwegian Institute of Public Health 29.04.20. Altogether we identified 366 systematic reviews and meta-analyses. The search was originally limited in time back to year 2010. However, we decided to include only articles from 2015 to the date of search, all together 252 articles. After blinded screening of these 252 articles in Rayyan by two independent reviewers, 44 papers were evaluated in full text.

After this selection process was finalized, we identified three recent governmental systematic reviews by British Scientific Advisory Committee on Nutrition (SACN) that were judged to cover our aim. We have therefore not proceeded with the results from the literature search for vitamin D and respiratory tract infection but based our evaluation upon the SACN reports. Twenty of the 44 papers selected in Rayyan, investigated vitamin D and birth outcomes, e.g., birth weight. A quality assessment with slightly adapted AMSTAR for systematic reviews, resulted in eight systematic reviews and meta-analyses for vitamin D and birth weight graded as A or B, one paper was graded C. Two papers were excluded for reasons given in Table 5.3.4-1, Chapter 5.

The results from the data extraction and weight of evidence from this literature search are described in Chapters 5.3.3 (respiratory tract infection) and 5.3.4 (birth weight).

A flow chart for selection of systematic reviews and meta-analyses on vitamin D and the outcome measures birth weight and respiratory tract infection is given in Figure 3.2.5-1.

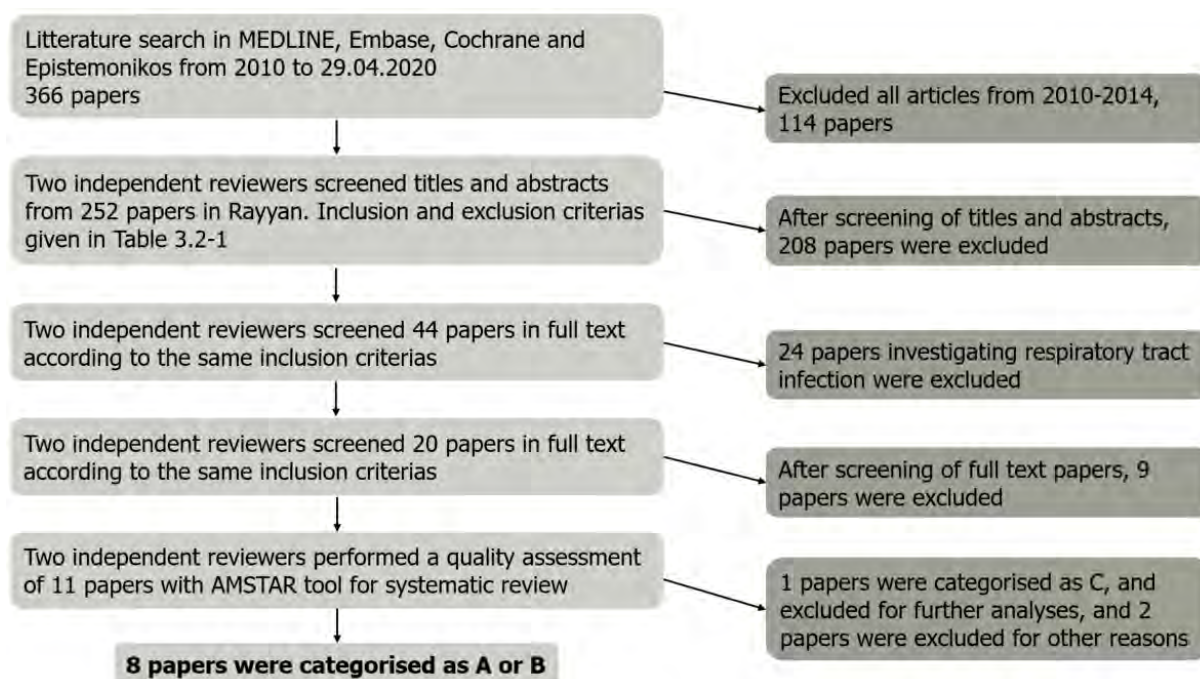


Figure 3.2.5-1 Flow chart for selection of systematic reviews and meta-analyses on vitamin D and the outcome measures birth weight and respiratory tract infection.

3.2.6 Data extraction from systematic reviews and meta-analyses of nutrient intake

Data were extracted from systematic reviews graded A or B that also included a quantitative meta-analysis. The extraction of review studies on nutrients was similar to that performed for review studies on fish (Chapter 3.1.4), but with some differences. The review studies on nutrients were to a large extent based on randomized controlled trials (RCTs) of dietary supplements and data were extracted for type of LC n-3 FA, dose, and comparison group (placebo or other).

3.3 Search for systematic reviews and meta-analysis on methyl mercury

Since EFSA's risk assessment of methylmercury from 2012, many publications have assessed the association between mercury exposure and different endpoints. Their findings may potentially indicate that there is a need to update the risk assessment of methyl mercury. VKM conducted a literature search for systematic reviews published after the EFSA risk assessment in 2012. In addition, a separate search was conducted in order to identify original publications addressing mercury exposure and health outcomes in the Norwegian population. This second search was done to capture new information of particular relevance to Norway, in view of a relatively high fish consumption combined with relatively low methyl mercury concentrations in the fish species most often consumed. VKM searched the databases Medline, Embase, PsycInfo, Web of Science and Epistemonikos. The search was

performed the 11th of January, 2021, and an updated search was performed the 4th of October, 2021. The search strategy and search terms are available in Appendix II, Chapter 15.

VKM obtained 106 hits in the search for systematic reviews and meta-analyses. The screening of title and abstract was done in accordance with criteria in Table 3.3-1 by two independent reviewers and resulted in 22 papers that were checked in full text. From these 22, 14 reviews fulfilled the inclusion criteria, and 10 were graded B. These are summarized in the narrative review of reviews in Appendix IV, Chapter 17. The quality of included reviews was assessed by use of AMSTAR (see section 3.1.3.2). The included studies comprised papers covering the topics autism, ADHD, neurodevelopment, neurological disorder, blood pressure/hypertension, foetal growth, birth outcomes, autoimmunity, diabetes and metabolic diseases.

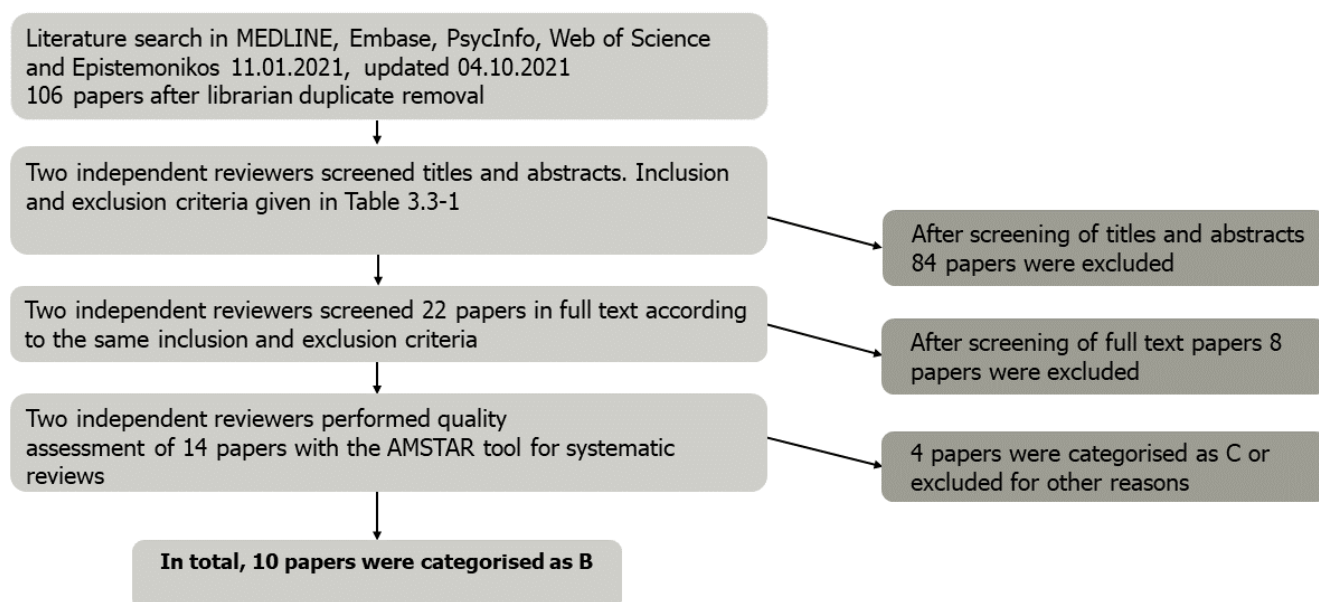


Figure 3.3-1 Flow chart for selection of systematic reviews and meta-analyses on methyl mercury and various outcomes.

In the separate search for studies in the Norwegian population described above, VKM obtained 148 hits, but only five studies fulfilled the inclusion criteria. Because the summary of the systematic reviews showed that there was no need for an update of the TWI, these five studies of the Norwegian population was not investigated further, and they are not included in the flow chart in Figure 3.3-1.

Table 3.3-1 Inclusion/exclusion criteria for reviews of human studies on methyl mercury and health effects.

Inclusion criteria	Exclusion criteria
• Measured levels of Hg in blood/hair/toenails • Association with health outcomes • Systematic reviews, meta-analyses	• Methyl mercury concentration not measured in blood/hair/toenails, only urinary Hg measured (inorganic Hg), thiomersal from vaccines • No assessment of exposure-health association (e.g., paper on disease burden, health costs) • Intermediate endpoint (e.g., blood pressure) • Non-human studies • Single cohort studies

3.4 Method for benefit and risk assessment – a tiered approach

EFSA's guidance on human health risk-benefit assessment of foods (EFSA, 2010) and the later more refined BRAFO tiered approach for benefit and risk assessment of foods by Hoekstra et al. (2012) suggest a stepwise tiered approach. Hoekstra et al. (2012) suggest an introductory pre-assessment and problem formulation, followed by four tiers; tier 1) Individual assessments of benefits and risks, tier 2) Qualitative Integration of benefits and risk, tier 3) Deterministic computation of common/composite health metric, and finally tier 4) Probabilistic computation.

Hoekstra et al. (2012) suggest that a consideration should be done at each initial tier level whether to proceed to the next tier. Such a refined benefit and risk assessment aims to provide, depending on the availability of data, semi-quantitative or quantitative estimates of benefits and risks at relevant exposures. The semi-quantitative assessments contain comparisons of, e.g., exposures to nutrients and contaminants to health-based guidance values (like average requirements (AR) for nutrients, and tolerable weekly intakes (TWI) for contaminants), and the probabilities of being below or exceeding these reference values. At tier 3, a quantitative approach is suggested to link the changes in intake to changes in occurrence of specific health outcomes. Common metrics, i.e., incidence/mortality can be used to quantify the impacts of current intake and intake scenarios, and composite metrics, i.e., DALY or QALY can be used to quantify the impacts taking both morbidity and mortality into account on the same scale of measurement. A quantitative methodology using either common or composite health metrics has the advantage that it allows for a quantitative expression of the overall health impact of a given change in diet. A quantitative expression provides the evidence, not only if, but by how much a change in diet impacts health.

Due to the new TWIs for PCDD/F and DL-PCBs, and PFASs it was not evident that the benefits would clearly outweigh the risks, or vice versa, prior to this present benefit and risk assessment of fish. The opinions on TWIs performed by EFSA had shown that fish is an important source for these contaminants, and that the general European population already exceed these TWIs. VKM therefore anticipated that it would not be an option to stop at

either tier 1 or tier 2, but rather that it would be necessary to proceed to tier 3 as described above.

Figure 3.4-1 illustrates the approach used for this benefit and risk assessment of fish consumption. The figure includes references to the chapters. This approach does not follow the described stepwise tiered approach strictly, but rather includes several tiers. The quantitative assessment integrates benefits and risks in a common metric (incidence and/or mortality) by a deterministic approach. VKM considers this the main part of this benefit and risk assessment of fish in the Norwegian diet. The quantitative assessment has been performed for health outcomes related to fish intake that was judged to have strong evidence ("probable" or "convincing"), with sufficient data available to quantify the increase/decrease in incidence and mortality. The methodology and data applied for the quantitative assessment is described in detail in Chapter 9.2.

VKM's systematic literature review on fish intake and health outcomes would potentially reveal both beneficial and adverse effects of increased fish consumption. However, in the assignment letter, VKM was specifically asked to consider the new TWIs set by EFSA for PCDD/Fs and DL-PCBs and PFASs.

Our quantitative analyses/model does not include critical health outcomes relevant for the contaminants due to limited available data. Moreover, a quantitative modelling approach with common metrics was not applied for contaminants and nutrients due to a limitation in available models. The dioxin (PCDD/Fs and DL-PCBs) model, published in the Global burden of foodborne disease project has not been updated with the 2018 EFSA Scientific opinion on TWIs for PCDD/Fs and DL-PCBs. For PFASs there is no existing model, and there is a lack of consensus for the use of linear no-threshold dose-response model for methyl mercury. Moreover, for the included nutrients in the present benefit and risk assessment there are models for LC n3 FA and vitamin B₁₂, but not for the other nutrients. To avoid possible imbalance from including some compounds (contaminants and/or nutrients) and not others, VKM decided not to integrate any single compounds found in fish in the quantitative modelling. The evaluation of all nutrients and contaminants relevant for fish intake has been performed using a semi-quantitative approach. The exposures to nutrients are evaluated as proportions of the populations with intakes below average requirements (ARs), and the exposures to contaminants are evaluated as proportions of the population with intakes above the TWIs.

Finally, we did a qualitative integration of the quantitative assessment of fish intake and the semi-quantitative assessments of nutrients and contaminants in fish.

In Hoekstra et al. (2012) it is suggested to transform incidence of different health outcomes, including mortality, onto a composite metric such as disability adjusted life years (DALY) at tier 3. This is neither a trivial task, nor an unchallenged approach. VKM considered the possibility of performing a full-scale benefit and risk assessment using DALYs, and critically evaluated the necessary assumptions for such an approach.

DALY is a metric developed for the World Health Organization in the 1990'ies and have been applied since then, as a metric to estimate the public health impact of diseases, injuries and

risk factors in the Global Burden of Disease Study (GBD). DALY relies on and integrates information on disease incidence, mortality, duration, and severity. Particularly, DALYs express how many healthy life years are lost due to a given disease in a population by estimating how many years are lived with the disease of a given severity and add them to the number of years lost due to death earlier than expected. The severity of a given disease (or health outcome) is expressed by a disability weight. The concept of DALY and disability weights have been disputed as a measure of public health, but also recognized as an indicator for impact comparable across diseases.

To properly implement DALY as a composite metric, a more rigorous modelling approach would be necessary, especially for health outcomes not included in GBD. For instance, incidence, duration and mortality for specific age-groups should be collated to quantify the number of years lived with a given health effect and years of life lost to premature death, which would require a substantial amount of work.

In addition, the communication of DALY can in some instances be challenging, and it was decided that the added value of estimating DALY on top of the quantitative assessment of change in incidence and mortality, was limited.

We did not have sufficient data to conduct probabilistic computation in the quantitative benefit and risk assessment of fish consumption.

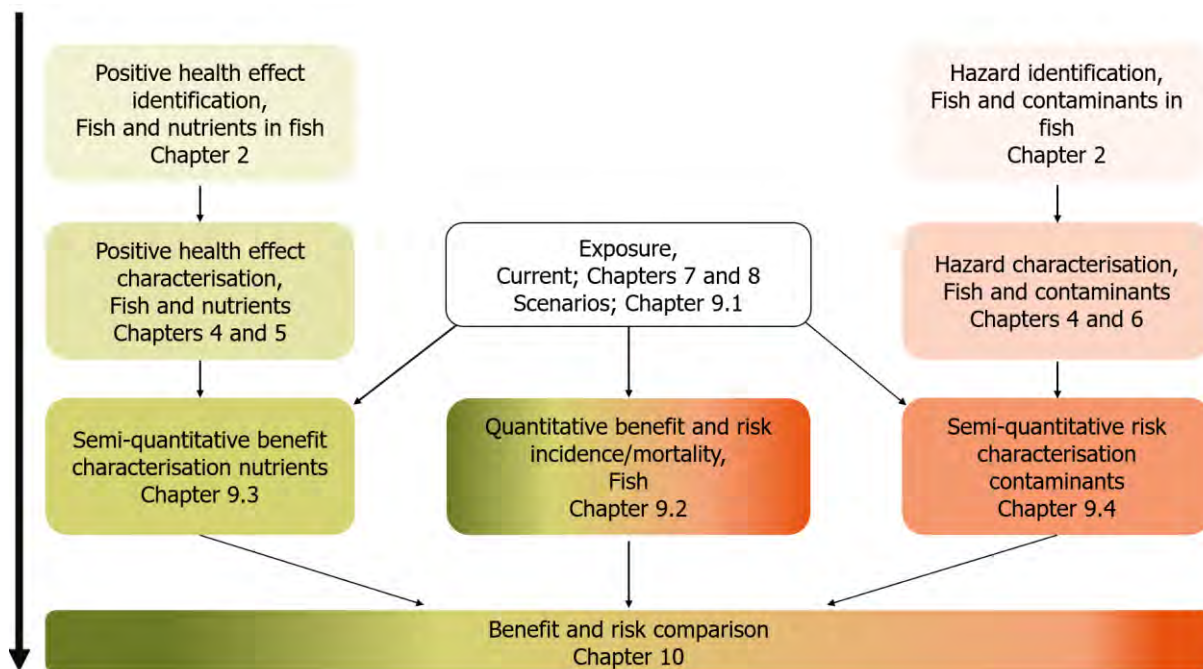


Figure 3.4-1 Illustration of approach used for this benefit and risk assessment including reference to chapters (inspired by Figure 2 in Hoekstra et al., 2012).

3.5 References

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4 Fish intake and health outcomes

This chapter summarizes the epidemiologic evidence for an association between fish consumption and selected health outcomes based on a systematic literature review performed by VKM for the current report (see Chapter 3.1 for description of methods). The literature review has a wide scope and includes systematic reviews and meta-analyses published since 2016, and primary studies without time restriction.

The health outcomes included are non-communicable diseases common in the Norwegian population for which fish, or compounds in fish (nutrients or contaminants) have an established or hypothesized role. Due to the length of the current chapter, the structure is explained here as a guide to the reader: The literature is summarized by health outcome. For diseases that are common causes of death, studies of incidence (risk of developing the disease) are summarized separately from studies of mortality (risk of dying from the disease).

First, we present studies of cardiovascular disease (CVD) incidence overall and by more specific sub-types (coronary heart disease, myocardial infarction, stroke, other CVD outcomes) followed by studies of mortality (cause specific in alphabetical order, and all-cause) in adults. The focus is on the general population, but for CVD (incidence or mortality) and all-cause mortality, some patient populations have been included, those with a history of CVD or at high risk of CVD due to vascular disease or type 2 diabetes (T2D). Next, we summarize neurological outcomes (neurodevelopmental outcomes in children, and cognition and mental health outcomes in adults) followed by T2D, rheumatoid arthritis, body weight and other anthropometric indicators in adults and children), bone health (limited to hip fractures), birth outcomes, asthma and allergies in children, and last multiple sclerosis (MS). Cancer was not included in the current literature review as the Third Expert Report from the World Cancer Research Fund (WCRF) was sufficiently comprehensive.

Under each outcome, previous systematic reviews are summarized before the primary studies. For some large outcome groups there are introductory chapters that give a brief overview of the outcomes. In some introductory chapters we also present all the included systematic reviews and meta-analyses at the beginning if the reviews contained multiple outcomes. These introductory chapters are 4.1 (CVD), 4.7 (Mortality), 4.10 (Neurodevelopment in children), 4.13 (Neurocognitive and psychiatric endpoints in adults), 4.18 (Anthropometric measures), 4.23 (Birth outcomes) and 4.29 (Asthma and allergies). The header level is the same as for the different outcome groups, but they should be read as overarching chapters.

The primary studies are first described under different sub-headings: included studies from the literature search; overlapping publications (may arise from studies publishing individually and as part of consortia, or studies publishing with varying lengths of follow-up) and any exclusions due to overlap; studies by design and geographic region; studies in patients (described in more detail, if included under outcome); study distribution by sex which was evaluated as a potential effect modifier, and other central sub-groups that were presented;

study distribution by fish exposure (fish overall and/or by sub-categories such as fatty- or lean fish, or other classifications) and for some outcomes also by exposure timing (e.g. for birth outcomes, maternal intake may be measured prior to pregnancy, during pregnancy, or during lactation). There is also a header for studies assessing potential non-linearity to alert readers to studies that have evaluated the shape of the dose-response relation in more detail, and that may include figures that are not shown in the current report.

Next, we present the study results, grouped under sub-headings for total fish, and other sub-classifications presented in the literature (mainly fatty- and lean, or fried vs non-fried fish intake). Results from patient studies are presented under a separate sub-heading, and there is a sub-heading where VKM's summary relative risk (RRs) are presented and compared with results from previous meta-analyses. Heterogeneity of results between studies and dose-response relationships are part of the weight of evidence and described under separate headings. Last, the weight of evidence criteria are summarized with an overall conclusion. A list of common abbreviations in tables within the current chapter is given below (Table 4.1).

Table 4-1 Overview of common abbreviations used in tables in Chapter 4.

Abbreviation in tables	Explanation
Cat.	Category or categories
CI	Confidence interval
d, wk, mo, yr(s)	Day, week, month, year(s)
FFQ, semi-quant.	Food frequency questionnaire, semi-quantitative
GW	Gestational week
M, W, M/W	Men, women, men and women combined
MD, β	Measures of difference or change for continuous health outcomes: mean difference (MD) or linear regression coefficient (β)
NA	Not available, or not applicable
Q1-Q4 or Q1-Q5	Quartiles for range Q1-Q4, quintiles for range Q1-Q5
RR, HR, IRR, OR	Measures of relative risk (RR) for binary health outcomes: hazard ratio (HR), incidence rate ratio (IRR), odds ratio (OR)
SD, SE	Standard deviation, standard error
Sig. assoc.	Statistically significant association

Although cancer was not included, the evidence for associations between fish intake and risk of disease or mortality is weighed according to the same criteria used by the WCRF (see Chapter, section 3.1.6). The following weighting criteria are used: the published evidence of fish intake and health outcome (number of studies for each study design), heterogeneity between studies, and evidence for biological plausibility. There is also possibly to upgrade the level of evidence according to the WCRF grading system. Examples of upgrading factors are evidence of a plausible biological gradient (dose-response) in the association, particularly large effect sizes, evidence from randomized controlled trials in humans, or robust evidence from experimental studies in appropriate animal models. In the current literature review, evidence of dose-response was the most applicable upgrading factor.

For an overview of the weight of evidence conclusions, see Chapter 4.37. Quantitative assessments were performed for health outcomes for which the evidence for an association

with fish intake was graded at least “probable” (see Chapter 9.2 for Quantitative benefit and risk characterization of fish intake).

4.1 Introduction fish intake and cardiovascular disease incidence

This chapter is an introduction to the weight of evidence analysis chapters for the included CVD outcomes (Chapters 4.2-4.6).

Overview of studies summarized according to cardiovascular disease outcomes

Cardiovascular disease (CVD) is a large group of diseases that involve the heart and blood vessels. Coronary heart disease (CHD) and cerebrovascular disease (stroke) are two common forms of CVD. CHD affect blood vessels supplying the heart muscle, and stroke affect blood vessels supplying the brain. The underlying reason for CHD and stroke is mainly a narrowing or blockage of the arteries (atherosclerosis), leading to restricted blood supply (ischemia), a shortage of oxygen, and tissue damage. Ischemic heart disease and myocardial infarction (MI or heart attack) are large components of CHD. Stroke can also be caused by bleeding from a blood vessel in the brain. Therefore, strokes may be defined as ischemic or hemorrhagic. Historically, hemorrhagic stroke has dominated in many Asian populations, and ischemic stroke in Western populations, but with the word wide spread of Western diet and lifestyle, ischemic strokes are now most common. Both MI and stroke are usually acute and may be fatal. Other forms of CVD include peripheral arterial disease (affecting blood vessels supplying the arms and legs), thromboembolic disease (from migrating blood clots in the circulation), abnormal heart rhythms such as atrial fibrillation, and different forms of heart failure (affecting the pumping capacity of the heart). Rheumatic heart disease (complication from rheumatic fever) and congenital heart disease (malformations at birth) are also part of the CVD group but less relevant in relation to fish intake.

The results are summarized separately for each outcome in sub-chapters within this chapter. We start by presenting the evidence for incidence of total CVD as a composite outcome (Chapter 4.2), followed by CHD (Chapter 4.3), MI (Chapter 4.4), stroke and stroke sub-types (Chapter 4.5) and other CVD outcomes (heart failure, atrial fibrillation, venous thromboembolism, Chapter 4.6). Although it may seem artificial to draw separate conclusions for outcomes nested within each other, the outcome classifications reflect those used in the literature. Because the number of included primary studies varies for each outcome, conclusions on CVD overall may differ from those on CVD sub-groups, depending on the published evidence (or lack of evidence) for each outcome.

Figure 4.1.-1 shows the CVD outcomes placed within a hierarchical classification scheme. Outcomes in white boxes (unstable angina, unspecified stroke) were not summarized separately but included in outcomes higher in the hierarchy. Acute coronary syndrome (one study) was grouped with studies of CHD, and all cerebrovascular disease (one study) was groups with studies of total stroke.

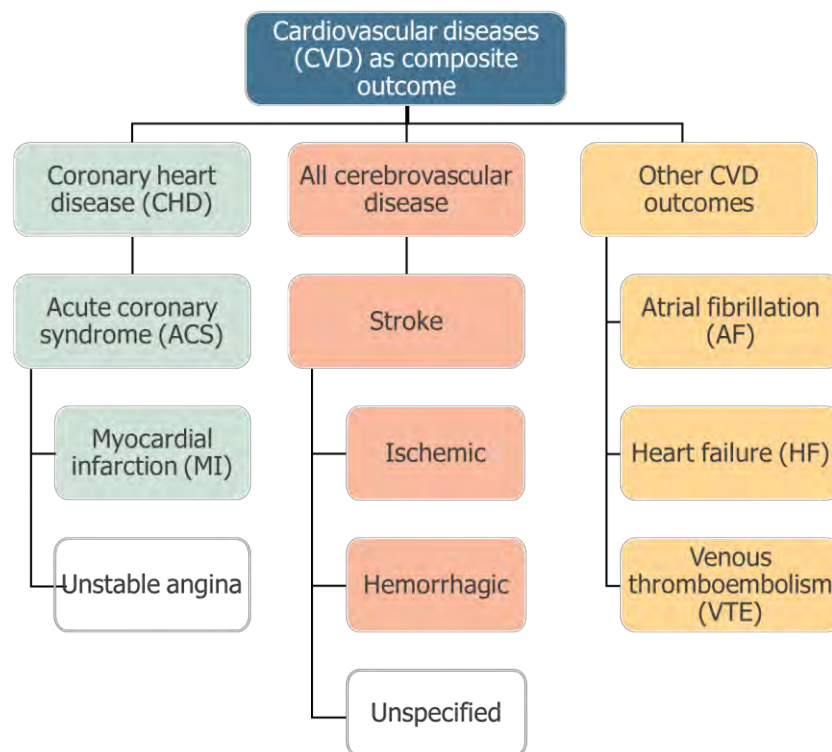


Figure 4.1-1 Overview of CVD outcomes included in primary studies.

Mechanisms CVD

The potential mechanisms for how fish consumption may prevent CVD, and all the underlying outcomes like CHD, stroke and MI, with fish consumption are quite well described. These mechanisms have been attributed to essential nutrients found in fish, including long chain n-3 fatty acids (LC n-3 FA), vitamin D, trace elements, and bioactive peptides. Mechanisms for LC n-3 FA influence on CVD is described in more detail in Chapter 5.2.

Fatty fish is also an important source of vitamin D. Vitamin D can directly regulate gene expression by acting as ligand for the vitamin D receptor, and in addition, vitamin D regulates the renin-angiotensin-aldosterone system (Mheid et al., 2017). It has also been suggested that vitamin D regulates blood pressure by influencing arterial stiffness and endothelial function, and vitamin D may inhibit inflammation. Experimental, cross-sectional, and prospective evidence suggest that vitamin D deficiency play a role in the pathogenesis of CVD, however, meta-analyses of RCTs have found no association between vitamin D intake and inflammation markers, blood pressure or arterial stiffness (Zittermann et al., 2019).

Fish contains trace elements, including selenium and iodine. Selenium can alleviate oxidative stress and inflammation in patients with CVD.

Fish is also considered an excellent source of protein and contain bioactive peptides that may inhibit the angiotensin-converting enzyme (ACE) and thereby have a blood pressure reducing effect (Kim et al., 2012).

Due to contamination of aquatic ecosystems and aquaculture feed ingredients, fish may also contain contaminants, and intake of fish could potentially cause adverse health effects. Moreover, contaminants present in fish may counteract mechanisms induced by nutrients, and hence modify potential beneficial health effects of fish intake. Compounds may also be added or lost from the fish during processing, packaging, and/or preparation. This can further affect the net health effect of fish intake (e.g Ho et al., 2021).

4.2 Fish intake and CVD incidence

4.2.1 VKM's search for previous systematic reviews and meta-analyses of fish intake and CVD incidence

4.2.1.1 Description of the identified publications

VKM's search for systematic reviews and meta-analyses (see Chapter 3.1.2.2) identified one publication on fish intake and incident CVD (Krittawong et al. 2021). This study was excluded after quality assessment (graded C by VKM using the AMSTAR tool).

4.2.2 VKM's systematic review of primary studies on fish intake and CVD incidence

4.2.2.1 Included studies from search

A total of 11 publications graded A or B, including one pooled analysis and one global, multicenter study, presented results on CVD incidence: Bonaccio et al., 2017, Erkkila et al., 2003, Morris et al., 1995, Nahab et al., 2016, Mohan et al., 2021; Rhee et al., 2017, Strom et al., 2012, Strom et al., 2011, Virtanen et al., 2008; Zhang et al., 2021; Zhong et al., 2020. One study was limited to CHD patients (Erkkila et al., 2003) and the global multicenter study (Mohan et al., 2021) assessed CVD in patient groups with a history of CVD or at high risk of CVD (all sub-cohorts) as well as in the general population (one sub-cohort). Thus, one of 11 studies was conducted in patients only, nine of 11 in general population, and one in both patients and the general population (as separate analyses).

Selected study characteristics (study name, design, time period, size and age of the study population and dietary assessment method) are presented in Table 4.2.2.1-1.

Table 4.2.2.1-1 Overview of primary studies included in weight of evidence analysis of fish intake and CVD incidence.

Author, year, country	Study name	Study design	Inclusion year(s), end, follow-up time	Study population	Dietary assessment method	Dietary assessment period
Bonaccio et al., 2017, Italy	Moli-sani study	Prospective observational	2005-2010 to 2011, 4.3 yrs follow-up (median)	24 325 (46% male), ≥35 yrs (mean age 55 yrs)	Italian version of EPIC FFQ, validated	Previous year, at baseline
Mohan et al., 2021, Global, 6 continents, 58 countries	PURE, ONTARGET, TRANSCEND, and ORIGIN, only data from PURE on general population	Prospective observational, multicenter	Follow-up to 2019 (PURE), Median follow-up (yrs) was 9.1 in PURE	191 558 (47.9% male), 51 731 with vascular disease and 139 827 generally healthy. PURE (n=147 541), Mean age PURE 51 (35-70) yrs	Country specific FFQs	Usual intake in previous year, at baseline
Morris et al., 1995, USA	Physicians' Health Study (PHS)	Prospective observational	1982, 4 yrs of follow-up	21 185 male physicians, 40-84 yrs	FFQ, semi quant, validated	Average intake, previous year, at 12-month follow-up
Nahab et al., 2016, USA	REasons for Geographic And Racial Differences in Stroke (REGARDS) study	Prospective observational	2003-2007 (inclusion yrs.) to 2010, 5.1 yrs follow up (median)	16 479 men and women (34% African Americans, 59% female, 74% were overweight or obese), 40-75 yrs	FFQ, Block98	Usual intake, past year, at baseline
Rhee et al., 2017, USA	Women's Health Study (WHS)	Prospective observational	1993 to 2014, 22 yrs follow-up	38 392 female health professionals, ≥45 yrs	FFQ, semi-quant, validated	Average intake, at baseline
Strom et al., 2012, Denmark	Danish National Birth Cohort	Prospective observational	1996-2002 to 2008, 8 yrs follow-up (median)	48 627 pregnant women, 15.7– 46.9 yrs (mean 29.9 yrs)	2 computer assisted telephone interviews and FFQ, semi-quant, validated	Telephone interviews in 1st and 3rd trimester (weeks 12 and 30). FFQ in 2nd trimester (week 25) covered previous month
Strom et al., 2011, Denmark	Aarhus Birth Cohort (ABC)	Prospective observational	1992-1997 to 2009, 15 yrs follow-up (median)	7429 pregnant women, <20 to 40+ yrs	Pregnancy questionnaires	Intake in pregnancy at week 16 (intake since pregnant) and 30 (intake since wk 16)

Author, year, country	Study name	Study design	Inclusion year(s), end, follow-up time	Study population	Dietary assessment method	Dietary assessment period
Virtanen et al., 2008, USA	Health Professionals Follow-Up Study (HPFS)	Prospective observational	1986 to 2004, 18 yrs follow-up	40 230 male health professionals, 40-75 yrs, mean age approx 53 yrs	Repeated FFQ (1986, 1990, 1994, 1998, and 2002), semi-quant, validated	Average intake, previous year
Zhang et al., 2021, UK	UK Biobank	Prospective observational	2006-2010 to 2020, follow-up 11.2 yrs (median)	462,155 (44% male), 40-69 yrs, mean 56.7 yrs	Touchscreen FFQ	NA, probably usual intake at baseline
Zhong et al., 2020, USA	Lifetime Risk Pooling Project: Atherosclerosis Risk in Communities study (ARIC), Coronary Artery Risk Development in Young Adults (CARDIA) study, Cardiovascular Health Study (CHS), Framingham Heart Study (FHS), Framingham Offspring Study (FOS), and Multi-Ethnic Study of Atherosclerosis (MESA)	Prospective cohorts, pooled	1985-2002 (inclusion 1986-1990 for FHS, 1991-1995 for FOS, 1986-1989 for ARIC, 1985-1986 for CARDIA, 1989-1990 for CHS, 2000-2002 for MESA) to 2016, follow-up 19 yrs (median)	29 682 (44.4% male), mean age 53.7 yrs	FFQ, validated or diet history, depending on study	NA, probably usual intake at baseline
Patient populations						
Erkkila et al., 2003, Finland	Finnish sub-cohort of European Action on Secondary Prevention through Intervention to Reduce Events (EUROASPIRE)	Prospective observational	1991-1994 (first hospitalization), baseline examination in 1995, 5 yrs of follow-up to 2000 (hospitalization) or 2001 (deaths)	285 men and 130 women with coronary artery disease, 33-74 yrs, mean age 61 yrs	4-d food record (3 weekdays and 1 weekend day) completed at home. Portion size booklet	Current intake at baseline, 4 days

Author, year, country	Study name	Study design	Inclusion year(s), end, follow-up time	Study population	Dietary assessment method	Dietary assessment period
Mohan et al., 2021, Global, 6 continents, 58 countries	PURE, ONTARGET, TRANSCEND, and ORIGIN	Prospective observational, multicentre	Follow-up to 2019 (PURE), Median follow-up (yrs) was 9.1 in PURE, 4.5 in ONTARGET and TRANSCEND, and 6.2 in ORIGIN	191 558 (47.9% male), 51 731 with vascular disease and 139 827 generally healthy. PURE (n=147 541), ONTARGET and TRANSCEND (n = 31 491), ORIGIN (n=12 422). Mean age PURE 51 (35-70) yrs, ONTARGET and TRANSCEND 67 yrs, ORIGIN 64 yrs	Country specific FFQs (no amounts in ONTARGET and TRANSCEND), validated in some countries	Usual intake in previous year, at baseline

4.2.2.2 Overlapping publications

No overlapping publications were identified among studies of CVD incidence.

4.2.2.3 Studies by design and geographic region

All eleven publications on CVD incidence were based on prospective, observational designs (cohort, birth cohort, or follow-up of RCT) and study samples from Western populations in Denmark (Strom et al., 2012, Strom et al., 2011), Italy (Bonaccio et al., 2017) or USA (Morris et al., 1995, Nahab et al., 2016, Rhee et al., 2017, Virtanen et al., 2008, Zhong et al., 2020). One study was a global multicenter study with data from 58 countries on 6 continents (Mohan et al. 2021).

Both Danish studies were based on birth cohorts, and fish intake during pregnancy was considered representative of habitual intake after pregnancy. The women were still relatively young, and cases of CVD were therefore largely non-fatal.

Two of the publications included multiple studies. Zhong et al., 2020 (Lifetime Risk Pooling Project) pooled data from 6 US cohort studies (ARIC, CARDIA, Cardiovascular Health Study; Framingham; Framingham Offspring; and Multi-Ethnic Study of Atherosclerosis), and Mohan et al. (2021) presented data from one prospective cohort and three follow-up studies of drug trials.

4.2.2.4 Studies in patient populations

Two studies were carried out in patient populations (Erkkila et al., 2003; Mohan et al. 2021). Erkkila et al., (2003) included 285 men and 130 women with coronary artery disease (CAD) from the Finnish sub-cohort of the European Action on Secondary Prevention through Intervention to Reduce Events (EUROASPIRE) (Erkkila et al., 2003). Fish intake was not part of the intervention, and the study used fish intake measured at baseline, which was after first hospital admission. In addition to the composite CVD outcome (CVD death, or non-fatal acute MI, or non-fatal stroke), the study also included CAD (CAD death, or non-fatal acute myocardial infarction), CAD mortality, and all-cause mortality. Therefore, this study is included multiple times under several outcomes.

Mohan et al. (2021) included data from four studies: one cohort study (PURE) and follow-up of three drug trials (ONTARGET, TRANSCEND, and ORIGIN). In addition to the composite outcome of major CVD events, the study also included MI, stroke, CVD death, cardiac death, and total mortality (and a composite of death and major CVD not summarized here). Therefore, this study is also presented multiple times under several outcomes. The different studies in Mohan et al. (2021) are described in more detail below:

The Prospective Urban Rural Epidemiology (PURE) study is a large cohort study conducted in 21 low-, middle-, and high-income countries on 5 continents. The analysis included 147 645 participants with complete information on diet, where 5.3% had a history of CVD. Only results in patients with a CVD history were considered here.

The Ongoing Telmisartan Alone and in Combination With Ramipril Global EndPoint Trial (ONTARGET) is an RCT of antihypertension medication (ramipril, telmisartan, or combination). All patients had vascular disease or diabetes which is a risk factor for vascular disease. The Telmisartan Randomized Assessment Study in ACEIntolerant Subjects With Cardiovascular Disease (TRANSCEND) was an RCT of telmisartan vs placebo. The analysis included 31 491 participants (ONTARGET and TRANSCEND combined) with dietary assessments in 40 countries on 6 continents. ONTARGET and TRANSCEND did not include low-income countries. Results were presented for ONTARGET and TRANSCEND combined.

The Outcome Reduction With Initial Glargine Intervention (ORIGIN) trial was an RCT of insulin glargine therapy or standard care and n-3 fatty acid or placebo supplementation (2 × 2 factorial design). The participants had cardiovascular risk factors plus impaired fasting glucose, impaired glucose intolerance, or diabetes. The analysis included 12 422 participants with dietary assessments in 40 countries (19 middle-income and one low-income) on 5 continents. Information about type of fish was only collected in ORIGIN.

4.2.2.5 Studies by sex, potential effect modification, and other sub-groups

Of the 11 studies on CVD, six included both men and women (Bonaccio et al. 2017, Erkkila et al. 2003; Mohan et al. 2021; Nahab et al. 2016, Zhang et al., 2021, Zhong et al., 2020), the remaining studies were conducted in males (Morris et al., 1995, Virtanen et al., 2008) or females (Rhee et al., 2017, Strom et al., 2012, Strom et al., 2011) only. Three US studies were conducted in male (Morris et al., 1995, Virtanen et al., 2008) or female (Rhee et al., 2017) health professionals. Among studies in both men and women, Bonaccio et al. (2017) reported to have examined potential effect modification by sex. The test of interaction was not statistically significant ($p > 0.05$). Zhang et al. (2021) reported a stronger association for total fish intake with CVD risk in women relative to men but without a test of interaction. VKM only calculated summary RRs for men and women combined.

Mohan et al., 2021 stratified results by CVD history in the study participants (PURE study only) whereas Zhang et al. (2021) stratified results by genetic CVD risk, defined as a family history of cardiovascular disease (CVD) or a CVD polygenic risk score (PRS) in the study participants. Associations in Mohan et al. 2021 differed by CVD history and are presented separately for the general population (Chapter 4.2.3.1) and patient populations (Chapter 4.2.3.4). Zhang et al. (2021) reported similar results for subgroups with and without a family history of CVD ($P_{\text{interaction}} = 0.13$) and for the highest versus lowest quartile of the PRS ($P_{\text{interaction}} = 0.77$) and only the combined results for all participants are presented in this report.

4.2.2.6 Studies by fish exposure

All studies except two (Nahab et al. 2016, Rhee et al. 2017) included a total fish exposure (sum of all fish, unspecified fish, or fish including shellfish). Several studies presented results by multiple fish classifications. The most common sub-classifications were fatty or lean (based on species mainly), or by flesh color (e.g. "dark fish"). When "dark fish" in Virtanen et al. (2008) (mackerel, salmon, sardines, bluefish, and swordfish given as examples in FFQ)

was categorized as fatty fish and remaining fish (not dark) as lean fish, we could study fatty fish in three studies and lean fish in two studies in the general population. Results were not summarized for categories of fish found in only one study; fried or non-fried fish (Nahab et al., 2016), tuna only (Virtanen et al., 2008), preserved fish (canned or dried, Bonaccio et al., 2017) or all fish excluding cod fish (Carballo-Casla et al., 2021). Both studies in patients (Erkkila et al. 2003; Mohan et al. 2021) included total fish, and Mohan et al. 2021 additionally presented results on lean fish and fatty fish in one sub-cohort.

4.2.2.7 Studies assessing potential non-linearity

One primary study presented a dose-response figure of fatty fish intake and risk of incident CVD (Bonaccio et al., 2017) based on restricted cubic spline regressions, which takes potential non-linearity into account.

4.2.3 Results from the included primary studies fish intake and CVD incidence

4.2.3.1 Studies of total fish intake and CVD incidence

We summarized eight publications with eight estimates of the association between total fish intake (excluding two studies with other fish exposures) and risk of developing CVD in the general population. Table 4.2.3.1-1 shows the exposure levels and results in these studies. The sex-specific estimates are included in addition to the overall estimate (men and women combined) for one study that reported statistically significant effect modification by gender (Zhang et al., 2021). All studies had a prospective, observational design.

Table 4.2.3.1-1 Results from prospective observational studies included in the weight of evidence analysis of total fish intake and CVD incidence.

Author, year, country	Fish exposure, sex	Intake unit	High-low intake	Total cases	HR high-low (95 % CI)	Overall result
Bonaccio, 2017, Italy	Fish, incl shellfish, M/W	Times/wk, 3 cat	>4 vs <2 times/wk, 92.5 vs 23.0 g (mean)	352	0.62 (0.42, 0.93)	Protective assoc. of highest vs lowest category, <i>P</i> -trend=0.008, or 10% lower risk per 1/wk increase
Mohan, 2021, global, 6 continents, 58 countries	Fish, incl shellfish, M/W, PURE study	g/mo or wk, 4 cat	≥350 g/wk vs <50 g/mo, median 594 vs 0.1 g/wk	6825	0.97 (0.88, 1.08)	No sig. assoc., <i>P</i> -trend 0.24
Morris, 1995, USA	Fish, M	Meals/wk, 4 cat	≥5 per wk vs <1 per mo	525	0.9 (0.6, 1.5)	No sig. assoc. overall. Sig. adverse assoc. for intake 1/wk vs <1/wk only, not higher. <i>P</i> -trend 0.65
Strom, 2012, Denmark	Fish, W	g/d, 5 cat	>30 vs 0-3 g/d	577	0.65 (0.47, 0.88), reported as 1.54 (1.13, 2.11) for low vs high	Protective assoc. of higher intakes, <i>P</i> -trend 0.024
Strom, 2011, Denmark	Fish, incl shellfish, W	g/d, 6 cat	No intake + quintiles among consumers, upper quintile (mean 39 g/d) vs no intake	263	1.30 (0.51, 3.33), reported as low vs high: 0.77 (0.30, 1.96)	No sig. assoc. Sig. adverse effect of intake in 2 nd quintile, <i>P</i> -trend 0.61
Virtanen, 2008, USA	Fish, M	Servings, 5 cat, cumulative average	≥5/wk vs <1/mo	3639	1.04 (0.87, 1.25)	Protective assoc. of 1 serving/wk and 2–4 servings/wk, but not ≥5/wk vs <1/mo, <i>P</i> -trend 0.24
Zhang, 2021, UK	Total fish, M/W	Times/wk, 4 cat	≥3 vs <1/wk	46164	0.92 (0.89, 0.96)	Protective assoc., <i>P</i> -trend <0.001, reported sex interaction.
	Total fish, M	Times/wk, 4 cat	≥3 vs <1/wk	27085	0.97 (0.92, 1.03)	No sig. assoc.
	Total fish, W	Times/wk, 4 cat	≥3 vs <1/wk	18570	0.85 (0.79, 0.91)	Protective assoc. in all cat. above reference
Zhong, 2020, USA	Fish, incl shellfish, M/W	Servings/d: quintiles (cohort specific)	Quintile 5 vs 1, 0.47 vs 0.02 serving/day/1000 kcal	6963	1.04 (0.96, 1.13)	No sig. assoc., <i>P</i> -trend 0.31

Among the eight studies of total fish intake in over 65,000 cases of incident CVD, three reported a statistically significant protective association, the remaining studies reported no association.

4.2.3.2 Studies of fatty fish intake and CVD incidence

As previously mentioned, three studies (all prospective, observational) analyzed the association of fatty fish with risk of incident CVD in the general population. The exposure levels and results are shown in Table 4.2.3.2-1. Two studies did not find a statistically significant association, while one reported a protective association of intake once or more per week.

Table 4.2.3.2-1 Results from prospective observational studies included in the weight of evidence analysis of fatty fish intake and CVD incidence.

Author, year, country	Fish exposure, sex	Intake unit	High-low intake	Total cases	HR high-low (95 % CI)	Overall result
Bonaccio, 2017, Italy	Fatty fish, M/W	Times/wk, 3 cat	≥1/wk vs none, 24.2 vs 0 g/d (mean)	352	0.56 (0.36, 0.87)	Protective assoc. of ≥1/wk vs none, inverse trend
Rhee, 2017, USA	Fatty fish, W	Servings/mo or wk, 4 cat	>1/wk vs <1/mo	1941	1.00 (0.86, 1.16)	No sig. assoc., <i>P</i> -trend 0.78
Virtanen, 2008, USA	Fatty fish, M	Servings, 5 cat, cumulative average	≥2/wk vs <1/mo	3639	1.10 (0.93, 1.29)	No sig. assoc., <i>P</i> -trend 0.35

4.2.3.3 Studies of lean fish intake and CVD incidence

Two studies of fatty fish intake also provided estimates for lean fish intake (Table 4.2.3.3-1). As shown below, one study did not find a statistically significant result, the other reported a protective association of intake without a significant trend.

Table 4.2.3.3-1 Results from prospective observational studies included in the weight of evidence analysis of lean fish intake and CVD incidence.

Author, year, country	Fish exposure, sex	Intake unit	High-low intake	Total cases	HR high-low (95 % CI)	Overall result
Bonaccio et al., 2017, Italy	Lean fish, M/W	Times/wk, 3 cat	≥1 vs none, 30.9 vs 0 g/d (mean)	352	1.25 (0.70, 2.23)	No sig. assoc.
Virtanen et al., 2008, USA	Lean fish, M	Servings, 5 cat, cumulative average	≥2/wk vs <1/mo,	3639	1.00 (0.88, 1.13)	Protective assoc. of 1 serving/wk and borderline for 1-3/mo vs <1/mo, <i>P</i> -trend 0.58

4.2.3.4 Studies of fish intake and CVD risk in patient populations

Both Erkkila et al. (2003) and Mohan et al. 2021 consistently reported association on the protective side, but not statistically significant, for total fish intake in patients with previous CVD, or at high risk of CVD. In the only study of fatty fish and lean fish (ORIGIN sub-cohort in Mohan et al. 2021), the association was protective and statistically significant for fatty fish. No association was seen for lean fish (Table 4.2.3.4-1).

Table 4.2.3.4-1 Results from prospective observational studies included in the weight of evidence analysis of fish intake and CVD risk in patients.

Author, year, country	Fish exposure, sex	Intake unit	High-low intake	Total cases	HR high-low (95 % CI)	Overall result
Prior CVD or high risk, total fish						
Erkkila, 2003, Finland	Fish, M/W	g/d, 3 cat (above/below median, null)	Cat 3 vs 1, >57 (above median) vs 0 g/d	44	0.45 (0.19, 1.09)	Suggestive, <i>P</i> -trend 0.12
Mohan, 2021, global, 6 continents, 58 countries	Fish, incl shellfish, M/W, PURE	g/mo or wk, 4 cat	≥350 g/wk vs <50 g/mo, median 594 vs 0.1 g/wk	1363	0.86 (0.69, 1.08)	No sig. assoc., <i>P</i> -trend 0.08
	Fish, incl shellfish, M/W, ONTARGET, TRANSCEND	g/mo or wk, 4 cat	≥350 g/wk vs <50 g/mo, median 450 vs 2.8 g/wk	5182	0.91 (0.81, 1.03)	Borderline protective assoc. in two highest categories, <i>P</i> -trend 0.02
	Fish, incl shellfish, M/W, ORIGIN	g/mo or wk, 4 cat	≥350 g/wk vs <50 g/mo, median 568 vs 2.2 g/wk	2020	0.87 (0.76, 1.01)	Protective or borderline protective assoc. in all categories, <i>P</i> -trend 0.02
Prior CVD or high risk, fatty and lean fish						
Mohan, 2021, global, 6 continents, 58 countries	Fatty fish, M/W, ORIGIN	g/d, continuous, per 5 g/d increment		2020	0.94 (0.92, 0.97)	Protective assoc.
	Lean fish, M/W, ORIGIN	g/d, continuous, per 5 g/d increment		2020	1.03 (0.98, 1.08)	No sig. assoc.

4.2.3.5 Summary relative risks (RRs) based on VKM's inclusion of primary studies

The summary relative risk (RR) for incident CVD in the general population (Table 4.2.3.1-1) suggested a protective but statistically non-significant association for the highest versus lowest intake of total fish (RR=0.94, 95% CI: 0.86, 1.02, eight studies). There was significant heterogeneity ($P_{\text{heterogeneity}}=0.01$), but no reports of a statistically significant adverse effect.

The summary RRs for fatty fish (three publications, Table 4.2.3.2-1) was similar to total fish in magnitude, but with a wider confidence interval (RR=0.93, 95% CI: 0.73, 1.19, $P_{\text{heterogeneity}}=0.018$). The association with lean fish (two publications, Table 4.2.3.3-1) was at unity (RR=1.01, 95% CI 0.90, 1.14, $P_{\text{heterogeneity}}=0.46$). VKM did not identify a previous meta-analysis for comparison.

The summary RR for CVD in high-risk patients with diabetes, a history of vascular disease or CVD (Table 4.2.3.4-1) indicated a protective association with total fish that was statistically significant overall: RR=0.88 (95% CI: 0.81, 0.96) without significant heterogeneity (two publications, four cohorts, $P_{\text{heterogeneity}}=0.47$).

4.2.4 Heterogeneity fish intake and CVD incidence

The summary relative risk estimated based on the included primary studies showed significant heterogeneity, but what explained this heterogeneity was not further explored.

4.2.5 Dose-response relationship fish intake and CVD incidence

No meta dose-response analysis was identified. The dose-response analysis in one primary study (Bonaccio et al. 2017) suggested a linear, inverse relationship for fatty fish (P -value for non-linearity = 0.99). Judging from the confidence interval of the line (figure not shown), the relationship was not statistically significant for intakes higher than 38 g/day.

In the 4 sub-cohorts of patients with vascular disease in Mohan et al. (2021), significant linear trends were reported across intake categories of total fish in ONTARGET/TRANSCEND (P -trend 0.02) and ORIGIN (P -trend 0.02) but somewhat weaker in PURE (P -trend 0.08).

4.2.6 Weight of evidence fish intake and CVD incidence

In this section, the evidence of the association between fish intake and CVD incidence is weighed according to the WCRF criteria presented in Chapter 3.1.6 (Box 2).

Published evidence of fish intake and CVD incidence

No relevant systematic reviews or meta-analyses of the association between fish intake and risk of incident CVD as a composite outcome, were identified. VKM identified 10 primary studies in the general population, and two (including one global, multicenter study with 4 sub-cohorts) in patients with a history of CHD or CVD. The summary RR for primary studies

included by VKM suggests reduced risk of CVD for the highest versus lowest intake of total fish (eight studies), but the association was only statistically significant in patients.

Heterogeneity

There was significant heterogeneity between studies included in VKM's summary RR for the general population, but the direction of the associations was generally consistent with no reports of statistically significant adverse associations. There was no significant heterogeneity between studies in patient populations.

Mechanism / biological plausibility

Plausible mechanisms for an effect of LC n-3 FA have been described.

Upgrading factors

Dose-response was evaluated but not found to be an upgrading factor in this case. No meta dose-response analysis was found. No other upgrading factors were evaluated.

4.2.6.1 Conclusion fish intake and CVD incidence

There is evidence from more than two independent and good quality cohort studies on total fish intake (VKM included eight publications on the general population, two on patients, but no previous meta-analysis). VKM's summary RR for primary studies in the general population is not statistically significant but suggests lower risk of CVD for the highest versus lowest intake of total fish. The direction of association is generally consistent, but with some heterogeneity between studies. There is evidence for biological plausibility.

In conclusion, the evidence was graded "limited, suggestive" for a protective effect of fish consumption on incident CVD in the general population. VKM's summary RR for studies in patients with prior CVD or at high risk was slightly stronger, but consistent with the summary RR for the general population.

There were fewer studies of fatty fish and lean fish than total fish, and the evidence was too limited to conclude on a differential effect of fatty and lean fish on risk of CVD. Therefore, the evidence was graded "limited, no conclusion" for an effect of fatty fish and lean fish on risk of incident CVD in the general population.

4.3 Fish intake and CHD incidence

4.3.1 VKM's search for previous systematic reviews and meta-analyses of fish intake and CHD incidence

4.3.1.1 Description of the identified publications

VKM's search for systematic reviews and meta-analyses (see Chapter 3.1.2.2) identified nine publications on the association between fish intake and CHD incidence that fulfilled the inclusion criteria and were read as full papers. Four papers were excluded (see Table 4.3.1.1-1 for reason for exclusions).

Table 4.3.1.1-1 Reasons for exclusion of identified papers from the search of systematic reviews and meta-analyses of fish intake and CHD incidence 2016-2021.

Included papers	Excluded paper and reasons for exclusions
Umbrella reviews Jayedi and Shab-Bidar, 2020 D'Alessandro et al., 2019	Kromhout et al., 2016: Type of umbrella review. No search strategy was presented, no description of quality assessment.
Systematic reviews Jayedi et al., 2020, patients with T2D Zhang et al., 2020 Bechthold et al., 2019	Schwingshackl et al., 2019: Comparative risk assessment modelling study, not a systematic literature review or a meta-analysis.
	Micha et al., 2017: Umbrella review combined with some meta-analysis, but selection of papers was only done by one person. No information about any quality assessment for the included meta-analysis. One meta-analysis of fish and CHD mortality was included (Zheng et al., 2012).
	Umesawa et al., 2020: Narrative review. Target group was only Japanese population.

The meta-analyses are described in more detail below; first a main description of the methods used and then main/selected results from each meta-analysis are presented (Table 4.3.1.2-1).

Two of the identified five studies were umbrella reviews (Jayedi and Shab-Bidar 2020; D'Alessandro et al., 2019). These umbrella reviews build on one relevant meta-analysis; Bechthold et al. (2019). Bechthold et al. (2019) was also identified in our search, and in addition, we identified two more recent meta-analyses; Zhang et al. (2020) and Jayedi et al. (2020), that were not included in the umbrellas (see flow-chart in Figure 4.3.1.1-1 below).

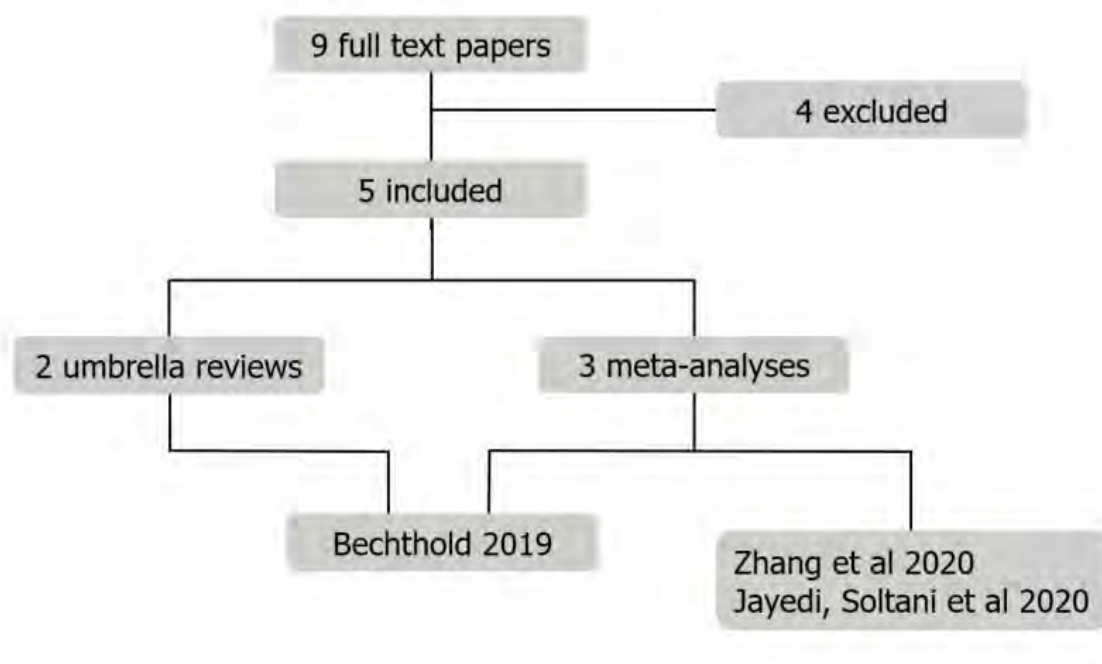


Figure 4.3.1.1-1 Flow-chart of the included meta-analyses for fish intake and CHD.

Umbrella reviews

Jayedi and Shab-Bidar (2020) is an umbrella review of meta-analyses of prospective studies investigating fish intake and different outcomes (CVD, T2D), site-specific cancers, neurological disorders, all cause and cause-specific mortality, and any other diseases. This umbrella review selected only the meta-analyses with the largest number of primary prospective cohort studies, one for each outcome. The quality of the meta-evidence was assessed using the NutriGrade scoring system. For total fish intake and risk of CHD Jayedi and Shab-Bidar (2020) included the meta-analysis by Bechthold et al. (2019) (described below).

D'Alessandro et al. (2019) is an umbrella review on the association between food groups and CVD, CHD, stroke, T2D, colorectal cancer and breast cancer risk. They identified one meta-analysis for fish intake and CHD as outcome; Bechthold et al. (2017) (see below for description of the study).

Meta-analyses

Jayedi et al. (2020) is a meta-analysis of prospective studies investigating fish intake and different cardiovascular outcome (CHD, stroke, MI) in patients with T2D. The authors did a systematic search in PubMed and Scopus databases up to June 2019. The quality of eligible papers was assessed with use of the 9-point Newcastle-Ottawa scale. Three prospective cohort studies were included in this meta-analysis of fish intake and the risk of CHD (Hu et al., 2003; Deng et al., 2018; Wallin et al., 2018), and they scored 8, 8, and 9 by the Newcastle-Ottawa scale. Jayedi et al. (2020) graded the quality of the meta-evidence for fish intake and CHD as low (score = 5.2) based on the NutriGrade scoring system.

Zhang et al. (2020) is a meta-analysis of prospective studies investigating the association between fish intake and CHD incidence and mortality. The authors performed a systematic literature search in the Web of Science, Embase, and PubMed databases until October 2019. The quality of the eligible papers included in the meta-analysis was assessed by The Newcastle-Ottawa Scale criteria. The criteria included nine aspects with a maximum of 9 points. Scores of 0–3, 4–6, and 7–9 points indicated low, medium, and high quality, respectively. Twenty-two studies looking into fish intake and CHD incidence were included. The quality of all the papers included in the meta-analysis were overall; 13 high-quality articles and 9 medium-quality articles.

Bechthold et al. (2019) performed a systematic literature search in PubMed and Embase until March 2017 and included 22 prospective studies on fish intake and risk of CHD. The aim of this meta-analysis was to synthesize the knowledge about the relation between intake of major food groups, including fish, and the risk of CHD, stroke, and heart failure. Bechthold et al. (2019) rated the quality of the meta-evidence of fish intake and CHD (based on n=15 studies) as moderate based on the NutriGrade scoring system.

4.3.1.2 Results from the meta-analyses

Below is a summary table for total fish intake and CHD based on the three identified meta-analyses.

Table 4.3.1.2-1 Summary of results from meta-analyses on total fish intake and risk of CHD incidence.

Author, year	Type of studies included	Total no studies	No of cases	Comparison	Summary RR (95% CI)	Heterogeneity	Overall conclusion
Jayedi, 2020	Prospective studies which evaluated fish (seafood) intake and CHD (incidence or mortality) in patients with T2D	3	791	Highest vs lowest	0.61 (0.29, 0.93)	$I^2=68\%$	Fish consumption was associated with a lower risk of CHD in patients with T2D. The quality of the meta-evidence was rated low
Zhang, 2020	Prospective cohort studies which evaluate the association between fish intake and CHD incidence	22	15 973	Highest vs lowest	0.91 (0.84, 0.97)	$I^2=47.4\%$	Higher fish consumption is inversely related to CHD incidence
		19		Per 20 g/d	0.96 (0.95, 0.97)		
Bechthold, 2019	Prospective cohort studies which evaluated the association between fish intake and CHD (excluding studies of mortality)	22	16 732	Highest vs lowest	0.94 (0.88 to 1.02)	$I^2=52\%$	Fish intake is associated with a decreased risk of CHD. The quality of the meta-evidence was rated moderate
		15		Per 100 g	0.88 (0.79 to 0.99)	$I^2=40\%$	

Both meta-analyses based on prospective studies from the general population concluded that fish intake is associated with a decreased risk of CHD. This was also observed in the meta-analysis of studies in T2D patients.

4.3.2 VKM's systematic review of primary studies on fish intake and CHD incidence

4.3.2.1 Included studies from search

A total of 14 publications graded A or B included CHD incidence as outcome; Ascherio et al. (1995); Bernstein et al. (2010); Bonaccio et al. (2017); Burr et al. (1989); Erkkila et al. (2003); Gillum et al. (2000); Hengeveld et al. (2018); Hu et al. (2002); Hu et al. (2003); Iso et al. (2006); Key et al. (2019); Manger et al. (2010); Osler et al. (2003); Zhang et al. (2021). One study (Bjerregaard et al., 2010) assessed acute coronary syndrome (ACS) as outcome, a composite of acute myocardial infarction and unstable angina (Figure 4.1.2-1), and was included with the other CHD studies, 15 in total.

There were overlapping publications from the same studies as described below, and one was excluded, leaving 14 for further analysis. Four of 14 publications concerned patient populations, one with type 2 diabetes (Hu et al. 2003) and three with CHD (Burr et al. 1989; Erkkila et al. 2003; Manger et al. 2010). Thus, 10 studies were included in the analysis of CHD in the general population.

A description of the studies (study name, design, time period, size and age of the study population, and dietary assessment method) can be found in Table 4.3.2.1-1.

Table 4.3.2.1-1 Overview of primary studies included in weight of evidence analysis of fish intake and incidence of CHD or acute coronary syndrome (ACS).

Author, year, country	Study name	Study design	Inclusion year(s), end, follow-up time	Study population	Dietary assessment method	Dietary assessment period
Ascherio, 1995, USA	Health Professionals Follow-Up Study (HPFS)	Prospective observational	1986, 6 yrs of follow-up	44 895 male health professionals, 40-75 yrs	FFQ, semi quant, validated	Average frequency during the previous year, at baseline
Bernstein, 2010, USA	Nurses' Health Study (NHS)	Prospective observational	1980 to 2006, 26 yrs follow-up	84 136 female nurses, 30–55 yrs	Repeated FFQ every 4 yrs (1980, 1984, 1986, 1990, 1994, 1998, and 2002), semi-quant, validated	Average intake during the previous year
Bjerregaard, 2010, Denmark	Diet, Cancer and Health cohort, Danish Cancer Society	Prospective observational	1993–97 to 2003, 7.6 yrs follow-up (mean)	25 573 men and 28 653 women, 50 -64 yrs	FFQ, semi quant, validated	Frequency during the previous year, at baseline
Bonaccio, 2019, Italy	Moli-sani study	Prospective observational	2005-2010 to 2011, 4.3 yrs follow-up (median)	24 325 (46% male), ≥35 yrs (mean age 55 yrs)	Italian version of EPIC FFQ, validated	Previous year, at baseline
Gillum, 2000, USA	National Health and Nutrition Examination Survey (NHANES I) follow-up study	Prospective observational	1971–1975 to 1992, 18.8 yrs follow-up (mean)	8825 (7421 white and 1404 black Americans). Oversampling of the elderly, women of childbearing age, and persons residing in poverty areas, 25–74 yrs	FFQ by interview	Usual intake, 3 month prior to interview
Hengeveld, 2018, the Netherlands	EPIC-Netherlands (Prospect and MORGEN sub-cohorts)	Prospective observational	1993-1997 to 2011, 18 yrs follow-up (median 15.1 yrs)	34 033 (25% male), 20-70 yrs, mean age 48.7 yrs	FFQ, semi quant, validated	Usual intake, previous year, at baseline
Iso, 2006, Japan	Japan Public Health Center-Based (JPHC) Study Cohort I	Prospective observational	1990-1992 to 2001, 11 yrs follow-up	41 578 (19 985 men and 21 593 women), 40-59 yrs	Repeated FFQ (1990, 1995), validated	Average intake previous month (1990) or previous year (1995)
Key, 2019, Europe (9 countries)	EPIC	Prospective observational, pooled	NA to 2003-2010, but mainly 2008 or 2009, 12.6 yrs follow-up (mean)	106 751 men (4608 cases) and 303 134 women (2590 cases), Mean 52.7 yrs (M) and 51.3 yrs (W)	Contry specific methods, mostly validated FFQs	Year before enrolment

Author, year, country	Study name	Study design	Inclusion year(s), end, follow-up time	Study population	Dietary assessment method	Dietary assessment period
Osler, 2003, Denmark	Copenhagen County Centre for Preventive Medicine (CPM) cohort (5 sub cohorts incl MONICA I-III)	Prospective observational	1982–1992 to 1997 (CHD incidence) or 2000 (mortality)	4007 men and 3533 women, incl priori defined CHD high risk group (981 men and 622 women), 30–70 yrs	FFQ, validated	Average intake, at baseline
Zhang, 2021, UK	UK Biobank	Prospective observational	2006-2010 to 2020, follow-up 11.2 yrs. (median)	462 155 (44% male), 40-69 yrs, mean 56.7 yrs	Touchscreen FFQ	NA, probably usual intake at baseline
Patient populations						
Hu, 2003, USA	Nurses' Health Study (NHS)	Prospective observational	1980 to 1996, 16 yrs follow-up	5103 female nurses with diagnosed type 2 diabetes, 30-55 yrs	Repeated FFQ (1980, 1984, 1986, 1990, and 1994), semi-quant, validated	Average intake during the previous year
Burr 1989, UK	Diet and Reinfarction trial (DART)	RCT	1983, 2 yrs follow-up	2033 men recovered from myocardial infarction, <70 yrs, (mean age 57 yrs)	Detailed dietary questionnaire at 6 months and 2 yrs after randomization	NA, baseline intake
Erkkila, 2003, Finland	Finnish sub-cohort of European Action on Secondary Prevention through Intervention to Reduce Events (EUROASPIRE)	Prospective observational	1991-1994 (first hospitalization), baseline examination in 1995, 5 yrs of follow-up to to 2000 (hospitalization) or 2001 (deaths)	285 men and 130 women with coronary artery disease, 33-74 yrs, mean age 61 yrs	4-d food record (3 weekdays and 1 weekend day) completed at home. Portion size booklet	Current intake at baseline, 4 days
Manger, 2010, Norway	Sub-study of Western Norway B Vitamin Intervention Trial (WENBIT)	Prospective observational	1999-2004 to 2006, 57 mo. follow-up (median)	2412 patients (80.5% men) with well-characterized and treated coronary artery disease (90% statin and users), ≥18 yrs, mean age 61.7 yrs	FFQ, semi quant, validated	Usual intake, previous year, at baseline

4.3.2.2 Overlapping publications

Three publications from the Nurses' Health Study (NHS) described fish intake in relation to risk of CHD in women: Bernstein et al. (2010); Hu et al. (2003); Hu et al. (2002). The most recent publication (Bernstein et al., 2010) had a longer follow-up period and more cases and was kept in the main analysis according to protocol (VKM, 2020). Hu et al. (2002) was excluded. The study by Hu et al. (2003) was limited to the sub-sample of women with T2D at baseline.

Bjerregaard et al. (2010) was based on the Diet, Cancer and Health cohort by the Danish Cancer Society, which is also the Danish sub-cohort in the EPIC study by Key et al. (2019). Bjerregaard 2010 was included in the analysis of total fish, which was not presented in Key et al. (2019) but excluded from the analysis of fatty fish and lean fish covered by Key et al. (2019).

4.3.2.3 Studies by design and geographic region

Among the ten included studies on CHD incidence in the general population, all studies had a prospective, observational design. Except one study from Japan (Iso et al., 2006), the study samples were from Western populations in Europe (Bjerregaard et al., 2010; Bonaccio et al., 2017; Hengeveld et al., 2018; Key et al., 2019; Osler et al., 2003; Zhang et al., 2021) or USA (Ascherio et al., 1995; Bernstein et al., 2010; Gillum et al., 2000). The countries included in the EPIC study by Key et al. 2019 were Denmark, Norway, Sweden, France, Netherlands, UK, Greece, Italy, and Spain.

Three of the US studies focused on health professionals, including the study in T2D patients. In addition, there were three more studies in patients (described in more detail below). These were based on trials or prospective observational studies conducted in Europe (Burr et al., 1989; Erkkila et al., 2003; Manger et al., 2010).

4.3.2.4 Studies in patient populations

Three publications assessed secondary prevention in patient with CHD, including MI. One RCT study included men recovered from myocardial infarction in the Diet and Reinfarction trial (DART) (Burr et al., 1989), the other two prospective cohorts included men and women with established or treated coronary artery disease; either from the Western Norway B Vitamin Intervention Trial (WENBIT) (Manger et al., 2010) or the Finnish sub-cohort of the European Action on Secondary Prevention through Intervention to Reduce Events (EUROASPIRE) (Erkkila et al., 2003). Only the DART trial included fish intake as part of the intervention; it was advised to eat at least two weekly portions of fatty fish. The other studies were originally designed for other purposes and assessed the effect of fish intake measured at baseline. One publication from the Nurses' Health Study (NHS) was limited to women with T2D at baseline (Hu et al. 2003).

4.3.2.5 Studies by sex, potential effect modification, and other sub-groups

Of the ten publications that did not concern patients, one included only men (Health Professionals Follow-Up study, Ascherio et al., 1995) and one included only women (Nurses' Health Study, Bernstein et al., 2010). The remaining included both men and women (Bjerregaard et al., 2010; Bonaccio et al., 2017; Gillum et al., 2000, Hengeveld et al., 2018; Iso et al., 2006; Key et al., 2019, Osler, et al., 2003, Zhang et al., 2021) and presented either sex-specific estimates and/or estimates for men and women combined. Gillum et al., (2000) presented sex specific estimates by race (white or black), and all estimates were included.

4.3.2.6 Studies by fish exposure

All but one study (Key et al., 2019) included a total fish exposure (sum of all fish, unspecified fish, or fish including shellfish and/or fish products). In studies that presented fish intake with and without the inclusion of shellfish (Hengeveld et al., 2018), the results without shellfish were considered the main result in line with VKM's protocol. The most common sub-classification of fish intake was by fat content (fatty or lean). More infrequent sub-classifications were by flesh color (e.g., white or dark fish), or by conservation method (canned or dried). The number of studies sufficed to summarize the evidence for total fish (n=9), fatty fish (n=4) and lean fish (n=4) for first event CHD. "Dark fish" (unspecified in Bernstein et al. 2010) was then categorized as fatty fish, and "light fish" (unspecified in Bernstein et al. 2010) and "white fish" (unspecified in Key et al. 2019) as lean fish. Several studies presented results by multiple fish classifications. The secondary prevention studies examined the effect of fatty fish (dietary advice intervention), usual intake of fish and fish products, or fish intake from a four-day dietary record.

4.3.2.7 Studies assessing potential non-linearity

None of the primary studies was found to include an analysis of fish intake and risk of CHD that assessed potential non-linearity of the association.

4.3.2.8 Studies with converted risk estimates

Osler et al. (2003) did not use the lowest intake category as reference, and the reported relative risk estimate was converted to high vs. low. Both the reported and converted estimates are presented (Table 4.3.3.1-1).

4.3.3 Results from the included primary studies of fish intake and CHD incidence

4.3.3.1 Studies of total fish intake and CHD incidence

Nine prospective studies (13 estimates) were included on total fish intake and CHD incidence in the general population. Table 4.3.3.1-1 shows the exposure levels and the results in these

studies. Case numbers were not available in the study by Gillum et al. (2000) which was carried out in 8825 (7421 white and 1404 black) Americans. Relative risk (RR) estimates were generally suggesting a protective association ($RR < 1$, statistically significant in three of ten studies) or no association (RR around 1).

Table 4.3.3.1-1 Results from prospective observational studies included in the weight of evidence analysis of total fish intake and CHD incidence.

Author, year, country	Fish exposure, sex	Intake unit	High-low intake	Total cases	HR high-low (95 % CI)	Overall result
Ascherio, 1995, USA	Fish, incl shellfish, M	Servings, 6 cat	≥6/wk vs 1/mo, 119 vs 0 g/d (mean)	1543	1.14 (0.86, 1.51)	No sig. assoc., <i>P</i> -trend 0.19
Bernstein, 2010, USA	Fish, W	Servings/d: quintiles, cumulative average	Quintile 5 vs 1, 0.45 vs 0.07 servings (median)	3162	0.81 (0.72, 0.90)	Protective assoc. from 2nd quintile (≥0.11 vs 0.07 serving/d), <i>P</i> -trend <0.001, or 20% risk reduction per serving/d
Bjerregaard, 2010, Denmark	Fish, incl shellfish, M	g/d, 5 cat	Quintile 5 vs 1, >64 vs 0–24 g/d	854	0.87 (0.69, 1.10)	No sig. assoc.
	Fish, incl shellfish, W	g/d, 5 cat	Quintile 5 vs 1, >55 vs 0–22 g/d	268	0.85 (0.55, 1.32)	No sig. assoc.
Bonaccio, 2017, Italy	Fish, M/W	Times/wk, 3 cat	≥4 vs <2 times/wk, 92.5 vs 23.0 g/d (mean)	287	0.62 (0.40, 0.98)	Protective assoc. of >4 vs <2 times/wk (92.5 vs 23.0 g/d), <i>P</i> -trend=0.029 or 9% risk reduction per time/wk
Gillum, 2000, USA	Fish, incl shellfish, M-white	Times/wk, 4 cat	>1/wk vs never	NA	0.86 (0.65, 1.13)	No sig. assoc.
	Fish, incl shellfish, M-black	Times/wk, 4 cat	>1/wk vs never,		1.05 (0.50, 2.19)	No sig. assoc.
	Fish, incl shellfish, W-white	Times/wk, 4 cat	>1/wk vs never,		0.97 (0.74, 1.28)	No sig. assoc.
	Fish, incl shellfish, W-black	Times/wk, 4 cat	>1/wk vs never,		0.90 (0.51, 1.60)	No sig. assoc.
Hengeveld, 2018, the Netherlands	Fish, M/W	Portions/wk, 3 cat	≥1/wk vs none, 28.7 g/wk fatty and 93.7 g/wk lean (median values)	2134	1.03 (0.94, 1.13)	No sig. assoc.
Iso, 2006, Japan	Fish, incl fish products, M/W	g/d, quintiles	Quintile 5 vs 1, 180 vs 23 g/d (median values)	258	0.63 (0.38, 1.04)	No sig. assoc., <i>P</i> -trend 0.25

Author, year, country	Fish exposure, sex	Intake unit	High-low intake	Total cases	HR high-low (95 % CI)	Overall result
Osler, 2003, Denmark	Fish, M/W	Times/mo or wk, 4 cat	≥2/wk vs ≤1/mo (cat 3: 1/wk is ref), NA	491	0.91 (0.61, 1.35), reported as 0.93 (0.68, 1.27) for ≥2/wk vs 1/wk (ref) and 1.02 (0.80, 1.30) for ≤1/mo vs 1/wk (ref)	No sig. assoc., <i>P</i> -trend 0.55
Zhang, 2021, UK	Total fish, M/W	Times/wk, 4 cat	≥3 vs <1/wk	NA, sample 458050	0.83 (0.79, 0.88)	Protective assoc. in all cat. above reference, <i>P</i> -trend <0.001

4.3.3.2 Studies of fatty fish intake and CHD incidence

We included four studies (all prospective, observational) that analyzed the association of fatty fish with risk of CHD incidence. The exposure levels and results for fatty fish are shown in Table 4.3.3.2-1.

Table 4.3.3.2-1 Results from prospective observational studies included in the weight of evidence analysis of fatty fish intake and CHD incidence.

Author, year, country	Fish exposure, sex	Intake unit	High-low intake	Total cases	HR high-low (95 % CI)	Overall result
Bernstein, 2010, USA	Fatty fish, W	Servings/d, binary (cumulative average)	Cat 2 vs 1: 0.07 vs 0.0 servings/d (median)	3162	0.92 (0.82,1.02)	No sig. assoc., <i>P</i> -trend 0.13
Bonaccio, 2017, Italy	Fatty fish, M/W	Times/wk, 3 cat	≥1 vs none, 24.2 vs 0 g/d	287	0.55 (0.34, 0.89)	Protective assoc. of ≥1 and <1 vs none
Hengeveld, 2018, the Netherlands	Fatty fish, M/W	Portions/wk, 3 cat	≥1/wk vs none, 10.7 g/wk (median) in consumers	2134	1.08 (0.90, 1.30)	No sig. assoc.
Key, 2019, Europe (9 countries)	Fatty fish, M/W	g/d, quintiles	Quintile 5 vs 1, 29 vs 0 g/d (median)	7198	0.92 (0.86, 0.99)	Protective assoc. of intake in Q5 vs Q1, <i>P</i> -trend 0.054

Among the four studies (providing four estimates and almost 13 000 cases), three reported a statistically significant protective association, or a suggestive protective association of fatty fish intake with risk of CHD.

4.3.3.3 Studies of lean fish intake and CHD incidence

The studies of fatty fish intake also included results on lean fish intake. As shown in (Table 4.3.3.3-1) below, none of the four studies (providing four estimates) of lean fish intake reported a statistically significant association with CHD incidence.

Table 4.3.3.3-1 Results from prospective observational studies included in the weight of evidence analysis of lean fish intake and CHD incidence.

Author, year, country	Fish exposure, sex	Intake unit	High-low intake	Total cases	HR high-low (95 % CI)	Overall result
Bernstein, 2010, USA	Lean fish, W	Servings/d, quintiles (cumulative average)	Quintile 5 vs 1, 0.29 vs 0.0 servings	3162	0.91 (0.75, 1.11)	No sig. assoc., <i>P</i> -trend 0.74
Bonaccio, 2017, Italy	Lean fish, M/W	Times/wk, 3 cat	≥1 vs none, 30.9 vs 0 g/d	287	1.38 (0.70, 2.71)	No sig. assoc.
Hengeveld, 2018, the Netherlands	Lean fish, M/W	Portions/wk, 3 cat	≥1/wk vs none, 32.9 g/wk (median) in consumers	2134	0.97 (0.88, 1.07)	No sig. assoc.
Key, 2019, Europe (9 countries)	Lean fish, M/W	g/d, quintiles	Quintile 5 vs 1, 44 vs 0 g/d (median values)	7198	1.02 (0.94, 1.11)	No sig. assoc., <i>P</i> -trend 0.93

4.3.3.4 Studies of fish intake and CHD risk in patient populations

None of the three studies on secondary prevention reported a statistically significant association of fish intake (assessed after disease) or dietary advice to eat fatty fish, with risk of recurrent CHD. The Nurses' Health Study (NHS) reported a protective association in the sub-population of women with T2D (Hu et al., 2003) and in all women (Bernstein et al., 2010, Table 4.3.3.1-1). The exposure levels and results have been summarized below.

Table 4.3.3.4-1 Results from studies included in the weight of evidence analysis for total fish intake and secondary prevention of CHD.

Author, year, country	Study design	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low (95 % CI)	Overall result
Prior CHD or MI/secondary prevention							
Burr, 1989, UK	Intervention, dietary advice	Fatty fish, M	Weekly portions	Fish advice (two weekly portions 200-400 g of fatty fish) vs no fish advice	276	0.84 (0.66, 1.07)	No sig. assoc.
Manger, 2010, Norway	Prospective observational	Fish, incl fish products, M/W	g/d, quartiles	Quartile 4 vs 1, or Q2-4 vs 1, 198 vs 41.1 g/d (mean)	292	1.04 (0.74, 1.45)	No sig. assoc., <i>P</i> -trend 0.86
Erkkila, 2003, Finland	Prospective observational	Fish, M/W	g/d, 3 cat (above/below median, null)	Cat 3 vs 1, >57 (above median) vs 0 g/d	34	0.49 (0.17, 1.41)	No sig. assoc., <i>P</i> -trend 0.209
Diabetes population							
Hu, 2003, USA	Prospective observational	Fish, incl shellfish, W	Servings/mo or wk, 5 cat, cumulative average	≥5/wk vs <1/mo	362	0.36 (0.20, 0.66)	Sig. protective assoc. of intake 1/wk or higher vs <1/mo, <i>P</i> -trend=0.002

4.3.3.5 Summary relative risks (RR) based on VKM's inclusions of primary studies

VKM's summary RR for the highest versus lowest intake of total fish in relation to CHD (Table 4.3.3.1-1) showed a protective association that was statistically significant (RR=0.89, 95% CI: 0.81, 0.98). There was significant heterogeneity ($P_{\text{heterogeneity}}=0.002$), but no reports of a statistically significant adverse effect among the primary studies.

Despite some differences in the selection of studies compared with previous meta-analyses (as described below), the summary RR (high-low analysis) based on VKM's study selection was similar to the results in Bechthold et al. (2019) (RR=0.94, 95% CI: 0.88 to 1.02) and Zhang et al. (2020) (RR=0.91, 95% CI 0.84, 0.97) (Table 4.3.1.2-1).

VKM's summary RR for fatty fish (four studies, Table 4.3.3.2-1) was on the protective side, but not statically significant (RR=0.93, 95 %: 0.83, 1.04 $P_{\text{heterogeneity}}=0.07$), and the summary RR for lean fish (four studies, Table 4.3.3.3-1) was close to unity (RR=0.99, 95 CI%: 0.93, 1.05, $P_{\text{heterogeneity}}=0.52$). For fatty and lean fish, there were no previous meta-analysis for comparison.

VKM's summary RR for secondary prevention of CHD (three studies, Table 4.3.3.4-1) was on the protective side, but not statically significant (RR=0.89, 95% CI: 0.71, 1.10). Heterogeneity was non-significant ($P_{\text{heterogeneity}}=0.32$). There was no previous meta-analysis for comparison.

VKM could not calculate a summary RR for CHD in patients with T2D as there was only one study. One previous meta-analysis (Jayedi et al. 2020, Table 4.3.1.2-1) included the same study of CHD incidence that was identified by VKM, and two studies on CHD mortality in patients with T2D. The reported association was protective (RR=0.61, 95%: 0.29, 0.93) but with substantial heterogeneity ($I^2=68\%$).

4.3.3.6 VKM's search compared to previous meta-analyses

Jayedi et al. (2020) included 3 prospective studies on CHD incidence or mortality in patients with T2D, all were identified by VKM, but two studies were summarized under CHD mortality (Deng et al. 2018, Wallin et al. 2018) and not CHD incidence.

Zhang et al. (2020) included 22 prospective studies, of which eight were not in VKM's systematic review. Five of these were identified but did not fulfil our eligibility criteria (Mozaffarian et al. 2003, Iajous et al. 2013, Haring et al. 2014, Ward et al. 2019) or a later publication from the same cohort was included (Hengeveld replaced de Goede et al. 2010, see Chapter 4.3.2.2 on Overlapping publications). The papers by Buckland et al. 2009, Fraser et al. 1992 and Martinez-Gonzales et al. 2011 did not appear in the VKM search.

One additional paper on fish intake and risk of CHD (Salonen et al., 1995) was only included by VKM. In addition, we included one paper on T2D patients and three papers on secondary prevention.

Bechthold et al. (2019) summarized 22 prospective studies, of which nine were not included by VKM. Five of nine studies were identified but did not fulfil VKM's eligibility criteria; Tektonidis et al. (2015) and Mozaffarian et al. (2003) were limited to non-fatal MI only. Two studies did not pass the quality assessment (Haring et al., 2014; Holmberg et al., 2009) and one study was replaced by a more recent publication from the same cohort (Hengeveld et al. 2018 replaced de Goede et al. 2010). The remaining studies did not appear in VKM's search. (Buckland et al. 2009; Dillis et al. 2012; Fraser et al. 1992; Martinez-Gonzalez et al. 2011). VKM identified two papers (Bonaccio et al. 2017; Hengeveld et al., 2018) published after the literature search done in Bechthold et al. (2017). In addition, we included one paper on T2D patients and three papers on secondary prevention of CHD.

An overview of primary studies included by VKM compared with two previous meta-analysis on incident CHD and/or myocardial infarction (MI) is given in Table 4.3.3.6-1.

Table 4.3.3.6-1 Overview of studies included by VKM compared with two identified meta-analyses on CHD incidence.

Publication	Included by VKM		Meta-analyses	
	Coronary heart disease	Myocardial infarction	Bechthold, 2019	Zhang, 2020
Albert, 1998		X	X	X
Ascherio, 1995	X	X	X	X
Bernstein, 2010	X		X	X
Bjerregaard, 2010	X (Acute Coronary Syndrome)		X	X
Bonaccio, 2017	X			X
Gammelmark, 2016		X	X	X
Gillum, 2000	X		X	X
Hengeveld, 2018	X	X		X
Iso, 2006	X	X	X	X
Key, 2019	X			
Kuhn, 2013		X	X	X
Lockheart, 2007		X		
Nahab, 2016		X	X	X
Osler 2003	X		X	X
Salonen, 1995		X	X	
Wennberg, 2011		X	X	X
Diabetes population				
Hu, 2003	X			
Secondary prevention				
Burr, 1989	X			
Erkkila, 2003	X			
Manger, 2010	X	X		
Overlapping				
Hu, 2002	X			X
Morris, 1995		X		

	Included by VKM		Meta-analyses	
Studies only in meta-analyses				
Buckland, 2009			X	X
De Goede, 2010			X	X
Dillis, 2012			X	X
Fraser, 1992			X	X
Haring, 2014			X	X
Holmberg, 2009			X	X
Iajous, 2013				
Martinez-Gonzalez, 2011			X	X
Mozaffarian, 2003			X	X
Tektonidis, 2015			X	
Ward, 2019				X
<i>Studies included</i>	<i>13</i>	<i>12</i>	<i>22</i>	<i>22</i>

4.3.4 Heterogeneity fish intake and CHD incidence

Heterogeneity was statistically significant for VKM's summary RR ($P_{\text{heterogeneity}}=0.002$). Two independent meta-analyses of CHD incidence have reported overall protective associations with moderate heterogeneity (I^2 around 50%, Table 4.3.1.2-1), but with little heterogeneity in the direction of association (evaluated by VKM from forest plots, not shown).

Zhang et al. (2020) reported that none of the included covariates in meta-regression (publication year, continent, sex, evaluation method of fish consumption, follow-up period, adjustment for BMI, and adjustment for alcohol) significantly influenced the heterogeneity between studies (results not shown).

Bechthold et al. (2019) performed sub-group analysis of heterogeneity by sex, region (Europe, America, Asia & Australia); follow-up duration (cut-point 10 years); number of cases (cut-point 1000); dietary assessment (validated or not). The only significant sub-group difference (high-low analysis) was found for region.

4.3.5 Dose-response relationship fish intake and CHD incidence

Zhang et al. (2020) performed a linear and non-linear dose-response analysis (19 prospective cohort studies, Table 4.3.1.2-1) of fish intake and CHD incidence. The results showed that an increase in fish intake by 20 g/day was associated with a 4% reduction in CHD incidence. However, based on the authors' report, the risk of CHD only decreased for intakes above 40 g/day.

Bechthold et al. (2019) performed found no departure from linearity in the dose-response analysis of fish intake and CHD ($n = 15$ studies, $P_{\text{non-linearity}}=0.10$) The risk of CHD decreased by approximately 15% with increasing intake of fish up to about 250 g/day.

4.3.6 Weight of evidence fish intake and CHD incidence

In this section, the evidence of the association between fish intake and CHD incidence is weighed according to the WCRF criteria presented in Chapter 3.1.6 (Box 2).

Published evidence of fish intake and CHD incidence

Two systematic reviews and meta-analyses of fish intake and risk of CHD (Zhang et al., 2020; Bechthold et al., 2019) have concluded that total fish consumption is associated with decreased risk of CHD. There was quite large overlap between the papers/studies in these two meta-analyses and the papers/studies included by VKM. However, as described above, the meta-analyses included several publications that were either excluded by VKM, or not identified in our search. VKM identified two publications after 2017 (Bonaccio et al., 2017, Hengeveld et al., 2018) that were not included in Bechthold et al. (2019). Hengeveld et al. (2018) did not find an association between fish consumption and risk of CHD, while Bonaccio et al. (2017) found a protective association. The summary RR from the primary studies included by VKM was consistent with results in Zhang et al. (2020) and Bechthold et al. (2019). Based on previous meta-analyses and VKM's pooled estimate (statistically significant) there seems to be evidence that total fish intake reduces the risk of CHD. For fatty fish and lean fish there were fewer studies than for total fish, and no previous meta-analyses.

Heterogeneity

Moderate heterogeneity was observed between studies in the two included meta-analyses, and significant heterogeneity was also observed for the pooled estimate based on the primary studies included by VKM. There may be some unexplained heterogeneity, mainly in the magnitude of associations, less in direction.

Mechanism / biological plausibility

There is evidence for several plausible mechanisms operating in humans.

Upgrading factors

Meta-analyses indicate a dose-response relation. No other upgrading factors were evaluated.

4.3.6.1 Conclusion weight of evidence fish intake and CHD incidence

There is evidence from more than two independent and good quality cohort studies on total fish intake (VKM included nine publications on the general population, four in patients, and three previous meta-analyses, including two dose-response meta-analyses). The published evidence indicates a protective association of fish intake with CHD incidence.

VKM's summary RR for primary studies in the general population shows statistically significant lower risk of incident CHD for the highest versus lowest intake of total fish and is supported by independent meta-analyses. The direction of association is generally consistent towards protective, but there is some heterogeneity between studies. There is evidence for biological plausibility, and a dose-response relationship.

In conclusion, the evidence was graded “probable” for a protective effect of fish consumption on incident CHD in the general population. VKM’s summary RR for secondary prevention of CHD is not statistically significant but consistent with the summary RR for the general population. No conclusion could be drawn for incident CHD in patients with T2D.

There were fewer studies of fatty fish and lean fish, and the evidence was graded “limited, suggestive” for a protective effect of fatty fish and “limited, suggestive” for no effect of lean fish on incident CHD in the general population.

4.4 Fish intake and myocardial infarction incidence

4.4.1 VKM's search for previous systematic reviews and meta-analyses of fish intake and myocardial infarction incidence

4.4.1.1 Description of the identified publications

VKM's search for systematic reviews and meta-analyses (see Chapter 3.1.2.2) identified three publications on the association between fish intake and myocardial infarction (MI) that all fulfilled the inclusion criteria.

Table 4.4.1.1-1 Identified papers from the search of systematic reviews and meta-analyses of fish intake and MI 2016-2021.

Included papers	Excluded paper and reasons for exclusions
Umbrella review Jayedi and Shab-Bidar (2020)	None
Systematic reviews Jayedi et al. (2020) patients with T2D Jayedi et al. (2019)	

The umbrella review by Jayedi and Shab-Bidar et al. (2020) and the systematic review by Jayedi et al. (2020) have previously been described in Chapter 4.3.1.

Jayedi et al. (2019) summarized fish consumption with MI (outcome included were total, fatal, or non-fatal MI) in prospective cohort studies (n=11). Literature searches were conducted in PubMed and Scopus databases through January 2018. The single studies included in Jayedi et al. (2019) had a good quality and scored 7-9 according to the 9-point Newcastle-Ottawa scale. Jayedi et al. (2019) graded the overall quality of the meta-evidence as moderate (7.5 out of 10 points) assessed by the NutriGrade scoring system.

4.4.1.2 Results from the meta-analyses

Below is a summary table for total fish and MI based on the identified meta-analysis including studies of incidence and mortality.

Table 4.4.1.2-1 Summary of results from meta-analyses on total fish intake and risk of MI.

Author, year	Type of study	Total no of studies	No of cases	Comparison	Summary RR (95 % CI)	Heterogeneity	Conclusion
Jayed, 2020	Prospective studies which evaluated fish (seafood) intake and MI (incidence or mortality) in patients with T2D	2	NA from paper	Highest vs lowest	0.80 (0.46, 1.14)	$I^2=83.9\%$	A non-significant association on the protective side was found between fish and MI in patients with T2D. The quality of the meta-evidence was rated very low.
Jayed, 2019	Prospective cohort studies which evaluated the association between fish intake and MI risk. Outcome is total, fatal or non-fatal MI	11	8468	Highest vs lowest	0.73 (0.59, 0.87)	$I^2=72\%$	Higher intake of fish was associated with a decreased risk of MI. Observed a significantly linear relationship between fish intake and MI (P-nonlinearity=0.64). The quality of meta-evidence was moderate.
				Per 15 g/d increment	0.96 (0.94, 0.99)	$I^2=65\%$	

4.4.2 VKM's systematic review of primary studies on fish intake and MI incidence

4.4.2.1 Included studies from search

A total of 14 publications graded B, including on global, multicenter study, presented results on incident myocardial infarction (MI): Albert et al. (1998); Ascherio et al. (1995); Gammelmarm et al. (2016); Hengeveld et al. (2018); Iso et al. (2006); Kuhn et al. (2013); Lockheart et al. (2007); Manger et al. (2010); Mohan et al. (2021); Morris et al. (1995); Nahab et al. (2016); Salonen et al. (1995); Wallin et al. (2018); Wennberg et al. (2011). Three studies included patient populations (Manger et al. 2010; Mohan et al. 2021; Wallin et al. 2018). One study was carried out in patients with T2D (Wallin et al. 2018), one in patients with a history of coronary artery disease (Manger et al., 2010), and the global multicenter study (Mohan et al., 2021) presented MI in participants with and without a family history of CVD (as separate analyses).

Thus, two of 14 studies were conducted in patients only. Among studies with result on the general population (n=12), there were two publications from the same study (as described below), and one was excluded, leaving 11 for further analysis.

Studies or results limited non-fatal MI only (Ascherio et al. 1995; de Goede et al. 2010; Hu et al. 2002; Kuhn et al. 2013; Morris et al. 1995; Mozaffarian et al. 2003; Sasazuki et al. 2001), were not summarized in this report, but were included in previous meta-analyses.

A description of the included studies (study name, design, time period, size and age of the study population, and dietary assessment method) can be found in Table 4.4.2.1-1.

Table 4.4.2.1-1 Overview of primary studies included in weight of evidence analysis of fish intake and MI incidence.

Author, year, country	Study name	Study design	Inclusion year(s), end, follow-up time	Study population	Dietary assessment method	Dietary assessment ref period
Albert, 1998, USA	Physicians' Health Study (PHS)	Prospective observational	1983 to 1995, 11 yrs follow-up	20 551 male physicians, 40-84 yrs	FFQ, semi quant, validated	Current, on average, at baseline
Ascherio, 1995, USA	Health Professionals Follow-Up Study (HPFS)	Prospective observational	1986, 6 yrs of follow-up	44 895 male health professionals, 40-75 yrs	FFQ, semi quant, validated	Average frequency during the previous year, at baseline
Gammelmark, 2016, Denmark	Danish Diet, Cancer and Health cohort	Prospective observational	1993–97, 17 yrs follow-up (median)	25 913 men and 28 991 women, 50 - 64 yrs (median age 55.9 yrs for males and 56.2 yrs for females)	FFQ, semi-quant, validated	Average/daily intake at baseline
Hengeveld, 2018, the Netherlands	EPIC-Netherlands (Prospect and MORGEN sub-cohorts)	Prospective observational	1993-1997 to 2011, 18 yrs follow-up (median 15.1 yrs)	34 033 (25% male), 20-70 yrs., mean age 48.7 yrs	FFQ, semi quant, validated	Usual intake, previous year, at baseline
Iso, 2006, Japan	Japan Public Health Center-Based (JPHC) Study Cohort I	Prospective observational	1990-1992 to 2001, 11 yrs follow-up	41 578 (19 985 men and 21 593 women), 40-59 yrs	Repeated FFQ (1990, 1995), validated	Average intake previous month (1990) or previous year (1995)
Kuhn, 2013, Germany	EPIC-Germany	Prospective observational	1994-1998 to 2006, 8.1 yrs follow-up (mean)	48 315 (42% male), 35-65 yrs, mean age 50.5 yrs.	FFQ, uncertain validity	Usual intake during the previous year, at baseline

Author, year, country	Study name	Study design	Inclusion year(s), end, follow-up time	Study population	Dietary assessment method	Dietary assessment ref period
Lockheart, 2007, Norway	Study of dietary patterns, food groups and myocardial infarction, South-east Norway	Case-control	1995-1997	106 cases and 105 controls (men and postmenopausal women), 45-75 yrs, mean age 62.5 (cases) and 62.2 (controls)	FFQ (validated) by 90 min interview. During interviews with the male patients their spouses or cohabitants were invited to participate.	Intake previous year assessed 3 d after incident MI in cases.
Mohan, 2021, global, 6 continents, 58 countries	PURE, ONTARGET, TRANSCEND, and ORIGIN, only data from PURE on general population	Prospective observational, multicenter	Follow-up to 2019 (PURE), Median follow-up (yrs) was 9.1 in PURE	191 558 (47.9% male), 51 731 with vascular disease and 139 827 generally healthy. PURE (n=147 541), Mean age PURE 51 (35-70) yrs	Country specific FFQs	Usual intake in previous year, at baseline
Nahab, 2016, USA	REasons for Geographic And Racial Differences in Stroke (REGARDS) study	Prospective observational	2003-2007 to 2010, 5.1 yrs of follow up (median)	16 479 men and women (34% African Americans, 59% female, 74% were overweight or obese), 40-75 yrs	FFQ, Block98	Usual intake, past year, at baseline
Salonen, 1995, Finland	Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD)	Prospective observational	1984-1989 to 1991 (acute MI) or 1992 (mortality), 5 yrs or 6 yrs of follow-up (mean values)	1833 men, 52.4 yrs	4-day dietary record	4 days at baseline
Wennberg, 2011, Sweden	Northern Sweden Health and Disease Study (NSHDS)	Prospective observational/ nested case-control	1987-1999	392 cases (317 male) and 474 (331 male) controls, Men: 30-77 (median 53) yrs; Women: 30-74 (median 58) yrs	Multiple versions of FFQ, differ by cohort (VIP, MONICA)	Average frequency during the previous year
Patient populations						
Manger, 2010, Norway	Sub-study of Western Norway B Vitamin Intervention Trial (WENBIT)	Prospective observational	1999-2004 to 2006, 57 mo. follow-up (median)	2412 patients (80.5% men) with well-characterized and treated coronary artery disease (90% statin and users), ≥18 yrs, mean age 61.7 yrs	FFQ, semi quant, validated	Usual intake, previous year, at baseline

Author, year, country	Study name	Study design	Inclusion year(s), end, follow-up time	Study population	Dietary assessment method	Dietary assessment ref period
Mohan, 2021, global, 6 continents, 58 countries	PURE, ONTARGET, TRANSCEND, and ORIGIN	Prospective observational, multicentre	Follow-up to 2019 (PURE), Median follow-up (yrs) was 9.1 in PURE, 4.5 in ONTARGET and TRANSCEND, and 6.2 in ORIGIN	191 558 (47.9% male), 51 731 with vascular disease and 139 827 generally healthy. PURE (n=147 541), ONTARGET and TRANSCEND (n=31 491), ORIGIN (n=12 422). Mean age PURE 51 (35-70) yrs, ONTARGET and TRANSCEND 67 yrs, ORIGIN 64 yrs	Country specific FFQs (no amounts in ONTARGET and TRANSCEND), validated in some countries	Usual intake in previous year, at baseline
Wallin, 2018, Sweden	Cohort of Swedish Men (COSM) and the Swedish Mammography Cohort (SMC)	Prospective cohort	1998 to 2012, mean follow-up 11.8 yrs for incidence and 13.2 yrs for mortality	2225 (912 women and 1313 men) with type 2 diabetes, 45-84 yrs	FFQ, validated in men	Average frequency during the previous year, at baseline (1997)
Excluded due to overlap						
Morris, 1995, USA	Physicians' Health Study (PHS)	Prospective observational	1982, 4 yrs of follow-up	21 185 male physicians, 40-84 yrs	FFQ, semi quant, validated	Average intake, previous year, at 12-month follow-up

4.4.2.2 Overlapping publications

Both Morris et al. (1995) and Albert et al. (1998) reported on fish intake and risk of MI incidence in the US Physicians' Health Study. Albert et al. (1998) had longer follow-up and more cases and was therefore kept in the main analysis according to protocol. However, the studies differ in the fish intake. Albert et al. (1998) included shellfish as part of fish intake, whereas Morris et al. (1995) did not, and Morris et al. (1995) also reported on sub-types of fish, which explains the inclusion of Morris et al. (1995) in the analyses of MI by lean and fatty fish.

4.4.2.3 Studies by design and geographic region

Among the 11 included studies on incident MI in the general population, there was one case-control study (Lockheart et al., 2007). Remaining studies (n=10) had a prospective, observational design. Wennberg et al. (2011) was a nested case-control study with dietary intake assessed prior to disease and counted among the prospective studies. All studies were based on European populations (Gammelmarm et al., 2016; Hengeveld et al., 2018; Kuhn et al., 2013; Lockheart et al., et al., 2007; Salonen et al., 1995; Wennberg et al., 2011) or USA (Albert et al., 1998; Ascherio et al., 1995; Nahab et al., 2016), except for one study from Japan (Iso et al., 2006), and Mohan et al. (2021) which is a global multicenter study with data from 58 countries on 6 continents.

4.4.2.4 Studies in patient populations

Two publications assessed MI in patients at high risk, either due to established or treated coronary artery disease (Manger et al., 2010), or a CVD history or treatment for vascular disease (Mohan et al. 2021). Manger et al. (2010) included participants from the Western Norway B Vitamin Intervention Trial (WENBIT). Mohan et al. (2021) was based on four studies: one cohort (PURE) where participants with a history of CVD were analyzed separately, and three follow-up studies of drug-trials (ONTARGET, TRANSCEND, and ORIGIN) where all participants were treated for vascular disease. Mohan et al. (2021) was described in more detail under CVD incidence (Chapter 4.2.2.4). One study assessed MI in cohort participants limited to those with T2D at baseline (Wallin et al. 2018).

4.4.2.5 Studies by sex and potential effect modification

Most studies included both women and men. Three older studies included only men (Albert et al., 1998; Ascherio et al., 1995; Salonen et al., 1995), see Table 4.4.2.1-1. Studies analyzing or testing for potential effect modification by sex (Iso et al., 2006, Kuhn et al., 2013; Wallin et al. 2018; Wennberg et al., 2011) reported a non-significant ($P>0.05$) test of interaction, or no such effect. Therefore, we present estimates in men and women combined when available.

4.4.2.6 Studies by fish exposure

All studies except three (Gammelmarm et al. 2016; Lockheart et al. 2007; Nahab et al. 2016) included a total fish exposure (sum of fish, fish without specifications, or fish including shellfish and/or fish products). In studies that presented fish intake with and without the inclusion of shellfish (e.g. Hengeveld et al., 2018), the results without shellfish were considered the main result in line with VKM's protocol. The most common sub-classification of fish was by fat content (fatty or lean). Two studies (Gammelmarm et al. 2016; Nahab et al. 2016) included fatty- and lean fish without total fish, and one study included supplemental cod-liver oil as part of fatty fish intake (Lockheart et al. 2007, case-control design). One study (Salonen et al. 1995) included a male study population from Eastern Finland, considered to be highly exposed to methyl mercury from consuming local nonfatty fish species. Other sub-classifications were by flesh color (white or dark fish), or preparation method (fried or non-fried), or by species (e.g. tuna only). Evidence was summarized for total fish (n=8), fatty fish (n=4), and lean fish (n=4) in general population studies. "Dark fish" was then categorized as fatty- and "white fish" as lean fish. Fried and non-fried fish (one study) could not be summarized.

4.4.3 Results from the included primary studies of fish intake and MI incidence

4.4.3.1 Studies of total fish intake and MI incidence

We included eight studies (with eight estimates) on total fish intake and MI incidence. All studies had a prospective, observational design, including one nested-case control study (Albert et al., 1998; Ascherio et al., 1995; Hengeveld et al., 2018; Iso et al., 2006; Kuhn et al., 2013; Mohan et al. 2021; Salonen et al., 1995; Wennberg et al., 2011) Table 4.4.3.1-1 shows the exposure levels and results in these studies.

Table 4.4.3.1-1 Results from studies included in the weight of evidence analysis of total fish intake and MI incidence.

Author, year, country	Study design	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low (95% CI)	Overall result
Albert, 1998, USA	Prospective observational	Fish, incl shellfish, M	Servings as main dish, 5 cat	≥5/wk vs <1/mo	737	HR 1.00 (0.62, 1.60)	No sig. assoc., <i>P</i> -trend 0.67
Ascherio, 1995, USA	Prospective observational	Fish, incl shellfish, M	Servings, 6 cat	≥6/wk vs 1/mo, 119 vs 0 g/d (mean)	812	HR 0.91 (0.64, 1.28)	No sig. assoc., <i>P</i> -trend 0.47
Hengeveld, 2018, the Netherlands	Prospective observational	Fish, M/W	Portions/wk, 3 cat	≥1/wk vs none, 28.7 g/wk fatty and 93.7 g/wk lean (median values)	693	HR 1.00 (0.86, 1.17)	No sig. assoc.
Iso, 2006, Japan	Prospective observational	Fish, incl fish products, M/W	g/d, quintiles	Quintile 5 vs 1, 180 vs 23 g/d (median values)	221	HR 0.47 (0.26, 0.85)	Protective assoc. for intake in Q5 vs Q1, <i>P</i> -trend 0.03
Kuhn, 2013, Germany	Prospective observational	Fish, M/W	g/d, quintiles	Quintile 5 vs 1, >31.1 (median 40.4) vs <7.5 (median 2.7) g/d	605	HR 0.84 (0.66, 1.08)	No sig. assoc., <i>P</i> -trend 0.21
Mohan, 2021, global, 6 continents, 58 countries	Prospective observational	Fish, incl shellfish, M/W, PURE study only	g/mo or wk, 4 cat	≥350 g/wk vs <50 g/mo, median 594 vs 0.1 g/wk	NA (no CVD history), 3806 (all)	HR 0.96 (0.82, 1.11)	No sig. assoc., <i>P</i> -trend 0.46
Salonen, 1995, Finland	Prospective observational	Fish, M	g/d, binary	≥30 vs <30 g/d	73	HR 1.87 (1.13, 3.09)	Adverse assoc. for intake ≥30 vs <30 g/d
Wennberg, 2011, Sweden	Prospective observational/ nested case-control	Fish, M/W	Meals/mo or wk, 4 cat	≥2/wk vs <1/mo	263	OR 1.21 (0.43, 3.33)	No sig. assoc.

There was one report of a statistically significant protective association (Iso et al., 2006), one report of a statistically significant adverse association (Salonen et al., 1995) for the highest vs lowest category. The remaining studies reported no significant association.

4.4.3.2 Studies of fatty fish intake and MI incidence

We included four observational studies, three with a prospective design (Gammemark et al., 2016; Hengeveld et al., 2018; Morris et al., 1995) and one case-control study (Lockheart et al., 2007) with five estimates of the association between fatty fish intake and risk of MI in the general population. Table 4.4.3.2-1 shows the exposure levels and results. The case-control study included fish oil as part of fatty fish intake and reported a statistically significant protective effect on the continuous scale (per SD of log food group intake, result not shown), but not for the highest versus lowest intake category. The other studies reported no statistically significant findings on MI.

Table 4.4.3.2-1 Results from studies included in the weight of evidence analysis of fatty fish intake and MI incidence.

Author, year, country	Study design	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low (95 % CI)	Overall result
Gammemark, 2016, Denmark	Prospective observational	Fatty fish, M	g/d, quintiles	Quintile 5 vs 1, >28 vs 0-8 g	2136	HR 0.93 (0.81, 1.07)	No sig. assoc., <i>P</i> -trend 0.38
	Prospective observational	Fatty fish, W	g/d, quintiles	Quintile 5 vs 1, >23 vs 0-6 g	892	HR 0.86 (0.69, 1.08)	No sig. assoc., <i>P</i> -trend 0.57
Hengeveld, 2018, the Netherlands	Prospective observational	Fatty fish, M/W	Portions/wk, 3 cat	≥1/wk vs none, 10.7 g/wk (median) in consumers	693	HR 1.12 (0.82, 1.52)	No sig. assoc.
Lockheart, 2007, Norway	Case-control	Fatty fish incl suppl, M/W	g/d, 3 cat	52 vs 12 g/d (median in controls)	111	OR 0.54 (0.23, 1.26)	No sig. assoc. as categories but protective as continuous (per SD of Ln food group intake (g/d))
Morris, 1995, USA	Prospective observational	Fatty fish, M	Meals/mo or wk, 4 cat	≥2/wk vs rarely/never	279	HR 0.90 (0.4, 1.8)	No sig. assoc., <i>P</i> -trend 0.72

4.4.3.3 Studies of lean fish intake and MI incidence

The four studies on fatty fish and MI summarized above, also included results on lean fish (Table 4.4.3.3-1). There were no statistically significant findings in any of the studies.

Table 4.4.3.3-1 Results from studies included in the weight of evidence analysis of lean fish intake and MI incidence.

Author, year, country	Study design	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low (95% CI)	Overall result
Gammelmark, 2016, Denmark	Prospective observational	Lean fish, M	g/d, quintiles	Quintile 5 vs 1, >39 vs 0-14 g	2136	HR 1.12 (0.97, 1.29)	No sig. assoc., <i>P</i> -trend 0.21
	Prospective observational	Lean fish, W	g/d, quintiles	Quintile 5 vs 1, >33 vs 0-13	892	HR 0.99 (0.79, 1.24)	No sig. assoc., <i>P</i> -trend 0.98
Hengeveld, 2018, the Netherlands	Prospective observational	Lean fish, M/W	Portions/wk, 3 cat	≥1/wk vs none, 32.9 g/wk (median) in consumers	693	HR 0.97 (0.82, 1.15)	No sig. assoc.
Lockheart, 2007, Norway	Case-control	Lean fish, M/W	g/d, 3 cat	99 vs 32 g/d (median in controls)	111	OR 0.70 (0.31, 1.59)	No sig. assoc.
Morris, 1995, USA	Prospective observational	Lean fish, M	Meals/mo or wk, 4 cat	≥2/wk vs rarely/never	272	HR 0.8 (0.3, 2.3)	No sig. assoc., <i>P</i> -trend 0.95

4.4.3.4 Studies of fish intake (total, fatty, lean) and risk of MI in patient populations

In two studies (four estimates) of total fish intake and risk of MI in patients with coronary artery disease (Manger et al., 2010) or with a history or high risk of CVD (Mohan et al., 2021), there was one report of a statistically significant protective association. One study in T2D patients only reported a statistically significant protective association for total fish. Estimates for fatty fish and lean fish were also on the protective side, but only significant for the fatty fish category herring and mackerel (analyzed separately from salmon, whitefish, char).

Table 4.4.3.4-1 Results from prospective observational studies included in the weight of evidence analysis for total fish intake and risk of MI in patient populations.

Author, year, country	Fish exposure, sex	Intake unit	High-low intake	Total cases	HR high-low (95% CI)	Overall result
Prior CHD, CVD or high risk						
Manger, 2010, Norway	Total fish, incl processed fish, M/W	g/d, quartiles	Quartile 4 vs 1, or Q2-4 vs 1, 201 vs 41.1 g/d (mean)	210	0.93 (0.63, 1.40)	No sig. assoc., <i>p</i> -trend 0.72

Author, year, country	Fish exposure, sex	Intake unit	High-low intake	Total cases	HR high-low (95% CI)	Overall result
Mohan, 2021, global, 6 continents, 58 countries	Fish, incl shellfish, M/W, PURE	g/mo or wk, 4 cat	≥350 g/wk vs <50 g/mo, median 594 vs 0.1 g/wk	NA (CVD history), 3806 (all)	0.71 (0.51, 0.99)	Protective assoc. in highest category, <i>P</i> -trend 0.07
	Fish, incl shellfish, M/W, ONTARGET, TRANSCEND	g/mo or wk, 4 cat	≥350 g/wk vs <50 g/mo, median 450 vs 2.8 g/wk	1552	0.86 (0.69, 1.06)	No sig. assoc., <i>P</i> -trend 0.34
	Fish, incl shellfish, M/W, ORIGIN	g/mo or wk, 4 cat	≥350 g/wk vs <50 g/mo, median 568 vs 2.2 g/wk	591	1.16 (0.90, 1.49)	No sig. assoc., <i>P</i> -trend 0.21
Diabetes population						
Wallin, 2018, Sweden	Total fish, M/W	Servings/mo or wk, 4 cat	>3/wk vs ≤3/mo, 3.5 vs 0.5 servings/wk (median)	333	0.60 (0.39, 0.92)	Protective assoc. (cat 2-4 vs 1, <i>p</i> -trend 0.08)
	Fatty fish (herring and mackerel), M/W	Servings/mo or wk, 3 cat	≥1/wk vs < 1/mo, 1.5 vs 0 servings/wk (median)	333	0.70 (0.50, 0.98)	Protective assoc. (cat 3 vs 1, <i>p</i> -trend 0.03)
	Fatty fish (salmon, whitefish, char), M/W	Servings/mo or wk, 3 cat	≥1/wk vs <1/mo, 1.5 vs 0 servings/wk (median)	333	0.86 (0.57, 1.31)	No sig. assoc
	Lean fish (cod, saithe, and fish fingers), M/W	Servings/mo or wk, 3 cat	≥1/wk vs <1/mo, 1.5 vs 0 servings/wk (median)	333	0.75 (0.53, 1.05)	Suggestive protective assoc. (limited to cat 2 vs 1, <i>p</i> -trend 0.89)

4.4.3.5 Summary relative risks (RR) based on VKM's inclusions of primary studies

VKM's summary RR for the highest versus lowest intake of total fish in relation to MI in prospective studies (eight studies, Table 4.4.3.1-1) was close to unity and not statistically significant (RR=0.96, 95% CI: 0.82, 1.12). One odds ratio (OR) from a nested-case control study (Wennberg et al., 2011) was combined with hazard ratios (HRs) in the summary RR and heterogeneity analysis. There was borderline statistically significant heterogeneity between studies ($P_{\text{heterogeneity}}=0.051$) and one report of a statistically significant adverse effect (Salonen et al. 1995, 7% relative weight). An influence analysis showed that the exclusion of Salonen et al. (1995) had some impact on the summary estimate and confidence interval (RR=0.93, 95% CI: 0.84, 1.04 after exclusion) and heterogeneity was reduced ($P_{\text{heterogeneity}}=0.32$).

Compared with VKM's summary estimate, the summary RR from the high-low meta-analysis by Jayedi et al. (2019) suggested a stronger protective association (RR=0.73, 95% CI: 0.59, 0.87), reflected in a statistically significant linear effect. The study selection in Jayedi et al. 2019 differed from VKM's selection (described in more detail below) in that Jayedi also included studies of MI mortality and did not include Salonen et al. (1995) (reporting an adverse association).

VKM's summary RRs for studies of total fish and risk of MI in patients with a history of CVD or at high risk of CVD (two studies with four estimates, Table 4.4.3.4-1, excluding one study in T2D patients only) was on the protective side, but not statistically significant (RR=0.91, 95% CI: 0.74, 1.12,) and without significant heterogeneity ($P_{\text{heterogeneity}}=0.11$).

The only primary study in T2D patients showed a statistically significant protective association (RR=0.60, 95% CI: 0.39, 0.92) that was stronger than the summary RR in the meta-analysis by Jayedi et al. (2020) of two studies in patients with T2D (RR=0.80, 95% CI: 0.46, 1.14).

VKM's summary RR for MI and high-low intake of fatty fish or lean fish (four studies including one Norwegian case-control study, Table 4.4.3.2-1), was on the protective side for fatty fish (RR=0.93, 95% CI: 0.82, 1.05, $P_{\text{heterogeneity}}=0.37$) and closer to unity for lean fish (RR=1.04, 95% CI 0.94, 1.14, $P_{\text{heterogeneity}}=0.54$), but neither estimate was statistically significant.

4.4.3.6 VKM's search compared to previous meta-analyses

The meta-analysis by Jayedi et al. (2020) reported on two studies of MI in patients with T2D. Both were identified by VKM, one was reported here under MI incidence (Wallin et al. 2018), the other (Zhang et al. 2018) was included under mortality.

Jayedi et al. (2019) included 11 prospective studies. All were identified by VKM. Five studies were included in the current section on incident MI, whereas six were not (Davignus et al., 1997; Yuan et al., 2001; Hu et al., 2002; Mozaffarian et al., 2003; Yamagishi et al., 2008; de

Goede et al., 2012). Of these, two were replaced by more recent publications identified by VKM: Hengeveld et al. (2018) replaced de Goede et al. 2012, and Bernstein et al. (2010) replaced Hu et al. (2002). The remaining four were included by VKM under MI mortality (Davignus et al., 1997; Yuan et al., 2001; Mozaffarian et al., 2003; Yamagishi et al., 2008). VKM included one study not included in the meta-analysis; Morris et al. (1995). However, this paper was not included in our final judgement except for fish sub-types since a later paper from the same study (Albert et al., 1998) was used on total fish. The nested case-control study by Wennberg et al., 2011 was not included in Jayedi et al. (2019). An overview of primary studies included by VKM and Jayedi et al. (2019) on incident CHD and/or myocardial infarction (MI) is given in Table 4.3.3.6-1.

4.4.4 Heterogeneity fish intake and MI incidence

Jayedi et al. (2019) reported moderate to high heterogeneity ($I^2 = 72\%$ in high-low analysis and $I^2 = 65\%$ in linear dose-response analysis). Potential sources of this heterogeneity were explored in sub-groups stratified by gender, region (USA & Europe, Asia), follow-up duration (cut-off 12 years.), number of cases (cut-off 500), and adjustment for alcohol, fruit and vegetable, energy intake and physical activity. Region, number of cases, and confounder adjustment (alcohol and energy intake) were found to be sources of heterogeneity. However, in the 11 prospective studies relative risks were below or around 1 (forest plot, not shown). Thus, the observed heterogeneity mainly seems to reflect differences in the magnitude and not the direction of associations. As previously noted, Jayedi et al. (2019) also included studies of MI mortality, which may be an additional source of heterogeneity. The summary RR calculated by VKM incorporated significant heterogeneity, but was explained by a single study showing an adverse association in a study population with high exposure to mercury (Salonen et al., 1995).

4.4.5 Dose-Response relationship fish intake and MI incidence

The meta-analysis by Jayedi et al. (2019) included a linear and non-linear dose-response analysis of total fish consumption and risk of MI. For all studies combined, there was evidence of a linear decrease in risk with higher intakes ($P_{\text{nonlinearity}} = 0.64$). When stratified by world region, relationships appeared less consistent, with a linear decrease reported for Asian studies, and a modest U-shaped association for Western studies. However, confidence limits were wide in stratified analyses, indicating high uncertainty (figures not shown).

One primary study (Gammelmarm et al. 2016) presented a non-linear dose-response analysis of fish intake (gram/day) with risk of incident MI in Danish men and women, with figures stratified by gender and by fatty- and lean fish intake. Figures (not shown) indicated decreased risk of MI with higher intakes, except for lean fish in men, but confidence limits were wide, indicating high uncertainty.

4.4.6 Weight of evidence for fish intake and MI incidence

In this section, the evidence of the association between fish intake and MI incidence is weighed according to the WCRF criteria presented in Chapter 3.1.6 (Box 2).

Published evidence of fish intake and MI

There is evidence from more than two independent and good quality cohort studies on total fish intake (VKM included eight studies on the general population, three in patients, and two meta-analyses).

VKM's summary RR for primary studies in the general population is not statistically significant but suggests lower risk of MI for the highest versus lowest intake of total fish when excluding a single study showing an adverse association. The study population was highly exposed to methyl mercury from consuming local nonfatty fish species. The summary RR calculated by VKM incorporates significant heterogeneity but is largely explained by this study. Unlike previous meta-analyses, VKM's summary RRs do not include studies of MI mortality. There is evidence for biological plausibility of a protective effect, and a linear meta dose-response relationship has been reported for studies of MI incidence and mortality combined.

In conclusion, the evidence is graded "limited, suggestive" for a protective effect of total fish intake on incident MI in the general population. VKM's summary RR for patients with a history of CVD or at high risk of CVD is not statistically significant but consistent with the summary RR for the general population. No conclusions can be drawn for risk of incident MI in patients with T2D due to limited evidence.

There are fewer studies of fatty fish and lean fish than of total fish and the evidence is graded "limited, suggestive" for a protective effect of fatty fish and "limited, suggestive" for no effect of lean fish on risk of incident MI in the general population.

Heterogeneity

Significant heterogeneity was observed between studies in previous meta-analyses, and between studies included by VKM. The heterogeneity mainly seems to reflect differences in magnitude of associations in previous meta-analysis. In VKM's analysis, one older study (Salonen et al. 1995) reported an adverse association.

Mechanism/biological plausibility

There is evidence for several plausible mechanisms operating in humans.

Upgrading factors

Both meta-analyses and primary studies indicate a dose-response. No other upgrading factors were evaluated.

4.4.6.1 Conclusion weight of evidence fish intake and MI incidence

There is evidence from more than two independent and good quality cohort studies on total fish intake (VKM included eight studies on the general population, three in patients, and two meta-analyses).

VKM's summary RR for primary studies in the general population is not statistically significant but suggests lower risk of MI for the highest versus lowest intake of total fish when excluding a single study in a population highly exposed to methyl mercury from consuming local nonfatty fish species. The summary RR calculated by VKM incorporated significant heterogeneity, but was largely explained by this study. Unlike previous meta-analyses, VKM's summary RRs do not include studies of MI mortality. There is evidence for biological plausibility of a protective effect, and a linear meta dose-response relationship has been reported for studies of MI incidence and mortality combined. In conclusion, the evidence is graded "limited, suggestive" for a protective effect of total fish intake on incident MI in the general population. VKM's summary RR for MI in patients with a history of CVD or at high risk of CVD is not statistically significant but consistent with the summary RR for the general population. No conclusions could be drawn for risk of incident MI in patients with T2D due to limited evidence.

There were fewer studies of fatty fish and lean fish (four in total) than of total fish and the evidence is graded "limited, suggestive" for a protective effect of fatty fish and "limited, suggestive" for no effect of lean fish on risk of incident MI in the general population.

4.5 Fish intake and stroke incidence

4.5.1 VKM's search for published systematic reviews and meta-analyses of fish and stroke incidence

4.5.1.1 Description of the identified publications

VKM's search for systematic reviews and meta-analyses (see Chapter 3.1.2.2) identified 13 publications on the association between fish intake and stroke incidence that were assumed to fulfil the inclusion criteria and were read as full papers. Three papers were excluded, see Table 4.5.1.1-1 for reason for exclusions. The included papers were graded B by VKM using the AMSTAR tool.

Table 4.5.1.1-1 Reasons for exclusion of identified papers from the search of systematic reviews and meta-analyses of fish intake and stroke incidence 2016-2021.

Included papers	Excluded paper and reasons for exclusions
Umbrella reviews Jayedi and Shab-Bidar, 2020 Altobelli et al., 2019 D'Alessandro et al., 2019 Deng et al., 2018	Kromhout et al., 2016: Type of umbrella review but unclear if systematic. No search strategy shown, or description of quality assessment. This does not include a meta-analysis.
Systematic reviews Chen et al., 2021 Jayedi et al., 2020, patients with T2D Bechthold et al., 2019 Zhao et al., 2019 Qin et al., 2018 Xun et al., 2012	Schwingshackl et al., 2019: This is not a systematic literature review of a meta-analysis. Micha et al., 2017: Umbrella review. Selection of papers was only done by one person. There was no information about any quality assessment for the included meta-analyses. Stroke was included in the search but no information about stroke and fish included in the paper.

The meta-analyses are described in more detail below; first main descriptions of the methods used and then the main/selected results from each review.

In total four of the identified eight studies were umbrella reviews (Jayedi and Shab-Bidar, 2020; Altobelli et al., 2019; D'Allesandro et al., 2019; Deng et al., 2018). These umbrella reviews build on three relevant meta-analyses; two of which were also identified in VKM's search, Bechthold et al. (2019) and Qin et al. (2018), and one that was older, Xun et al. (2012). Two additional meta-analyses, not included in any of the umbrella reviews, were identified in the VKM search; Zhao et al. (2019) and Jayedi et al. (2020) (see flow-chart below, Figure 4.5.1.1-1).

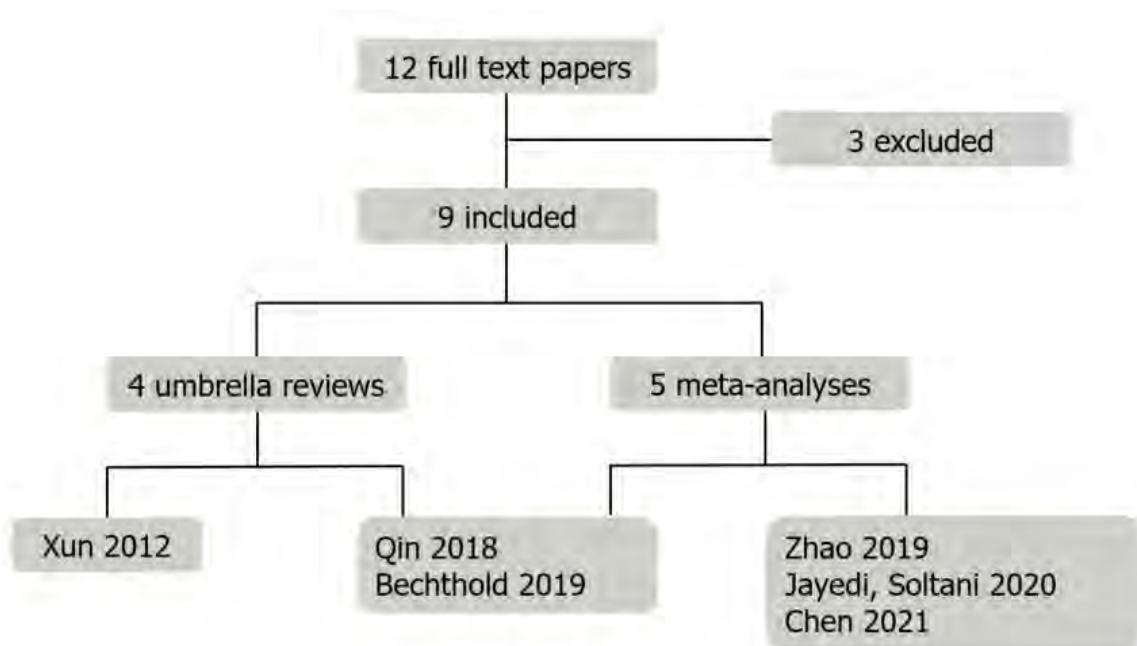


Figure 4.5.1.1-1 Flow-chart of the included meta-analyses of fish and stroke incidence.

Umbrella reviews

The umbrella reviews by Jayedi and Shab-Bidar (2020) and D'Allesandro et al. (2019) are described more in detail in Chapter 4.3.1. Both identified one meta-analysis looking at the association between fish intake and stroke; Bechthold et al. (2019).

Altobelli et al. (2019) is an umbrella review of meta-analyses looking at the impact of different foods and/or drinks in relationship to the risk of stroke events (ischemic/hemorrhagic). The authors did a search covering the last 10 years in MEDLINE, EMBASE, Scopus, Clinicaltrials.gov, Web of Science, and Cochrane Library databases up until 31 December 2018. Methodological quality assessments of the meta-analyses were made according to the AMSTAR 2 scale. All primary studies in this umbrella review came from countries with high income levels. Two meta-analyses studying fish intake and stroke; Qin et al. (2018) and Xun et al. (2012) were included in the umbrella review.

Deng et al. 2018 is an umbrella review of meta-analyses of the associations of different food groups with stroke risk (incident or mortality). The authors searched PubMed, EMBASE and Cochrane Library databases up to September 2015 for systematic reviews and meta-analyses of prospective studies. The methodological quality of the included meta-analyses was assessed with AMSTAR. Deng et al. (2018) identified three reviews/meta-analyses reporting on the relationship between fish and risk of stroke. Only one (Xun et al., 2012) was included, because it had the largest number of primary studies with individual studies' effect sizes. The meta-analysis by Xun et al. (2012) was not identified in the literature search performed by VKM (from 2016 onwards), but it is included in our overview below.

Meta-analyses

Chen et al. (2021) conducted systematic literature searches to identify prospective cohort studies that reported on fish consumption or LC n-3 PUFAs intake and risk of stroke. PubMed, EMBASE and the Cochrane Library were searched up to May 2019. The reference lists of previous systematic reviews were also searched. The study quality was assessed with the 9-point Newcastle-Ottawa Scale (NOS). Scores (range 0 to 9) of included primary studies ranged from 6 to 8, with a median of 7. A total of 10 studies were included on fish intake.

The meta-analysis by Bechthold et al. (2019) and Jayedi et al. (2020) have previously been described in detail in Chapter 4.3.1.

Zhao et al. (2019) conducted a meta-analysis of 31 prospective studies on fish consumption with total stroke (fatal and non-fatal). Literature searches were conducted in PubMed and Embase through March 23, 2018. If multiple publications from the same population or overlapping data were found, they included the study with the longest follow-up, or the most informative regarding both exposure and outcome. Zhao et al. (2019) rated the quality of the meta-evidence as moderate (NutriGrade score=7.7 points).

Qin et al. (2018) summarized studies of fatty and lean fish (not total fish) and stroke. The authors performed a literature search in PubMed, Embase, Scopus, and Cochrane Library through February 1, 2018. The 9-point Newcastle-Ottawa scale (NOS9) was used to evaluate the quality of each cohort. They included five prospective studies with quality scores ranging from 6 to 9.

Xun et al. (2012) summarized studies of fish consumption and incidence of stroke. They performed literature searches in Medline and Embase through April 2012 and included 16 prospective studies.

4.5.1.2 Results from the meta-analyses

Below are summary tables for total fish and stroke (Table 4.5.1.2-1), total fish and ischemic stroke (Table 4.5.1.2-2), total fish and hemorrhagic stroke (Table 4.5.1.2-3), fatty fish and stroke (Table 4.5.1.2-4), and lean fish and stroke (Table 4.5.1.2-5), based on the five identified meta-analyses.

Table 4.5.1.2-1 Summary of results from meta-analyses on total fish intake and risk of stroke incidence.

Author, year	Type of studies included	Total no studies	No of cases	Comparison	Summary RR (95% CI)	Heterogeneity	Overall conclusion
Chen, 2021	Prospective cohort studies of fish intake and stroke risk (incidence or mortality)	8 studies (10 estimates)	4164	Highest vs lowest	0.87 (0.78, 0.97)	$I^2=0\%$	Higher fish consumption significantly associated with lower risk of stroke
Jayedi, 2020	Prospective studies of fish (seafood) intake and MI (incidence or mortality) in patients with T2D	2	NA from paper	Highest vs lowest	0.65 (-0.08, 1.37), error in reported CI (neg. value)	$I^2=82.9\%$	A non-sig. protective association between fish and stroke in patients with T2D. The quality of the meta-evidence was rated very low
Bechthold, 2019	Prospective cohort studies of fish intake and stroke incidence. Studies including fatal cases only were excluded.	20	14360	Highest vs lowest	0.95 (0.89, 1.01)	$I^2=37\%$	Fish intake is associated with a decreased risk of stroke. The quality of meta-evidence was moderate
		5		Per 100 g/d	0.86 (0.75, 0.99)	$I^2=25\%$	
Zhao, 2019	Prospective cohort studies of fish intake and stroke risk (incidence or mortality)	31	32708	Highest vs lowest	0.90 (0.85, 0.96)	$I^2=39.2\%$	Higher intake of fish was associated with a decreased risk of stroke. The quality of meta-evidence was moderate
		NA	NA	Per 700g/wk	0.88 (0.80, 0.97)		
Xun, 2012	Prospective cohort studies of fish intake and stroke risk (incidence or mortality)	16	10568	Cat 1, never consumed	1	$I^2=20.1\%$	A modest protective association of fish with stroke risk, P -trend 0.09
				Cat 2, <1 fish serv/mo	0.97 (0.87, 1.08)		
				Cat 3, 1 fish serv/wk	0.86 (0.80, 0.93)		
				Cat 4, 2–4 fish serv/wk	0.91 (0.85, 0.98)		
				Cat 5, ≥5 fish serv/wk	0.87 (0.79, 0.96)		

Table 4.5.1.2-2 Summary of results from meta-analyses on total fish intake and ischemic stroke incidence.

Author	Type of studies included	Total no studies	No of cases	Comparison	Summary RR/HR (95 % CI)	Heterogeneity	Overall conclusion
Chen, 2021	Prospective cohort studies of fish intake and ischemic stroke risk (incidence or mortality)	7	NA for stroke sub-types	Highest vs lowest	0.81 (0.70, 0.94)	$I^2=15\%$, $P=0.31$	Higher fish consumption significantly associated with lower risk of ischemic stroke
Zhao, 2019	Prospective cohorts	15	NA	Highest vs lowest	0.96 (0.89, 1.03)	$I^2=27.9\%$	No sig. assoc.
Xun, 2012	Prospective cohort	11	5406	Cat 1, never consumed	1	NA in paper	Protective association (significant modest assoc. between fish intake and incidence of ischemic stroke), P -trend 0.07
				Cat 2, <1 fish serv/mo	0.96 (0.84, 1.11)		
				Cat 3, 1 fish serv/wk	0.82 (0.73, 0.93)		
				Cat 4, 2–4 fish serv/wk	0.89 (0.81, 0.97)		
				Cat 5, ≥ 5 fish serv/wk	0.83 (0.75, 0.92)		

Table 4.5.1.2-3 Summary of results from meta-analyses on total fish intake and hemorrhagic stroke incidence.

Author, year	Type of studies included	Total no studies	No of cases	Comparison	Summary RR/HR (95 % CI)	Heterogeneity	Overall conclusion
Chen, 2021	Prospective cohort studies of fish intake and hemorrhagic stroke risk (incidence or mortality)	6	NA for stroke sub-types	Highest vs lowest	1.01 (0.76, 1.34)	$I^2=0\%$, $P=0.55$	Higher fish consumption significantly associated with lower risk of hemorrhagic stroke
Zhao, 2019	Prospective cohorts	13	NA from paper	Highest vs lowest	0.88 (0.75, 0.96)	0%	Protective effect of total fish on hemorrhagic stroke

Author, year	Type of studies included	Total no studies	No of cases	Comparison	Summary RR/HR (95 % CI)	Heterogeneity	Overall conclusion
Xun, 2012	Prospective cohorts	11	1764	Cat 1, never consumed	1	NA in paper	No significant association, P -trend 0.28
				Cat 2, <1 fish serv/mo	1.08 (0.85, 1.39)		
				Cat 3 1 fish serv/wk	0.96 (0.83, 1.11)		
				Cat 4, 2-4 fish serv/wk	0.97 (0.84, 1.10)		
				Cat 5, ≥ 5 fish serv/wk	0.92 (0.80, 1.07)		

For fatty fish intake and stroke we identified two meta-analyses from 2018 and 2019 respectively (Qin et al 2018, Zhao et al 2019).

Table 4.5.1.2-4 Summary of results from meta-analyses on fatty fish intake and stroke incidence.

Author, year	Type of studies included	Total no studies	No of cases	Comparison	Summary RR/HR (95 % CI)	Heterogeneity	Overall conclusion
Zhao, 2019	Prospective cohort	6	NA from paper	Highest vs lowest	0.89 (0.79, 1.01)	$I^2=0\%$	Suggestive protective association
Qin, 2018	Prospective cohort	5	3066	Highest vs lowest	0.88 (0.75, 1.04)	$I^2=26.2\%$	No sig. association

For lean fish intake and stroke we identified two meta-analyses from 2018 and 2019, respectively (Qin et al 2018; Zhao et al 2019).

Table 4.5.1.2-5 Summary of results from meta-analyses on lean fish intake and stroke incidence.

Author, year	Type of studies included	Total no studies	No of cases	Comparison	Summary RR/HR (95 % CI)	Heterogeneity	Overall conclusion
Zhao, 2019	Prospective cohort	5	NA from paper	Highest vs lowest	0.84 (0.70, 1.00)	$I^2=0\%$	Suggestive protective association
Qin, 2018	Prospective cohort	4	2645	Highest vs lowest	0.81 (0.67, 0.99)	$I^2=0\%$	Lean fish reduces the risk of stroke

Zhao et al. (2019) and Bechthold et al. (2019) reported that fish consumption is associated with a lower risk of stroke, while Xun et al. (2012) concluded with a modest beneficial association. In Jayedi et al. (2020), a small meta-analysis of two studies in patients with type 2 diabetes, no association was found.

When looking at stroke sub-types, Zhao et al. (2019) found a significant protective association between fish consumption and hemorrhagic stroke, but not with ischemic stroke. Xun et al. (2012) observed the opposite; they found a statistically significant but modest protective association between fish consumption and ischemic stroke, but not hemorrhagic stroke.

Looking at fish type, Zhao et al. (2019) found no significant associations between stroke risk and any specific type of fish (lean or fatty fish). While Qin et al. (2018) concluded that lean fish were significantly associated with a decreased risk of stroke.

4.5.2 VKM's search for primary studies of fish intake and stroke incidence

4.5.2.1 Included studies from search

A total of 22 publications, graded A or B in VKM's quality assessment, included total stroke incidence (sum of ischemic, hemorrhagic, and unspecified strokes, or all cerebrovascular disease, see Figure 4.1-1) as outcome: Amiano et al., 2016; Atkinson et al., 2011; Bernstein et al., 2012; Bonaccio et al., 2017; de Goede et al., 2012; Gillum et al., 1996; He et al., 2002; Hengeveld et al., 2018; Iso et al., 2001; Keli et al., 1994; Kuhn et al., 2013; Larsson et al., 2011; Mohan et al. 2021, Montonen et al., 2009; Morris et al., 1995; Mozaffarian et al., 2005b; Myint et al., 2006; Orenca et al., 1996; Tong et al. 2021; Wallin et al. 2018; Wennberg et al., 2016; Zhang et al. 2021 . One additional study assessed ischemic stroke only (Nahab et al., 2016). Thus, 23 unique studies were evaluated on stroke. There were multiple publications from the same studies and three were excluded (see section "Overlapping publications" below), leaving 20 for further analysis, of which one was conducted in patients with T2D (Wallin et al. 2018) and one was conducted in patients with and without a history of CVD as separate analyses (Mohan et al. 2021). This study contributed results on patients and the general population (non-patients).

A description of the studies (study name, design, time period, size and age of the study population, and dietary assessment method) can be found in Table 4.5.2.1-1.

Table 4.5.2.1-1 Overview of primary studies included in weight of evidence analysis of fish intake and stroke incidence.

Author, year, country	Study name	Study design	Inclusion year(s), end, follow-up time	Study population	Dietary assessment method	Dietary assessment ref period
Amiano, 2016, Spain	EPIC-Spain	Prospective observational	1992- 1996 to 2008, 13.8 yrs follow-up (mean)	15 490 men and 25 530 women, 20–69 yrs	Face-to-face interview, computerized questionnaire based on a previously validated dietary history instrument	Usual intake, previous year, at baseline
Atkinson, 2011, UK	Caerphilly Prospective Study (CaPS)	Prospective observational	1979-1983, 18 yrs follow-up (median)	2710 men, 45-59 yrs	Repeated FFQ, semi-quant	Most recent diet (from the phase immediately preceding the stroke event or censoring): baseline (Phase I), and at 5 (Phase II) and 10 (Phase III) years post baseline
Bernstein, 2012, USA	Health Professionals Follow-Up Study (HPFS) and Nurses' Health Study (NHS)	Prospective observational	1980 to 2006 (NHS), 26 yrs follow-up; 1986 to 2008 (HPFS), 20 yrs follow-up	84 010 female nurses and 43 150 male health professionals, 30–55 yrs, (W), 40–75 year (M)	Repeated FFQ every 4 yrs., semi-quant, validated	Frequency during the previous year
Bonaccio, 2017, Italy	Moli-sani study	Prospective observational	2005-2010 to 2011, 4.3 yrs follow-up (median)	24 325 (46% male), ≥35 yrs (mean age 55 yrs)	Italian version of EPIC FFQ, validated	Previous year, at baseline
Gillum, 1996, USA	National Health and Nutrition Examination Survey (NHANES) I Epidemiologic Follow-up Study	Prospective observational	1971-1975 to 1987, 12 yrs follow-up (mortality)	5192 (4410 white persons and 782 black persons), 45-74 yrs	FFQ by interview	Usual intake previous 3 months, at baseline
Hengeveld, 2018, the Netherlands	EPIC-Netherlands (Prospect and MORGEN sub-cohorts)	Prospective observational	1993-1997 to 2011, 18 yrs follow-up (median 15.1 yrs)	34 033 (25% male), 20-70 yrs, mean age 48.7 yrs	FFQ, semi quant, validated	Usual intake, previous year, at baseline

Author, year, country	Study name	Study design	Inclusion year(s), end, follow-up time	Study population	Dietary assessment method	Dietary assessment ref period
Keli, 1994, the Netherlands	Zutphen study	Prospective observational	1960-1970 to 1985, 15 yrs. follow-up	552 men, 50-69 yrs (in 1970)	Repeated cross-check dietary history method (1960, 1965, 1970), adapted to Dutch situation, interview with wife and with husband for consumption away from home	Usual intake 6-12 months prior to interview
Kuhn, 2013, Germany	EPIC-Germany	Prospective observational	1994-1998 to 2006, 8.1 yrs follow-up (mean)	48 315 (42% male), 35-65 yrs, mean age 50.5 yrs	FFQ, uncertain validity	Usual intake during the previous year, at baseline
Larsson, 2011, Sweden	Swedish Mammography Cohort (SMC)	Prospective observational	1998 to 2008, 10.4 yrs follow-up (mean)	34 670 women, 49-83 yrs	FFQ, validated	Average frequency during the previous year, at baseline
Mohan, 2021, global, 6 continents, 58 countries	PURE, ONTARGET, TRANSCEND, and ORIGIN, only data from PURE on general population	Prospective observational, multicenter			Mohan et al., 2021, Global, 6 continents, 58 countries	PURE, ONTARGET, TRANSCEND, and ORIGIN, only data from PURE on general population
Montonen, 2009, Finland	Finnish Mobile Clinic	Prospective observational	1966-1972 to 1994, 28 yrs follow-up	3958 (52% male), 40-79 yrs	Dietary history interview	Usual intake, previous year, at baseline
Morris, 1995, USA	Physicians' Health Study (PHS)	Prospective observational	1982, 4 yrs of follow-up	21 185 male physicians, 40-84 yrs	FFQ, semi quant, validated	Average intake, previous year, at 12 month follow-up
Mozaffarian, 2005b, USA	Cardiovascular Health Study	Prospective cohort	1989-90 to 2001, 12 yrs follow-up	4778 men and women, 65-98 yrs, mean age 72.7 yrs	FFQ, picture sort version of the National Cancer Institute FFQ	Average intake during the previous year, at baseline
Myint, 2006, UK	EPIC-Norfolk	Prospective observational	1993-1997 to 2004, 8.5 yrs follow-up (mean)	10 972 men and 13 340 women, 40-79 yrs	FFQ	Average intake, previous year, at baseline

Author, year, country	Study name	Study design	Inclusion year(s), end, follow-up time	Study population	Dietary assessment method	Dietary assessment ref period
Nahab, 2016, USA	REasons for Geographic And Racial Differences in Stroke (REGARDS) study	Prospective cohort	2003-2007 to 2010, 5.1 yrs of follow up (median)	16 479 men and women (34% African Americans, 59 % female, 74% were overweight or obese), 40-75 yrs	FFQ, Block98	Usual intake, past year, at baseline
Orencia, 1996, USA	Chicago Western Electric Study	Prospective observational	1957-1958, 30 yrs follow-up	1847 men, 40-55 yrs	Standardized interviews and questionnaires based on Burke's diet history method	Previous 28 days, at baseline and 1 year later
Tong, 2021, Europe	EPIC (9 countries: Denmark, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden, and the UK)	Prospective cohort	1992–2000 to 2003-2012 (variations by study center), follow-up 12.7 yrs (mean)	418 329 (140,117 men and 278 212 women), Mean 52.0 (M) and 51.4 (W) yrs	Dietary history or FFQ, country specific, validated and calibrated against 24h recall	Year before enrolment
Wennberg, 2016, Sweden	Northern Sweden Health and Disease Study (NSHDS)	Prospective observational, nested case-control	1986–2005 to 1987-2007	735 cases (446 male) and 2698 (1633 male) controls, 25-74 yrs (mean 55 yrs)	Multiple versions of FFQ, differ by cohort (VIP, MONICA)	Average frequency during the previous year
Zhang, 2021, UK	UK Biobank	Prospective cohort	2006-2010 to 2020, follow-up 11.2 yrs (median)	462,155 (44% male), 40-69 yrs, mean 56.7 yrs	Touchscreen FFQ	NA, probably usual intake at baseline
Patient populations						

Author, year, country	Study name	Study design	Inclusion year(s), end, follow-up time	Study population	Dietary assessment method	Dietary assessment ref period
Mohan, 2021, global, 6 continents, 58 countries	PURE, ONTARGET, TRANSCEND, and ORIGIN	Prospective cohorts	Follow-up to 2019 (PURE), Median follow-up (yrs) was 9.1 in PURE, 4.5 in ONTARGET and TRANSCEND, and 6.2 in ORIGIN	191 558 (47.9% male), PURE (n = 147 541), ONTARGET and TRANSCEND (n = 31 491), ORIGIN (n=12 422), 51 731 with vascular disease, and 139 827 generally healthy individuals, Mean age PURE 51 yrs (range 35-70 yrs), ONTARGET and TRANSCEND 67 yrs, ORIGIN 64 yrs	Country specific FFQs (no amounts in ONTARGET and TRANSCEND), validated in some countries	Usual intake in previous year, at baseline
Wallin, 2018, Sweden	Cohort of Swedish Men (COSM) and the Swedish Mammography Cohort (SMC)	Prospective cohort	1998 to 2012, mean follow-up 11.8 yrs for incidence and 13.2 yrs for mortality	2225 (912 women and 1313 men) with type 2 diabetes, 45-84 yrs	FFQ, validated in men	Average frequency during the previous year, at baseline (1997)
Excluded due to overlap						
de Goede, 2012, the Netherlands	Monitoring Project on Risk Factors for Chronic Diseases (MORGEN)	Prospective cohort	1993-1997 to 2006, 10.5 yrs follow-up (median)	20 069 men and women, 20-65 yrs (mean age 40-43 yrs)	FFQ, validated	Usual freq of consumption in previous year, at baseline
He, 2002, USA	Health Professionals Follow-Up Study (HPFS)	Prospective cohort	1986 to 1988, 12 yrs follow-up	43 671 male health professionals, 40-75 yrs, mean age approx 53 yrs	Repeated FFQ (1986, 1990, 1994), validated	Average intake during the previous year
Iso, 2001, USA	Nurses' Health Study (NHS)	Prospective cohort	1980 to 1994, 14 yrs follow-up	79 839 female nurses, 34 -59 yrs	Repeated FFQ (1980, 1984, 1986, and 1990), semi-quant, validated	Average intake during the previous year

4.5.2.2 Overlapping publications

According to the protocol, the most recent publication with the longest follow-up time and/or largest number of events was included. Iso et al. (2001) (Nurses' Health Study, USA) and He et al. (2002) (Health Professionals Follow-Up Study, USA) are covered by Bernstein et al. (2012) (NHS and NPFS studies combined), and de Goede et al. (2012) (MORGEN study) is covered by Hengeveld et al. (2018) (EPIC-Netherlands, including the sub-cohorts MORGEN and Prospect). All three papers are therefore excluded from the weight of evidence analysis.

4.5.2.3 Studies by design and geographic region

All studies of incident stroke (total or sub-types) had a prospective, observational design, including one nested case-control study (Wennberg et al., 2016). The study samples were generally from Western populations in Europe or USA. Several of the US cohorts were based on specific occupational groups (health professionals or industrial workers). Tong et al (2021) is based on the EPIC study (multicenter, 9 European countries included) and Mohan et al. (2021) is a global multicenter study with data from 58 countries on 6 continents.

4.5.2.4 Studies in patient populations

Two publications included patient populations. Mohan et al. (2021) was based on four studies: one cohort (PURE) where participants with a history of CVD were analyzed separately, and three follow-up studies of drug-trials (ONTARGET, TRANSCEND, and ORIGIN) where all participants were treated for vascular disease. Mohan et al. (2021) was previously described in more detail under CVD incidence (Chapter 4.2.2.4). One study assessed stroke in cohort participants limited to those with T2D at baseline (Wallin et al. 2018).

4.5.2.5 Studies by stroke sub-types (ischemic or hemorrhagic)

Among 19 publications on total stroke (excluding overlapping studies and one in T2D patients), nine presented results on ischemic stroke: Amiano et al. (2016); Bernstein et al. (2012); Hengeveld et al. (2018); Kuhn et al. (2013); Larsson et al. (2011); Montonen et al. (2009); Mozaffarian et al. (2005b); Tong et al. 2021; Wennberg et al. (2016). In addition, Nahab et al. (2016) presented results on ischemic stroke only. All ten studies on ischemic stroke except Nahab et al. 2016 also presented results on hemorrhagic stroke. Thus, there were ten publications on ischemic stroke, and nine on hemorrhagic stroke.

4.5.2.6 Studies by sex and potential effect modification

Of the 19 studies on total stroke, most studies included both women and men, but four studies included men only (Atkinson et al., 2011; Keli et al., 1994; Morris et al., 1995; Orenca et al., 1996) and one study women only (Larsson et al., 2011), see Table 4.5.2.1-1. Studies testing for potential effect modification by sex (Bonaccio et al., 2017; Kuhn et al., 2013; Mozaffarian et al., 2005b) generally reported a non-significant test of interaction

($p > 0.05$), or no such effect. Therefore, we present estimates in men and women combined when available.

As described under CVD incidence (Chapter 4.2.2.5), Mohan et al., 2021 stratified results by CVD history in the study participants (PURE study only) whereas Zhang et al. (2021) stratified results by genetic CVD risk, defined as a family history of cardiovascular disease (CVD) or a CVD polygenic risk score (PRS) in the study participants. Associations in Mohan et al. 2021 differed by CVD history and are presented separately for the general population (Chapter 4.5.2.1) and patient populations (Chapter 4.5.2.6). In Zhang et al. (2021) stratified results were similar to overall results for CVD and subtypes of CVD, including cerebrovascular disease. Therefore, only the overall results are presented here.

4.5.2.7 Studies by stroke type and fish exposure

Several included studies presented results by multiple outcomes and/or fish classifications. Most studies had a total fish exposure (sum of fish, fish without specifications, or fish including shellfish and/or fish products). Hengeveld et al. (2018) presented fish intake with shellfish (main analysis) and without shellfish (sensitivity analysis), and the results without shellfish were included according to the study protocol which emphasizes fish intake. Other classifications were by fat content (fatty or lean), less commonly by preparation method (fried or non-fried) or conservation method (canned, smoked, salted, or dried fish).

Results could be summarized for total fish ($n=15$), fatty fish ($n=7$) and lean fish ($n=7$) in relation to total stroke (19 studies in general population). "Dark fish" was then categorized as fatty fish and "white fish" as lean fish. Four studies did not contribute with results on total fish; two studies only included fried and non-fried fish (Mozaffarian et al., 2005b; Nahab et al., 2016), and two studies used a binary categorization intake into no/yes (Myint et al., 2006, oily fish), or none/any (Gillum et al., 1996, fish intake in black men and women) without amount or frequency and were excluded according to protocol.

Results on total fish were also summarized in relation to ischemic stroke ($n=8$) and hemorrhagic stroke ($n=7$).

4.5.2.8 Studies assessing potential non-linearity

Three primary studies performed a non-linear dose-response analysis of fish and risk of stroke using restricted cubic spline regression (He et al., 2002; Larsson et al., 2011; Kuhn et al., 2013) of which two included figures (He et al., 2002; Larsson et al., 2011) not shown in this report.

4.5.3 Results from the included primary studies fish intake and stroke incidence

4.5.3.1 Studies of total fish intake and total stroke incidence

We included 15 prospective, observational studies (14 cohorts and one nested case-control) and 18 estimates (sex-specific, or for men and women combined) of the association between total fish intake and stroke. Table 4.5.3.1-1 shows the exposure levels and results in these studies.

Table 4.5.3.1-1 Results from studies included in the weight of evidence analysis of total fish intake and total stroke incidence.

Author, year, country	Study design*	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low (95% CI)	Overall result
Amiano, 2016, Spain	Prospective cohort	Fish, incl fish products and shellfish, M	g/d, quintiles	Quintile 5 vs 1, ≥ 111 vs < 38.6 g/d	373	0.77 (0.51, 1.16)	Suggestive protective trend, P -trend 0.06
	Prospective cohort	Fish, incl fish products and shellfish, W	g/d, quintiles	Quintile 5 vs 1, ≥ 77.8 vs < 26.1 g/d	301	1.07 (0.68, 1.69)	No sig. assoc., P -trend 0.56
Bernstein, 2012, USA	Prospective cohort	Fish, M/W	Servings/d, cumulative average, quintiles	Quintile 5 vs 1	4030	0.92 (0.82, 1.04)	No sig. assoc., P -trend 0.33
Bonaccio, 2017, Italy	Prospective cohort	Fish, M/W	Times/wk, 3 cat	> 4 vs < 2 times/wk, 92.5 vs 23.0 g/d (mean)	66	0.63 (0.26, 1.51)	No sig. assoc., P -trend 0.15
Gillum, 1996, USA	Prospective cohort	Fish, incl shellfish, W - white	Times/wk, 4 cat	> 1 /wk vs never	251	0.55 (0.32, 0.93)	Sig. protective assoc. of intake > 1 /wk vs never, P -trend 0.01
	Prospective cohort	Fish, incl shellfish, M - white	Times/wk, 4 cat	> 1 /wk vs never	262	0.85 (0.49, 1.46)	No sig. assoc.
Hengeveld, 2018, the Netherlands	Prospective cohort	Fish, M/W	Portions/wk, 3 cat	≥ 1 /wk vs none, 28.7 g/wk fatty and 93.7 g/wk lean (median values)	753	0.89 (0.77, 1.03)	No sig. assoc.
Keli, 1994, the Netherlands	Prospective cohort	Fish, M	g/d, binary	≥ 20 vs < 20 g/d, 35.4 vs 6.3 g/d (mean values)	42	0.49 (0.24, 1.01)	Borderline protective assoc. of intake ≥ 20 vs < 20 g/d ($P=0.052$)
Kuhn, 2013, Germany	Prospective cohort	Fish, M/W	g/d, quintiles	Quintile 5 vs 1, > 31.1 (median 40.4) vs < 7.5 (median 2.7) g/d	525	0.96 (0.73, 1.26)	No sig. assoc., P -trend 0.67

Author, year, country	Study design*	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low (95 % CI)	Overall result
Larsson, 2011, Sweden	Prospective cohort	Fish, W	Servings/wk, 5 cat	>3.0/wk vs <1.0/wk	1680	0.84 (0.71, 0.98)	Sig. protective assoc. of intake >3 vs <1 serving/wk, <i>P</i> -trend 0.049.
Mohan, 2021, global, 6 continents, 58 countries	Prospective cohort	Fish, incl shellfish, M/W, PURE	g/mo or wk, 4 cat	≥350 g/wk vs <50 g/mo, median 594 vs 0.1 g/wk	NA (no CVD history), 3925 (all)	0.97 (0.84, 1.11)	No sig. assoc., <i>P</i> -trend 0.22
Montonen, 2009, Finland	Prospective cohort	Fish, M/W	g/d, quartiles	Quartile 4 vs 1, 72 vs 6 (median values)	659	1.01 (0.81, 1.27)	No sig. assoc., <i>P</i> -trend 0.80
Morris, 1995, USA	Prospective cohort	Fish, M	Meals/wk, 4 cat	≥5/wk vs <1/mo	173	0.6 (0.3, 1.6)	No sig. assoc., <i>P</i> -trend 0.13
Myint, 2006, UK	Prospective cohort	Fish, incl fish products and shellfish, M	Portions/wk, 3 cat	≥2 vs <1/wk	217	1.34 (0.93, 2.93)	No sig. assoc., <i>P</i> -trend 0.26
	Prospective cohort	Fish, incl fish products and shellfish, W	Portions/wk, 3 cat	≥2 vs <1/wk	204	0.86 (0.60, 1.24)	No sig. assoc., <i>P</i> -trend 0.29
Orencia, 1996, USA	Prospective cohort	Fish, M	g/d, 4 cat	Cat 4 vs 1, ≥35 g/d vs none	222	1.26 (0.74, 2.16)	No sig. assoc.
Wennberg, 2016, Sweden	Case-control, nested	Fish, M/W	Times/mo or wk, 5 cat	>3/wk vs <1/mo	712	1.05 (0.60, 1.82)	No sig. assoc.
Zhang, 2021, UK	Prospective cohort	Total fish, M/W	Times/wk, 4 cat	≥3 vs <1/wk	NA, sample 458 050	0.92 (0.88, 0.96)	Sig. protective assoc. in two highest cat above reference, <i>P</i> -trend <0.001

Among the 15 studies, there were three reports of a statistically significant protective association, and no reports of a statistically significant adverse association (see Chapter 4.5.2.7 for the overall summary estimate).

4.5.3.2 Studies of total fish intake and ischemic stroke incidence

We included eight observational studies (seven prospective cohorts and one nested case-control) with nine estimates (sex-specific, or for men and women combined) of the association between total fish intake and ischemic stroke. Table 4.5.3.2-1 shows the exposure levels and results in these seven studies.

Table 4.5.3.2-1 Results from studies included in the weight of evidence analysis of total fish intake and ischemic stroke incidence.

Author, year, country	Study design*	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low (95 % CI)	Overall result
Amiano, 2016, Spain	Prospective cohort	Fish, incl fish products and shellfish, M	g/d, quintiles	Quintile 5 vs 1, ≥ 111 vs < 38.6	302	1.13 (0.68, 1.88)	No sig. assoc., P -trend 0.83
		Fish, incl fish products and shellfish, W	g/d, quintiles	Quintile 5 vs 1, ≥ 77.8 vs < 26.1	229	1.31 (0.69, 2.47)	No sig. assoc., P -trend 0.89
Bernstein, 2012, USA	Prospective cohort	Fish, M/W	Servings/d, cumulative average, quintiles	Quintile 5 vs 1	2212	0.94 (0.81, 1.11)	No sig. assoc., P -trend 0.66
Hengeveld, 2018, the Netherlands	Prospective cohort	Fish, M/W	Portions/wk, 3 cat	≥ 1 /wk vs none, 28.7 g/wk fatty and 93.7 g/wk lean (median values)	413	0.79 (0.65, 0.97)	Protective association of ≥ 1 vs null portion/wk
Kuhn, 2013, Germany	Prospective cohort	Fish, M/W	g/d, quintiles	Quintile 5 vs 1, > 31.1 (median 40.4) vs < 7.5 (median 2.7) g/d	407	0.87 (0.64, 1.19)	No sig. assoc., P -trend 0.66
Larsson, 2011, Sweden	Prospective cohort	Fish, W	Servings/wk, 5 cat	> 3.0 /wk vs < 1.0 /wk,	1310	0.87 (0.73, 1.04)	No sig. assoc.- borderline protective in cat 2-5, P -trend 0.19
Montonen, 2009, Finland	Prospective cohort	Fish, M/W	g/d, quartiles	Quartile 4 vs 1, 72 vs 6 (median values)	364	0.99 (0.73, 1.35)	No sig. assoc., P -trend 0.96
Shao, 2021, China	Prospective cohort	Fish, M/W	Servings/wk, 4 cat	≥ 11 vs 0-3 servings/wk	111	0.70 (0.40, 1.22)	Null, p -trend 0.21

Author, year, country	Study design*	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low (95 % CI)	Overall result
Wennberg, 2016, Sweden	Case-control, nested	Fish, M/W	Times/wk	Continuous	607	1.02 (0.92, 1.13)	No sig. assoc.

Among the eight studies, there was one report of a statistically significant protective association, and no reports of a statistically significant adverse association (see Chapter 4.5.2.7 for the overall summary estimate).

4.5.3.3 Studies of total fish intake and hemorrhagic stroke incidence

We included seven observational studies (six prospective cohorts and one nested case-control) and seven estimates (sex-specific, or for men and women combined) of the association between total fish intake and hemorrhagic stroke. Table 4.5.3.3-1 shows the exposure levels and results in these six.

Table 4.5.3.3-1 Results from studies included in the weight of evidence analysis of total fish intake and hemorrhagic stroke incidence.

Author, year, country	Study design*	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low (95 % CI)	Overall result
Bernstein, 2012, USA	Prospective cohort	Fish, M/W	Servings/d, cumulative average, quintiles	Quintile 5 vs 1	400	0.79 (0.55, 1.12)	No sig. assoc., <i>P</i> -trend 0.38
Hengeveld, 2018, the Netherlands	Prospective cohort	Fish, M/W	Portions/wk, 3 cat	≥1/wk vs none, 28.7 g/wk fatty and 93.7 g/wk lean (median values)	220	0.87 (0.66, 1.15)	No sig. assoc.
Kuhn 2013, Germany	Prospective cohort	Fish, M/W	g/d, quintiles	Quintile 5 vs 1, >31.1 (median 40.4) vs <7.5 (median 2.7) g/d	95	1.46 (0.77, 2.78)	No sig. assoc., <i>P</i> -trend 0.16
Larsson, 2011, Sweden	Prospective cohort	Fish, W	Servings/wk, 5 cat	>3.0 vs <1.0/wk	233	0.67 (0.42, 1.08)	No sig. assoc. Borderline protective in cat 5, <i>P</i> -trend 0.08

Author, year, country	Study design *	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low (95 % CI)	Overall result
Montonen, 2009, Finland	Prospective cohort	Fish, M/W	g/d, quartiles	Quartile 4 vs 1, 72 vs 6 (median values)	80	1.23 (0.63, 2.42)	No sig. assoc., <i>P</i> -trend 0.41
Shao, 2021, China	Prospective cohort	Fish, M/W	Servings/wk, 4 cat	≥11 vs 0-3 servings/wk	97	1.04 (0.59, 1.81)	Null, <i>P</i> -trend 0.72
Wennberg, 2016, Sweden	Case-control, nested	Fish, M/W	Times/wk, continuous		95	0.84 (0.63, 1.11)	No sig. assoc.

Among the seven prospective observational studies analyzing the association between total fish intake and hemorrhagic stroke, there was one report of a suggestive linear trend in the protective direction (*P*-trend 0.08). No adverse association was reported (see Chapter 4.5.2.7 for the overall summary estimate).

4.5.3.4 Studies of fatty fish intake and total stroke incidence

We included seven prospective, observational studies (six cohorts and one nested case-control) with nine estimates (sex-specific, or for men and women combined) of the association between fatty fish intake and total stroke. Table 4.5.3.4-1 shows the exposure levels and results in these six.

Table 4.5.3.4-1 Results from studies included in the weight of evidence analysis of fatty fish intake and total stroke incidence.

Author, year, country	Study design	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low (95 % CI)	Overall result
Amiano, 2016, Spain	Prospective cohort	Fatty fish, M	g/d, quintiles	Quintile 5 vs 1, ≥34.9 vs <2.6 g/d	373	0.97 (0.67, 1.42)	No sig. assoc., <i>P</i> -trend 0.65
		Fatty fish, W	g/d, quintiles	Quintile 5 vs 1, ≥22.7 vs <1.6 g/d	301	1.30 (0.87, 1.94)	No sig. assoc., <i>P</i> -trend 0.14

Author, year, country	Study design	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low (95 % CI)	Overall result
Atkinson, 2011, UK	Prospective cohort	Fatty fish, M	Residuals from regression on energy intake, most recent FFQ, 5 cat	Quintile 5 vs 1	225	0.69 (0.43, 1.11)	No sig. assoc., <i>P</i> -trend 0.13
Bonaccio, 2017, Italy	Prospective cohort	Fatty fish, M/W	Times/wk: 3 cat	≥1 vs none, 24.2 vs 0 g/d	66	0.69 (0.24, 1.94)	No sig. assoc.
Hengeveld, 2018, the Netherlands	Prospective cohort	Fatty fish, M/W	Portions/wk, 3 cat	≥1/wk vs none, 10.7 g/wk (median) in consumers	753	0.64 (0.45, 0.92)	Protective assoc. of ≥1 vs null portion/wk
Larsson, 2011, Sweden	Prospective cohort	Fatty fish (salmon, whitefish, char), W	Servings/mo or wk, 4 cat	≥3.0 wk vs 0/wk	1680	0.93 (0.59, 1.48)	Sig. protective assoc. of 1-3 servings/mo vs 0, no sig. trend (<i>P</i> =0.33), possible threshold
	Prospective cohort	Fatty fish (herring and mackerel), W	Servings/mo or wk, 4 cat	≥3.0/wk vs 0/wk	1680	0.94 (0.68, 1.29)	No sig. assoc., <i>P</i> -trend 0.57
Wennberg, 2016, Sweden	Case-control, nested	Fatty fish, M/W	Times/mo or wk, 4 cat	>2/wk vs <1/mo	720	0.97 (0.57, 1.65)	No sig. assoc.
Tong, 2021, Europe	Prospective cohort, multicenter	Fatty fish, M/W	g/d, quintiles	Quintile 5 vs 1, ≥16.4, 27.8 (median) vs <0.8, 0.0 (median) g/d	7100	0.98 (0.91, 1.06)	No sig. assoc., <i>P</i> -trend 0.38

Among the seven prospective observational studies analyzing the association between fatty fish intake and total stroke, there were two reports of a statistically significant protective association. No adverse associations were reported (see Chapter 4.5.2.7 for the overall summary estimate).

4.5.3.5 Studies of lean fish intake and total stroke incidence

We included seven observational studies (six prospective cohorts and one nested case-control) and seven estimates (sex-specific, or for men and women combined) of the association between lean fish intake and total stroke. Table 4.5.3.5-1 shows the exposure levels and results in these six studies.

Table 4.5.3.5-1 Results from studies included in the weight of evidence analysis of lean fish intake and total stroke incidence.

Author, year, country	Study design*	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low (95 % CI)	Overall result
Amiano, 2016, Spain	Prospective cohort	Lean fish, M	g/d, quintiles	Quintile 5 vs 1, ≥ 68.9 vs < 7.1 g/d	373	0.84 (0.55, 1.29)	Suggestive protective assoc., P -trend 0.06
		Lean fish, W	g/d, quintiles	Quintile 5 vs 1, ≥ 46.4 vs < 0.8 g/d	301	1.03 (0.65, 1.65)	No sig. assoc., P -trend 0.56
Atkinson, 2011, UK	Prospective cohort	Lean fish, M	Residuals from regression on energy intake, most recent FFQ, 5 cat	Quintile 5 vs 1	225	0.90 (0.55, 1.46)	No sig. assoc., P -trend 0.97
Bonaccio, 2017, Italy	Prospective cohort	Lean fish, M/W	Times/wk, 3 cat	≥ 1 vs none, 30.9 vs 0 g/d	66	0.91 (0.30, 2.75)	No sig. assoc.
Hengeveld, 2018, the Netherlands	Prospective cohort	Lean fish, M/W	Portions/wk, 3 cat	≥ 1 /wk vs none, 32.9 g/wk (median) in consumers	753	0.92 (0.79, 1.07)	No sig. assoc.
Larsson, 2011, Sweden	Prospective cohort	Lean fish, W	Servings/mo or wk, 4 cat	≥ 3.0 /wk vs 0/wk	1680	0.67 (0.49, 0.93)	Sig. protective assoc. of ≥ 3.0 /wk vs 0/wk, P -trend 0.07
Wennberg, 2016, Sweden	Case-control, nested	Lean fish, M/W	Times/ mo or wk, 4 cat	> 2 /wk vs < 1 /mo	725	0.99 (0.63, 1.54)	Borderline protective assoc. in categories ≤ 2 times/wk vs < 1 /mo

Author, year, country	Study design*	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low (95% CI)	Overall result
Tong, 2021, Europe	Prospective cohort, multicentre	Lean fish, M/W	g/d, quintiles	Quintile 5 vs 1, ≥ 16.4 , 27.8 (median) vs < 0.8 , 0.0 (median) g/d	7100	0.98 (0.91, 1.06)	No sig. assoc., P -trend 0.38

Among the seven prospective observational studies analyzing the association between lean fish intake and total stroke, there was one report of a statistically significant protective association, and no reports of a statistically significant adverse association (see Chapter 4.5.2.7 for the overall summary estimate).

4.5.3.6 Studies of fish intake and stroke risk in patient populations

In one study (tree estimates) of total fish intake and risk of stroke in patients with a history CVD or at high risk of CVD (Mohan et al. 2021), associations were inconsistent (borderline significant for adverse or protective associations, or no association). One study in T2D patients (Wallin et al 2018) found no associations for intake of total fish, fatty fish, or lean fish with risk of stroke.

Table 4.5.3.6-1 Results from prospective observational studies included in weight of evidence analysis for fish intake and risk of stroke in patients.

Author, year, country	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low (95 % CI)	Overall result
Prior CVD or high risk						
Mohan, 2021, global, 6 continents, 58 countries	Fish, incl shellfish, M/W, PURE	g/mo or wk, 4 cat	≥350 g/wk vs <50 g/mo, median 594 vs 0.1 g/wk	NA (CVD history), 3925 (all)	0.91 (0.66, 1.27)	No sig. assoc., <i>P</i> -trend 0.22
	Fish, incl shellfish, M/W, ONTARGET, TRANSCEND	g/mo or wk, 4 cat	≥350 g/wk vs <50 g/mo, median 450 vs 2.8 g/wk	1395	1.25 (1.00, 1.58)	Borderline adverse assoc. in highest category only, <i>P</i> -trend 0.20
	Fish, incl shellfish, MW, ORIGIN	g/o or wk, 4 cat	≥350 g/wk vs <50 g/mo, median 568 vs 2.2 g/wk	533	0.82 (0.62, 1.09)	Borderline protective assoc. in two lowest, but not highest category, <i>P</i> -trend 0.09
Diabetes population						
Wallin, 2018, Sweden	Total fish, M/W, diabetics	Servings/mo or wk, 4 cat	>3/wk vs ≤3/mo, 3.5 vs 0.5 servings/wk (median)	321	1.04 (0.66, 1.64)	No sig. assoc., <i>P</i> -trend 0.86
	Fatty fish (herring and mackerel), M/W, diabetics	Servings/mo or wk, 3 cat	≥1/wk vs <1/mo, 1.5 vs 0 servings/wk (median)	321	0.89 (0.63, 1.25)	No sig. assoc., <i>P</i> -trend 0.67
	Fatty fish (salmon, whitefish, char), M/W - diabetics	Servings/mo or wk, 3 cat	≥1/wk vs <1/mo, 1.5 vs 0 servings/wk (median)	321	1.05 (0.71, 1.55)	No sig. assoc., <i>P</i> -trend 0.80
	Lean fish (cod, saithe, and fish fingers), M/W, diabetics	Servings/mo or wk, 3 cat	≥1/wk vs <1/mo, 1.5 vs 0 servings/wk (median)	321	0.94 (0.64, 1.39)	No sig. assoc., <i>P</i> -trend 0.71

4.5.3.7 Summary relative risks (RR) based on VKM's inclusions of primary studies

VKM's summary RR for the highest versus lowest intake of total fish in relation to total stroke (Table 4.5.3.1-1) indicated a significant protective association (RR=0.92, 95% CI: 0.89, 0.95) without significant heterogeneity (15 studies, $P_{\text{heterogeneity}}=0.67$). One prospective study (Wennberg et al., 2016) reporting odds ratios (ORs) was included with reported hazard ratios (HRs) in the summary RR and heterogeneity analysis.

Despite some differences in the selection of studies compared with previous meta-analyses (as described below), the summary RR (high-low analysis) based on VKM's study selection was almost identical to the result in the previous meta-analysis by Zhao et al. (2019) (RR=0.90, 95% CI: 0.85, 0.96) and slightly lower (more protective) than the results in Bechthold et al. (2019) (RR=0.95, 95% CI: 0.89, 1.01). Xun et al. (2012) did not perform a high-low meta-analysis, but the summary estimates indicated a protective association. Chen et al 2021 was the most recent meta-analysis but only based on seven studies (RR=0.87, 95% CI: 0.78, 0.97).

Among the previous meta-analyses, the eligibility criteria in Bechthold et al. (2019) were similar to VKM's criteria (studies of stroke incidence, but not mortality). In a re-analysis of Bechthold et al. (2019), VKM added two primary studies of stroke incidence (Bonaccio et al., 2017; Hengeveld et al., 2018) not covered by Bechthold et al. (2019). The addition of studies had little impact on the summary result in Bechthold et al. (RR=0.95 before, and RR=0.94, 95% CI 0.89, 1.00 after VKM added studies) using a random effects model.

For ischemic stroke, VKM's summary RR for the highest versus lowest intake of total fish (Table 4.5.3.2-1) indicated a protective effect (RR= 0.93, 95% CI: 0.86, 1.02) without significant heterogeneity (eight studies, $P_{\text{heterogeneity}}=0.27$), quite similar to total stroke. The only high-low meta-analysis total fish intake and ischemic stroke was Zhao et al. (2019). Their summary RR was consistent with VKM's estimate, but slightly weaker (RR=0.96, 95% CI: 0.89, 1.03). As previously mentioned, Zhao et al. (2019) combined estimates of incidence and mortality.

For hemorrhagic stroke, VKM's summary RR for the highest versus lowest intake of total fish (Table 4.5.2.3-1) showed an association on the protective side, but not statistically significant (RR=0.88, 95% CI: 0.71, 1.09) and without significant heterogeneity (seven studies, $P_{\text{heterogeneity}}=0.29$). Zhao 2019 included a high-low summary estimate for hemorrhagic stroke that was quite similar (RR=0.88, 95% CI: 0.75, 0.96).

The summary RR was also calculated for the risk of total stroke in relation to the highest versus lowest intake of fatty fish (Table 4.5.2.4-1). VKM's summary RR was on the protective side, but not statistically significant (RR=0.92, 95% CI 95%: 0.80, 1.05) and without significant heterogeneity (seven studies, $P_{\text{heterogeneity}}=0.21$). Larsson et al. (2011) provided estimates for two categories of fatty fish (salmon, whitefish and char vs. herring and mackerel). Amounts were unavailable, and the estimate for herring and mackerel was chosen for the pooled effect to not include the same study population twice. In comparison,

the summary RR from previous high-low meta-analyses of stroke and fatty fish was 0.89 (95% CI: 0.79, 1.01) in Zhao et al. (2019) and 0.88 (95% CI: 0.75, 1.04) in Qin et al. (2018).

The summary RR for stroke in relation to the highest versus lowest intake of lean fish (seven studies, Table 4.5.2.5-1) suggested a weak protective association (RR=0.95, 95% CI: 0.89, 1.01) without significant heterogeneity ($P_{\text{heterogeneity}} = 0.51$). The summary RRs from previous high-low meta-analyses of lean fish with total stroke were slightly stronger: RR=0.84 (95% CI: 0.70, 1.00) in Zhao 2019 and RR=0.81 (95% CI: 0.67, 0.99) in Quin et al. (2018).

The summary RR for total fish and total stroke in one study of patient populations with a history or at high risk of CVD from vascular disease (3 estimates, Table 4.5.3.6-1) was close to unity, but with borderline significant heterogeneity (RR=0.99, 95% CI: 0.75, 1.30, $P_{\text{heterogeneity}} = 0.06$).

4.5.3.8 VKM's search compared to previous meta-analyses

The six included meta-analyses of fish intake and stroke (Xun et al., 2012; Bechthold et al., 2019; Qin et al., 2018; Zhao et al., 2019; Jayedi et al., 2020) except Chen et al. 2021 included some papers not included by VKM. Table 4.5.3.8-1 shows the overlap and differences in papers included.

Of 16 prospective studies in Xun et al. (2012), only one (Kurth et al., 2011) was not identified by VKM, but five studies were summarized by VKM under stroke mortality (Sauvaaget al., 2003; Folsom et al., 2004; Nakamura et al., 2005; Yamagishi et al., 2008). Xun et al. 2012 was the oldest meta-analysis and VKM identified several primary studies published after Xun et al. (Bernstein et al., 2012; Amiano et al., 2016; Bonaccio et al., 2017; Hengeveld et al., 2018).

Qin et al. (2018) included five prospective cohort studies looking at fatty fish, all were identified by VKM (Amiano et al., 2016; Atkinson et al., 2011; Bonaccio et al., 2017; Larsson et al., 2011; Myint et al., 2006). Qin et al. (2018) included four prospective cohort studies on lean fish, all were also identified (Amiano et al., 2016, Atkinson et al., 2011, Bonaccio et al., 2017, Larsson et al., 2011). We included an additional primary study on fatty fish and lean fish (Hengeveld et al., 2018) published after Qin et al. (2018).

Bechthold et al. (2019) included 20 prospective studies, of which four were not included by VKM (Misirli et al., 2012; Tognon et al., 2014; Haring et al., 2016; Hansen et al., 2017). Misirli et al. (2012) and Hansen et al. (2017) appeared in our search but did not fulfil our inclusion criteria. The papers by Tognon et al. (2014) and Haring et al. (2016) did not appear in our literature search. In our systematic review we included three studies not included in this meta-analysis, one older study (Kelli et al., 1994) and two studies published after 2017 (Bonaccio et al., 2017; Hengeveld et al., 2018).

Zhao et al. (2019) included 31 prospective studies and covered most studies in the above-mentioned meta-analyses by Bechthold et al. (2019) and Xun et al. (2012) and those identified by VKM. Zhao et al. (2019) included studies of both stroke incidence and mortality

which party explains the higher number of studies. VKM identified one study (Hengeveld et al. 2018) not covered by Zhao et al. (2019).

Chen et al. (2021) was the most recent meta-analysis but not the most comprehensive, seven studies were included on stroke incidence or mortality (one study), all were identified by VKM.

Table 4.5.3.8-1 Overview of papers included in VKM's literature review of fish and stroke incidence compared with previous meta-analyses.

	Included by VKM			Included in meta-analyses			
Publications	Total stroke	Hemorrhagic	Ischemic	Chen, 2021	Zhao, 2019	Bechthol, 2019	Xun, 2012
Amiano, 2016	X		X	X	X	X	na
Atkinson, 2011	X				X	X	na
Bernstein, 2012	X	X	X		X	X	na
Bonaccio, 2017	X				X	na	na
Gillum, 1996	X				X	X	X
Hengeveld, 2018	X	X	X		na	na	na
Keli, 1994	X				X	X	X
Kuhn, 2013	X	X	X	X	X	X	
Larsson, 2011	X	X	X		X	X	X
Montonen, 2009	X	X	X	X	X	X	X
Morris, 1995	X			X		X	
Mozaffarian, 2005b	X	X	X	X	X	X	X
Myint, 2006	X				X	X	X
Nahab, 2016			X		X	X	na
Orencia, 1996	X				X	X	X
Wennberg, 2016	X	X	X			X	na
Overlapping							
de Goede, 2012	X	X	X	X	X	X	
He, 2002	X	X	X				X
Iso, 2001	X	X	X				X
Papers in meta-analyses							
Farvid, 2017 (mortality)					X		na
Folsom, 2004 (mortality)					X		X
Hansen, 2017					X	X	na
Haring, 2015					X	X	na
Kaushik, 2008					X		
Kinjo, 1999 (mortality)					X		
Kurth, 2011							X
Misirli, 2012					X	X	na
Moms, 1995					X		
Nakamura, 2005 (mortality)					X		X

	Included by VKM			Included in meta-analyses			
Sauvaguet, 2003 (mortality)					X		X
Takachi, 2010					X		
Takata, 2013 (mortality)					X		na
Tognan, 2014					X	X	na
Wallin, 2018 (T2D)					X	na	na
Yamagishi, 2008 (mortality)				X	X		X
Yuan, 2001 (mortality)					X		X
Zhuan, 2018 (mortality)					X	na	na
<i>Studies included</i>	15	7	9		31	20	16
<i>Studies evaluated</i>	18	10	12				

*de Goede is covered by Hengeveld et al. (2018); He et al. (2002) and Iso et al. (2001) are covered by Bernstein et al. (2012).

Jayedi et al. (2020) was limited to patients with T2D and included two studies that were also identified by VKM (Deng et al., 2018; Wallin et al., 2018).

4.5.4 Heterogeneity fish intake and stroke incidence

Previous meta-analyses of fish intake and stroke risk have found overall protective associations (Chen et al. 2021; Zhao et al. 2019; Bechthold et al. 2017; Qin et al. 2018; and Xun et al. 2012) with low to moderate ($I^2 < 40\%$) between study heterogeneity (Table 4.5.1.2-1). The meta-analysis by Jayedi et al. (2020) in patients with T2D found a I^2 higher than 50%, indicating potentially important heterogeneity, but based on few studies. VKM found no significant heterogeneity between included studies of total fish, fatty fish or lean fish intake and incident stroke risk in the general population. Most primary studies have estimates on the protective side or close to null, but within the range of effects, there is still some heterogeneity. Zhao et al. (2019) identified stroke subtype, sex and geographic region as sources of heterogeneity. Heterogeneity is high in-patient studies, but studies are few.

4.5.5 Dose-response relationship fish intake and stroke incidence

Both Zhao et al. (2019) and Bechthold et al. (2019) performed meta dose-response analyses of fish consumption and risk of stroke.

Zhao et al. (2019) reported no significant departure from linearity (31 studies, $P=0.45$). Similarly, Bechthold et al. (2019) found no departure from linearity (15 studies, $P=0.37$). The risk of stroke decreased by 12-14% per 100 g daily increase in fish intake for the observed intake ranges.

Consistent with meta-analyses, the primary study by Kuhn et al. (2013) concluded with a linear relationship (German men and women). Two other primary studies in US men (He et

al., 2002) and Swedish women (Larsson et al., 2011) suggested a threshold where the risk reduction was most pronounced at lower intakes (between no intake and 1-2 servings per week based on visual inspection of the curves).

4.5.6 Weight of evidence for fish intake and stroke incidence

In this section, the evidence of the association between fish intake and stroke incidence is weighed according to the WCRF criteria presented in Chapter 3.1.6 (Box 2).

Published evidence of fish intake and stroke

VKM's summary RR for total fish and total stroke in 15 studies, shows a protective association that is statistically significant and supported by previous meta-analyses (Zhao et al. 2019; Bechthold et al. 2019). There was large overlap between the primary studies included in these two meta-analyses and by VKM, especially for Bechthold et al (2019). VKM included two studies published after 2017 (Bonaccio et al. 2017, Hengeveld et al. 2018) which were not included in Bechthold et al. (2019), but summary estimates were little affected by the addition of these studies.

When it comes to stroke type (hemorrhagic and ischemic) the results are less clear. The newest meta-analysis from 2019 (Zhao et al 2019) found a significant protective association between fish consumption and hemorrhagic stroke, and a borderline significant protective association with ischemic stroke, but incorporated studies of both stroke incidence and mortality. VKM's summary RRs for total fish intake by stroke sub-types were quite similar for ischemic and hemorrhagic stroke, but was less precise (wider CI) for hemorrhagic stroke.

When it comes to type of fish (fatty-lean) and risk of stroke, the most comprehensive meta-analysis (Zhao et al 2019) found suggestive protective associations for both fatty and lean fish. Similarly, the summary RRs calculated by VKM did not support differential effects of fatty and lean fish.

Patient studies remain limited. One global multicenter study (Mohan et al. 2021) reported heterogenous associations.

Heterogeneity

Some heterogeneity was observed between studies in the included meta-analyses. There was no significant heterogeneity between studies included in VKM's summary RRs for total fish, fatty fish or lean fish, with total stroke or stroke sub-types in the general population. Limited patient studies show heterogenous results.

Mechanism / biological plausibility

There is evidence for several plausible mechanisms operating in humans.

Upgrading factors

Two independent meta-analyses have reported linear, inverse dose-response relationships.

4.5.6.1 Conclusion weight of evidence fish intake and stroke incidence

There is evidence from more than two independent and good quality cohort studies on total fish intake (VKM included 15 publications on the general population, two in patients, and six meta-analyses including dose-response analyses).

VKM's summary RR for primary studies in the general population shows statistically significant lower risk of incident stroke for the highest versus lowest intake of total fish and is supported by independent meta-analyses. The direction of the effect is generally consistent. There is evidence for biological plausibility and a dose-response relation.

In conclusion, the evidence that consumption of fish reduces risk of total stroke is graded "probable". Due to limited and inconsistent evidence on patient populations, no conclusions can be drawn for risk of incident stroke in patients with a history of CVD or at high risk of CVD, or in patients with T2D.

The evidence is graded "limited, suggestive" for a protective effect of total fish on sub-types of stroke (ischemic or hemorrhagic) and also "limited, suggestive" for a protective effect of fatty fish and lean fish on total stroke, due to fewer studies and results that are not statistically significant.

4.6 Fish intake and other CVD outcomes

The current section summarizes the epidemiological evidence on fish intake and risk of CVD outcomes identified in the literature search that have not been summarized in previous sections, that is heart failure (HF), atrial fibrillation (AF) and venous thromboembolism (VTE). Due to few studies per outcome, they are summarized within the same chapter.

Heart failure may be fatal or non-fatal depending on stage. Some studies have focused on hospitalized HF, whereas other studies have included earlier stages that may have been diagnosed and treated in outpatient care. We have included both. The onset of AF is rarely fatal. Thus, studies of incident AF can be assumed to include predominantly non-fatal events. VTE comprises deep vein thrombosis and pulmonary embolism and may be fatal or non-fatal. VTE is commonly classified as unprovoked or provoked, i.e. subsequent to provoking clinical conditions such as surgery.

4.6.1 VKM's search on previous systematic reviews and meta-analyses or on fish intake and other CVD outcomes

VKM's search for systematic reviews and meta-analyses (see Chapter 3.1.2.2) identified seven publications on the association between fish intake and other CVD outcomes that fulfilled the inclusion criteria and were read as full papers. Five papers were excluded, see Table 4.6.1-1 for reason for exclusions.

Table 4.6.1-1 Reasons for exclusion of identified papers from the search of systematic reviews and meta-analyses of fish intake and other CVD outcome 2016-2021.

Included papers	Excluded paper and reasons for exclusions
Bechthold et al., 2019 (heart failure) Li et al., 2017 (atrial fibrillation)	Kerley et al., 2017 (HF): Not duplicate study selection in the review. Graded C. Kerley et al., 2019 (HF). Graded C. Kromhout et al 2016: Type of umbrella review. No search strategy was presented, no description of quality assessment. Graded C. Mattiuzzi et al., 2016 (VTE): Graded C. Umesawa et al., 2020: Narrative review. Target group -was only Japanese population.

4.6.1.1 Description of the identified publications on heart failure (HF)

Bechthold et al. (2019) conducted literature searches in PubMed and Embase through March 2017 and included eight prospective observational studies on fish intake and heart failure. The study by Bechthold et al. (2019) had a high quality (AMSTAR assessment done by Jayedi and Shab-Bidar (2020) assigned 10 out of 11 points). The quality of the meta-evidence of fish intake and HF (based on n=7 studies) was rated moderate based on the NutriGrade score (NutriGrade scoring were done by Bechthold et al. (2019)).

4.6.1.2 Description of the identified publications on atrial fibrillation (AF)

Li et al. (2017) conducted literature searches in PubMed and Embase from inception to 18 May 2017. The quality of the eligible papers included in the meta-analysis was assessed by the 9-point Newcastle-Ottawa Scale criteria. Six prospective observational studies on fish intake and atrial fibrillation were included in their meta-analyses. The quality of all the papers included in the meta-analysis were overall of high quality (scoring 7-8). The AMSTAR tool was used to assess the methodological quality of Li et al. (2017), and the study was found to have a moderate-low quality (AMSTAR assessment done by VKM project group and assigned quality level B).

4.6.1.3 Results from the meta-analyses

Below is a summary table for meta-analyses on fish intake and risk of other CVD outcomes.

Table 4.6.1.3-1 Summary of results from meta-analyses on fish intake and risk of other CVD outcomes.

Author, year	Type of studies included	Total no studies	No of cases	Comparison	Summary RR (95 % CI)	Hetero-geneity	Overall conclusion
Becthold, 2017	Prospective studies (heart failure)	8	7945	Highest vs lowest	0.89 (0.80 to 0.99)	$I^2=18\%$	Fish intake is associated with a decreased risk of HF. The quality of the meta-evidence was rated moderate
				Per 100 g	0.80 (0.67 to 0.95)	$I^2=20\%$	
Li, 2017	Prospective studies (atrial fibrillation)	6	6355	Highest vs lowest	1.01 (0.94 to 1.09)	$I^2=0\%$	No sig. assoc.

4.6.2 VKM's systematic review of primary studies on fish intake and incidence of other CVD outcomes

4.6.2.1 Included studies from search on other CVD outcomes

A total of eight publications included incident heart failure as outcome, of which four focused on hospitalized cases (Belin et al., 2011; Levitan et al., 2010; Levitan et al., 2009; Nettleton et al., 2008), and three included cases in different stages of inpatient and/or outpatient care (Dijkstra et al., 2009; Mozaffarian et al., 2005a; Wilk et al., 2012; Zhang et al. 2021). Wilk et al. (2012) used a self-reported, validated diagnosis of HF, else the diagnoses involved clinical data and/or data confirming treatment.

Six publications included incident atrial fibrillation (Berry et al., 2010; Brouwer et al., 2006; Gronroos et al., 2012; Larsson et al., 2017; Shen et al., 2011; Zhang et al. 2021), and three incident venous thromboembolism (Hansen-Krone et al., 2014; Lutsey et al., 2009; Zhang et al. 2021).

The publications were from unique studies without overlap, so all were retained for further analysis. Study characteristics are shown in Table 4.6.2.1-1.

Table 4.6.2.1-1 Overview of primary studies included in weight of evidence analysis of fish intake and other CVD outcomes (incident heart failure, atrial fibrillation, and venous thromboembolism).

Author, year, country	Study name	Study design	Inclusion year(s), end, follow-up time	Study population	Dietary assessment method	Dietary assessment period
Heart failure						
Belin, 2011, USA	Women's Health Initiative Observational Study (WHI-OS)	Prospective observational	1993-1998 to 2008, 10 yrs follow-up (average)	84 493 postmenopausal women, 50-79 yrs	FFQ -WHI, validated	NA, baseline intake
Dijkstra, 2009, the Netherlands	Rotterdam Study	Prospective observational	1993-1997 to 2006, 10.5 yrs follow-up (median)	2164 men and 3135 women, ≥55 yrs (mean age of 67.5 yrs)	FHI by interview (assumed face to face), semi-quant, validated	NA, baseline intake, probably previous year
Levitan, 2009, Sweden	Cohort of Swedish Men (COSM)	Prospective observational	1997 to 2004, 7 yrs follow-up (median)	39 367 men, 45-79 yrs	FFQ, validated	Average frequency during the previous year, at baseline
Levitan, 2010, Sweden	Swedish Mammography Cohort (SMC)	Prospective observational	1998 to 2006, 9 yrs of follow-up	36 234 women, 48-83 yrs	FFQ, validated	Usual freq of consumption in previous year, at baseline
Mozaffarian, 2005a, USA	Cardiovascular Health Study	Prospective observational	1989-90 to 2001, 12 yrs follow-up	4738 men and women, ≥65, mean age 73 yrs	FFQ, picture sort version of the National Cancer Institute FFQ	Average intake during the previous year, at baseline
Nettleton, 2008, USA	Atherosclerosis Risk in Communities (ARIC) Study	Prospective observational	1987–1989 to 2003, 13.3 yrs follow-up (mean)	14 153 (55% female) African-American (25%) and white men and women, 45-64 yrs	Repeated FFQ, semi-quant, by interview - modified from validated Willett 61-item FFQ	Not specified, probably usual intake
Wilk, 2012, USA	Physicians' Health Study (PHS) I + II	Prospective observational	1997-2001 to 2010, approx 8 yrs of follow-up	18 968 male health professionals, 67 yrs (mean)	FFQ, semi quant, validated	NA, baseline intake
Atrial fibrillation						
Berry, 2010, USA	Women's Health Initiative (WHI) clinical trials	Prospective observational	1993-1998 to 2008, 6 yrs follow-up	44 720 postmenopausal women, 50-79 yrs	FFQ -WHI, validated	NA, baseline intake

Author, year, country	Study name	Study design	Inclusion year(s), end, follow-up time	Study population	Dietary assessment method	Dietary assessment period
Brouwer, 2006, the Netherlands	Rotterdam Study	Prospective observational	1990-1993 to 1999; 6.4 yrs follow-up (mean)	5184 (40.6% males), ≥55 yrs (mean age 67.4 yrs)	FHI by interview (assumed face to face), semi-quant, validated	NA, baseline intake
Gronroos, 2012, USA	Atherosclerosis Risk in Communities (ARIC) Study	Prospective observational	1987–1989 to 2008, 17.6 yrs follow-up (mean)	14 222 men (55%) and women, 45-64 yrs (mean age 54 yrs)	Repeated FFQ (baseline 1987–1989 and 1993–1995) by interview - modified from validated Willett 61-item FFQ with biomarker DHA and EPA in subsample	Past year
Larsson, 2017, Sweden	Cohort of Swedish Men (COSM) and the Swedish Mammography Cohort (SMC)	Prospective observational	1997 to 2009, 12 yrs of follow-up	72 984 (38,960 men and 34 024 women), 45-83 yrs	FFQ, semi quant, validated in men	Average frequency during the previous year, at baseline
Shen, 2011, USA	Framingham Heart Study (FHS). Original and Offspring cohort	Prospective observational	1986–1989, 1991–1993, 1991–1995, 1996–1997, 1998–2001 (inclusion yrs), 4 yrs follow-up	4526 men and women (56%), ≥45 yrs, mean age 62 yrs	FFQ, semi-quant, validated	Past year
Venous thromboembolism						
Hansen-Krone, 2014, Norway	Tromsø IV study	Prospective observational	1994-95 to 2010, 15.8 yrs follow-up (median)	23 621 (47.6% males), 25-97 yrs (mean age 47 yrs)	Self-administered questionnaire, food frequency	Usual intake at baseline
Lutsey, 2009, USA	Iowa Women's Health Study (IWHS)	Prospective observational	1986 to 2004, 19 yrs. follow-up (median 13 yrs)	37 393 older women, 55–69 yrs, mean age 62 yrs (baseline), survivors enrolled in follow-up at 65 yrs	FFQ, semi quant, validated (from Willett)	Usual intake, at baseline
All outcomes (HF, AF, VTE)						

Author, year, country	Study name	Study design	Inclusion year(s), end, follow-up time	Study population	Dietary assessment method	Dietary assessment period
Zhang, 2021, UK	UK Biobank	Prospective cohort	2006-2010 to 2020, follow-up 11.2 yrs (median)	462 155 (44% male), 40-69 yrs, mean 56.7 yrs	Touchscreen FFQ	NA, probably usual intake at baseline

4.6.2.2 Study description by design, geographic region, and sex

The body of evidence (eight publications) on heart failure was prospective, observational studies (cohort or cohort based on RCT) with four conducted in US populations (Belin et al., 2011, Mozaffarian et al., 2005a, Nettleton et al., 2008, Wilk et al., 2012) and four in European populations in Sweden (Levitan et al., 2009, Levitan et al., 2010), the Netherlands (Dijkstra et al., 2009), and a large UK population (Zhang et al. 2021). One study (Women's Health Initiative) was conducted in postmenopausal women (Belin 2011), and one study (Physicians' Health Study) only included male health professionals, and one study (Atherosclerosis Risk in Communities (ARIC) Study) included around 25% African Americans.

The body of evidence on atrial fibrillation (AF) was similar to that on heart failure. All publications except one (Shen et al., 2011, Framingham Heart Study) were based on studies that were also used to examine heart failure (Atherosclerosis Risk in Communities (ARIC) Study; Cohort of Swedish Men (COSM) and Swedish Mammography Cohort (SMC); Rotterdam Study; Women's Health Initiative; UK Biobank). All studies on AF had a prospective, observational design (cohort or cohort based on RCT). The Cohort of Swedish Men and the Swedish Mammography Cohort were pooled in the analysis of AF but presented in separate publications on heart failure (Levitan et al., 2009; Levitan et al., 2010).

The three studies of VTE were prospective, observational studies carried out in US women (Lutsey et al., 2009), Norwegian men and women (Tromsø IV study, Hansen-Krone et al., 2014), or UK men and women (UK Biobank, Zhang et al 2021).

None of the studies concerned patients or secondary prevention.

Selected study characteristics (study name, design, time period, size and age of the study population, and dietary assessment method) are presented in Table 4.6.2.1-1.

4.6.2.3 Studies by fish exposure

Of the eight studies of heart failure, four studies (Dijkstra et al., 2009; Nettleton et al., 2008; Wilk 2012; Zhang et al. 2021) included a measure of total fish intake (sum of fish, fish without specifications, or fish including shellfish), two studies included fatty fish only (Levitan et al., 2009, Levitan et al., 2010) and two studies (Belin et al., 2011; Mozaffarian et al., 2005a) categorized fish according to preparation method (fried and non-fried, or fried and baked/broiled).

Of the six studies of atrial fibrillation, five assessed risk in relation to overall fish intake (Brouwer et al., 2006; Gronroos et al., 2012; Larsson et al., 2017; Shen et al., 2011; Zhang et al. 2021), of which three (except Brouwer et al., 2006) also included fatty and lean fish intake. Two studies looked at canned tuna specifically (Gronroos et al., 2012; Shen et al., 2011) and one study (Berry 2010) assessed risk in relation to non-fried fish only (non-fried overall, and non-fried fatty fish). "Dark fish" in Shen et al. (2011) (salmon, swordfish, bluefish, mackerel, and sardines) was classified as fatty fish, and "other fish" (other than

dark fish, and canned tuna) was classified as lean fish. Non-fried fatty fish in Berry et al. (2010) was included in the analysis of fatty fish.

The three studies of venous thromboembolism assessed risk in relation to total fish (Hansen-Krone et al., 2014; Lutsey et al., 2009; Zhang et al. 2021). In Hansen-Krone et al. (2014), fish intake was fish for dinner, which was also categorized as fatty or lean.

4.6.3 Results from the included primary studies fish intake and other CVD outcomes

4.6.3.1 Studies of fish intake and risk of incident heart failure

The exposure levels for total fish (n=4), fatty fish (n=2), and fried or non-fried (n=2) and results in the seven studies of HF are shown below (Table 4.6.3.1-1).

Table 4.6.3.1-1 Results from prospective observational studies included in the weight of evidence analysis of total fish intake and heart failure.

Author, year, country	Fish exposure, sex	Intake unit	High-low intake	Total cases	HR high-low (95% CI)	Overall result
Total fish						
Dijkstra, 2009, the Netherlands	Fish, M/W	g/d, 3 cat	Cat 3 vs cat 1, ≥ 20 vs 0	669	0.96 (0.78, 1.18)	No sig. assoc., P -trend 0.39
Nettleton, 2008, USA	Fish, incl shellfish, M/W	Servings/d	Continuous,	1140	0.99 (0.81, 1.22)	No sig. assoc.
Wilk, 2012, USA	Fish, M	Times/mo or wk, 4 cat	≥ 2 /wk vs < 1 /mo	695	0.72 (0.54, 0.96)	Sig. protective assoc. (30% lower risk) of intake 1-3/mo, 1/wk and ≥ 2 /wk vs < 1 /mo
Zhang, 2021, UK	Total fish, M/W	Times/wk, 4 cat	≥ 3 vs < 1 /wk		0.91 (0.78, 1.07)	Protective assoc. in cat 1-2 above reference, but not highest, P -trend 0.009
Fatty fish						
Levitan, 2009, Sweden	Fatty fish, M	Servings/wk, 5 cat	≥ 3 /wk vs never	597	0.97 (0.61, 1.55)	No sig. assoc.
Levitan, 2010, Sweden	Fatty fish, W	Servings/wk, 4 cat	≥ 3 /wk vs < 1 /wk	651	0.91 (0.59, 1.40)	Protective assoc. of 1 and 2, but not ≥ 3 servings/wk, P -trend 0.049
Non fried fish						
Belin, 2011, USA	Non-fried fish, incl shellfish, W	Servings/mo or wk, 5 cat	≥ 5 /wk vs < 1 /mo	1858	0.70 (0.51, 0.95)	Protective assoc. of ≥ 5 /wk vs < 1 /mo, P -trend 0.022
Mozaffarian, 2005a, USA	Non-fried fish, M/W	Times/mo or wk, 5 cat	≥ 5 /wk vs < 1 /mo	955	0.68 (0.45, 1.03)	Sig. protective assoc. of intake 1-2 times/wk or more, P -trend 0.009
Fried fish						
Belin, 2011, USA	Fried fish, incl shellfish, W	Servings/mo or wk, 3 cat	≥ 1 /wk vs < 1 /mo	1858	1.48 (1.19, 1.84)	Adverse assoc. of ≥ 1 /wk vs < 1 /mo, P -trend 0.005
Mozaffarian, 2005a, USA	Fried fish, M/W	Times/mo or wk, 3 cat	≥ 1 -2/wk vs < 1 /mo	955	1.35 (1.12, 1.62)	Sig. adverse assoc. of intake ≥ 1 -2 times/wk, P -trend 0.005

Overall fish intake was associated with a significantly lower risk of heart failure in one of four studies. This study was conducted in male health professionals. In the two studies of fried and non-fried fish, findings were consistent: intake of non-fried (including baked/broiled) fish was associated with lower risk, and fried fish with a higher risk of heart failure in both studies. These studies were conducted in postmenopausal

women, or in men and women combined. Fatty fish was associated with a statistically significant lower risk (except for the highest intake category) in women, but not in men in the two studies from Sweden.

4.6.3.2 Studies of fish intake and risk of incident atrial fibrillation (AF)

The exposure levels and results in the six studies of atrial fibrillation are shown below (Table 4.6.3.2-1) for total fish (n=5), fatty fish (n=4), lean fish (n=3), canned tuna (n=2), and non-fried (n=1) fish.

Table 4.6.3.2-1 Results from prospective cohort studies included in the weight of evidence analysis of total fish intake and atrial fibrillation.

Author, year, country	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low (95% CI)	Overall result
Total fish						
Brouwer, 2006, the Netherlands	Fish, M/W	g/d, 3 cat	Cat 3 vs cat 1, ≥ 20 vs 0 g/day	312	1.16 (0.84, 1.62)	No sig. assoc.
Gronroos, 2012, USA	Fish, M/W	Servings/wk, 4 cat	>2 vs 0 servings/wk	1604	1.00 (0.81, 1.24)	No sig. assoc., P -trend 0.15
Larsson, 2017, Sweden	Fish, M/W	Servings/mo or wk, 4 cat	≥ 5 servings/wk vs 0-3/mo	6095	1.01 (0.90, 1.13)	No sig. assoc., P -trend 0.65
Shen, 2011, USA	Fish, M/W	Servings/wk, 3 cat	>4 vs never or <1 serving/wk	296	1.25 (0.84, 1.86)	No sig. assoc., P -trend 0.81
Zhang, 2021, UK	Total fish, M/W	Times/wk, 4 cat	≥ 3 vs <1 /wk	NA	1.09 (1.00, 1.18)	Borderline adverse assoc. in two highest categories, P -trend 0.05
Fatty fish						
Berry, 2010, USA	Non-fried fatty fish, W	Servings/wk, quartiles	Quartiles 4 vs 1: ≥ 2 vs <0.5	378	0.92 (0.64, 1.32)	No sig. assoc., P -trend 0.57
Gronroos, 2012, USA	Fatty fish, M/W	Servings/wk, 4 cat	>2 vs 0 servings/wk	1604	0.83 (0.58, 1.21)	No sig. assoc., P -trend 0.44
Larsson, 2017, Sweden	Fatty fish - herring and mackerel, M/W	Servings/mo or wk, 3 cat	≥ 3 /wk vs 0/mo	6095	1.12 (0.95, 1.32)	Suggestive trend for adverse assoc., P -trend 0.05

Author, year, country	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low (95% CI)	Overall result
	Fatty fish – salmon, whitefish, char, M/W	Servings/mo or wk, 3 cat	≥3/wk vs 0/mo	6095	1.14 (0.92, 1.42)	No sig. assoc., (borderline protective for 1-3 servings/month, but not higher intakes), <i>P</i> -trend 0.21
Shen, 2011, USA	Fatty fish, M/W	Servings/wk, 3 cat	>4 vs never or <1 serving/wk	296	6.53 (2.65, 16.06)	Sig. adverse assoc. for highest intake only, <i>P</i> -trend 0.21
Lean fish						
Gronroos, 2012, USA	Lean fish, M/W	Servings/wk	>2 vs 0 servings/wk	1604	0.98 (0.76, 1.27)	No sig. assoc., <i>P</i> -trend 0.68
Larsson, 2017, Sweden	Lean fish, M/W	Servings/mo or wk, 3 cat	≥3/wk vs 0/mo	6095	0.79 (0.65, 0.95)	Protective association of intake ≥ 3/wk vs 0/mo, <i>P</i> -trend 0.19
Shen, 2011, USA	Lean fish, M/W	Servings/wk	>4 vs never or <1 serving/wk	296	1.16 (0.16, 8.29)	No sig. assoc., <i>P</i> -trend 0.16
Canned tuna						
Gronroos, 2012, USA	Preserved, M/W	Servings/wk, 4 cat	>2 vs 0 servings/wk	1604	0.85 (0.68, 1.06)	Suggestive protective, <i>P</i> -trend 0.17
Shen, 2011, USA	Preserved, M/W	Servings/wk, 3 cat	>4 vs never or <1 serving/wk	296	1.81 (0.79, 4.11)	No sig. assoc., <i>P</i> -trend 0.46
Non-fried fish						
Berry, 2010, USA	Non-fried fish, W	Servings/wk, quartiles	Quartiles 4 vs 1, ≥2 vs <0.5	378	1.02 (0.73, 1.42)	No sig. assoc., <i>P</i> -trend 0.916

Total fish was not associated with risk of atrial fibrillation in any of the five studies. A borderline adverse association was observed in one study (Zhang et al. 2021). For fatty fish (three studies) two studies reported either an adverse association (Shen et al., 2011), or a suggestive trend (*P*-trend 0.05) for an adverse association (Larsson et al., 2017), but only for intake of herring and mackerel, not other fatty fish (salmon, whitefish, char). The confidence interval in Shen et al. (2011) was extremely wide due to few cases in the highest intake categories of fatty fish (five cases) and lean fish (one case) and these sub-group analyses were considered exploratory by the authors. For lean fish (three studies) there was one report of a protective association, else null findings. Canned tuna (two studies) was not associated with risk of atrial fibrillation.

4.6.3.3 Studies of fish intake and risk of venous thromboembolism (VTE)

Three studies of venous thromboembolism (VTE, provoked and unprovoked combined) were included. Table 4.6.3.3-1 shows the exposure levels and results. The study from Norway found no significant association between fish intake for dinner (fatty or lean) and risk of VTE in participants that did not take fish oil supplements (stratified analysis). The US study reported a statistically significant adverse association for the highest vs lowest intake level, and the UK study reported a statistically significant protective association.

Table 4.6.3.3-1 Results from prospective cohort studies included in the weight of evidence analysis of total fish intake and venous thromboembolism.

Author, year, country	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low (95% CI)	Overall result
Hansen-Krone, 2014, Norway	Fish (dinner), M/W	Times/wk, 4 cat	≥3 vs <1, ref 1-1.9	536	0.76 (0.44, 1.32), reported as 0.96 (0.66, 1.39) for ≥3 vs 1-1.9/wk and 1.26 (0.84, 1.89) for <1/wk vs 1-1.9/wk	No sig. assoc. (non-users of fish oil supplements, stratified analysis)
Lutsey, 2009, USA	Fish, W	Servings/wk, 5 cat	≥2.5 vs <0.5 servings/wk	1950	1.22 (1.03, 1.46)	Sig. adverse assoc. of intake ≥2.5 vs <0.5 times/wk, <i>P</i> -trend 0.12
Zhang, 2021, UK	Total fish, M/W	Times/wk, 4 cat	≥3 vs <1 time/wk	NA	0.86 (0.76, 0.98)	Sig. protective assoc. in two highest cat above reference, <i>P</i> -trend <0.001

4.6.3.4 Summary relative risks (RR) based on VKM's inclusions of primary studies: heart failure and atrial fibrillation

Heart failure

VKM's summary RR for the highest versus lowest intake of total fish was on the protective side for heart failure (four studies, Table 4.6.3.1-1), and borderline statistically significant (RR=0.91, 95% CI: 0.82, 1.02), and without significant heterogeneity ($P_{\text{heterogeneity}}=0.33$).

Compared to VKM's summary estimate (total fish), the summary RR for heart failure from the high-low meta-analysis by Bechthold et al (2017) was of similar magnitude (RR=0.89, 95% CI: 0.80 to 0.99). Bechthold et al. (2017) included more primary studies, which may explain the increased precision, but the estimate was not limited to total fish: two studies of fatty fish and two studies of fried/non-fried fish as well as one study that did not appear in VKM's search, as described below) were also included. Bechthold et al. (2017) selected the estimates for non-fried fish rather than fried fish in studies that stratified results by preparation method (Belin et al., 2011, Mozaffarian et al., 2005a). The same studies reported statistically significant increased risk of heart failure for fried fish intake ≥ 1 or 1-2 times per week.

Atrial fibrillation

VKM's summary RR for the highest versus lowest intake of total fish in relation to atrial fibrillation (five studies, Table 4.6.3.2-1) suggested an adverse association (RR=1.06, 95% CI: 1.00, 1.13) that was borderline statistically significant without significant heterogeneity ($P_{\text{heterogeneity}}=0.66$).

The summary RR for fatty fish (four studies, Table 4.6.3.2-1) was not statistically significant (RR=1.26, 95% CI: 0.80, 1.97), but with significant heterogeneity ($P_{\text{heterogeneity}}<0.001$). When the exploratory analysis by Shen et al. (2011) (14% relative weight) was removed from the summary RR (influence analysis) of fatty fish, the RR was close to no association (RR=1.02, 95% CI: 0.85, 1.22), and heterogeneity became non-significant ($P_{\text{heterogeneity}}=0.27$). The summary RR for lean fish (three studies, Table 4.6.3.2-1) suggested a protective association (RR=0.85, 95% CI: 0.73, 0.99) without significant heterogeneity ($P_{\text{heterogeneity}}=0.39$). Removing the exploratory analysis by Shen et al. (2011) from the summary RR for lean fish (1% relative weight only) had little effect on the magnitude of the RR (RR=0.86, 95% CI: 0.70, 1.06) but the estimate lost statistical significance. Heterogeneity among the two studies was non-significant ($P_{\text{heterogeneity}}=0.18$).

In contrast to VKM's summary estimate for total fish, the summary RR for atrial fibrillation in the high-low meta-analysis by Li et al. (2017) indicated no association (RR= 1.01, 95% CI: 0.94, 1.09). Heterogeneity was not significant in any of the analyses. For fatty fish and lean fish, VKM did not identify any previous meta-analyses for comparison.

Venous thromboembolism

VKM's summary RR for total fish intake and VTE (three studies, Table 4.6.3.3-1) suggested an association close to unity (RR=0.97, 95% CI: 0.72, 1.29, $P_{\text{heterogeneity}}=0.006$) with significant heterogeneity. This reflected reports of both a protective association (UK study) and adverse association (US study) that were statistically significant.

4.6.3.5 VKM's search compared to previous meta-analyses: heart failure and atrial fibrillation

Heart failure

Bechthold et al. (2019) included eight prospective, observational studies of heart failure in relation to different fish exposures (overall fish, fatty fish, or non-fried fish but not fried fish), of which one study was not included in our systematic review (del Gobbo et al., 2015). This study did not focus specifically on fish, but on lifestyle factors, and was therefore not detected in the search. Our search identified one recent publication (Zhang, 2021) not included in Bechthold.

Atrial fibrillation

Li et al. (2017) included six prospective studies of atrial fibrillation in relation to fish exposure. Details about the fish exposure were not available in the paper but estimates from primary studies were generally the same as those extracted by VKM for fish overall. All studies but one (Mozaffarian et al. 2004) were identified by VKM. Unlike VKM, Li et al. (2017) included non-fried fish (Berry et al., 2010) in the analysis of overall fish intake. Li et al. (2017) also included the risk estimate from Brower et al. (2006) before excluding participants with previous myocardial infarction (MI), whereas VKM extracted the estimate after exclusion to avoid atrial fibrillation secondary to MI. Estimates were similar, apart from a slightly wider confidence interval after exclusion.

Venous thromboembolism

VKM did not identify any previous meta-analyses of fish intake and risk of VTE for comparison.

An overview of papers included by VKM on other CVD outcomes (HF, AF, VTE) compared with previous meta-analyses is presented below (Table 4.6.3.5-1).

Table 4.6.3.5-1 Overview of publications on other CVD outcomes included in VKM's review compared with previous meta-analyses.

Publications	Included by VKM			Included in meta-analyses	
	Heart failure	Atrial fibrillation	Venous thrombo-embolism	Bechthold, 2019: Heart failure	Li, 2017: Atrial fibrillation
Heart failure					
Belin, 2011	X			X	
Dijkstra, 2009	X			X	
Levitan, 2009	X			X	
Levitan, 2010	X			X	
Mozaffarian, 2005a				X	
Nettleton, 2008	X			X	
Wilk, 2012	X			X	
Zhang, 2021	X				
Atrial fibrillation					
Berry, 2010		X			X
Brouwer, 2006		X			X
Gronroos, 2012		X			X
Larsson, 2017		X			X
Shen, 2011		X			X
Zhang, 2021		X			
Venous thromboembolism					
Hansen-Krone, 2014			X		
Lutsey, 2009			X		
Zhang, 2021			X		
Only in meta-analysis					
Del Gobbo, 2015				X	
Mozaffarian, 2004					X
<i>Studies included</i>	<i>7</i>	<i>6</i>	<i>3</i>	<i>8</i>	<i>6</i>

4.6.4 Heterogeneity fish intake and other CVD outcomes

Heart failure

Bechthold et al. (2019) observed low heterogeneity between studies. No sub-group analyses were performed. Heterogeneity was nonsignificant for VKMs summary RR.

Atrial fibrillation

Li et al. (2017) observed low heterogeneity between studies. The observed null association persisted in their subgroup analyses (by geographical region, length of follow-up, gender, dietary exposure, baseline age). Heterogeneity was nonsignificant for VKMs summary RR.

Venous thromboembolism

Heterogeneity was highly significant for VKMs summary RR based on three prospective studies. There was no meta-analysis for comparison.

4.6.5 Dose-response relationship fish intake and other CVD outcomes

Heart failure

Bechthold et al. (2019) found no departure from linearity in a non-linear dose-response analysis of fish intake and HF (n=6 studies). The risk of HF decreased by approximately 80% with increasing intake of fish up to about 80-100 g/day.

Atrial fibrillation

Li et al. (2017) found that the summary RR was 0.99 (95% CI: 0.96, 1.02) for each one serving/week increase in fish intake. A restricted cubic spline random-effects meta-analysis, and a test for nonlinearity, did not support deviations from linearity.

Venous thromboembolism

No meta dose-response analysis was identified for VTE. The largest primary study with over 400,000 participants (Zhang 2021, UK Biobank) reported a statistically significant linear trend.

4.6.6 Weight of evidence for fish intake and other CVD outcomes

In this section the evidence for an association of fish intake with risk of heart failure (HF), atrial fibrillation (AF) is weighed separately according to the WCRF criteria presented in Chapter 3.1.6 (Box 2).

Published evidence of fish intake and HF, AF, VTE

Heart failure

The meta-analyses on fish intake and risk of HF by Bechthold et al. (2019) concluded that fish consumption is associated with a lower risk of heart failure based on eight primary studies of total fish, fatty fish, or non-fried fish intake. VKMs' summary RR (four studies) is not statistically significant but suggests lower risk of HF for the highest versus lowest intake of total fish. As described, there was almost complete overlap between the publications included in the meta-analysis and by VKM, but VKM did not identify one study, and limited the summary RR to total fish.

Atrial fibrillation

The meta-analyses on fish intake and risk of AT by Li et al. (2017) concluded with no association between fish intake and AF based on six studies. In contrast, VKM's summary RR suggests a potential adverse association. As described above, Li et al. included one study not identified by VKM (Mozaffarian et al., 2004), as well as the estimate for non-fried fish from an identified study (Berry et al., 2010). VKM on the other hand, included a primary study (Zhang, 2021, UK) published after Li et al. 2017 that reported a borderline adverse association. Limited evidence from VKM's review suggests no association for the sub-categories fatty fish, lean fish, and canned tuna with risk of AF.

Venous thromboembolism

VKM included three primary studies on fish intake and VTE. The largest study (UK Biobank) reported a statistically significant association based on over 400 000 participants.

Heterogeneity

No significant heterogeneity was observed between studies in previous meta-analyses. VKM found significant heterogeneity between three studies on VTE.

Mechanism / biological plausibility

There is evidence for several plausible mechanisms operating in humans. Mechanisms for effects of LC n-3 FAs, including on heart arrhythmias, are described in Chapter 5.2.

Upgrading factors

No substantial upgrading factors were evaluated.

4.6.6.1 Conclusions weight of evidence fish intake and other CVD outcomes

Heart failure

There is evidence from more than two independent and good quality prospective cohort studies for total fish (in total VKM identified 3 studies and one previous meta-analysis). VKM's summary RR is not statistically significant but suggest lower risk of HF for the highest versus lowest intake of total fish. One previous meta-analysis also included fatty- and non-fried fish and reported statistically significant lower risk of HF and a dose-response relationship. The direction of the effect is generally consistent (towards protective), and there is low heterogeneity. There is evidence for biological plausibility. Based on one previous meta-analysis and VKM's summary RR there seems to be evidence that fish intake has a protective effect on HF except when the fish is served as fried fish. In conclusion, the evidence that consumption of fish reduces the risk of HF is graded "limited, suggestive".

Atrial fibrillation

There is evidence from more than two independent prospective cohort studies for total fish (VKM included five studies, and one meta-analysis). In contrast with a previous meta-analysis, VKM's summary RR suggest a small increased risk of AF for the highest versus lowest intake of total fish. The summary RR is borderline statistically significant without significant heterogeneity. The mechanism for a potential adverse effect is uncertain. In conclusion, the evidence is graded "limited, suggestive" for a potential adverse effect of fish intake on risk of AF.

Venous thromboembolism

There is evidence from three independent prospective cohort studies showing heterogenous results. The evidence that consumption of fish reduces risk of venous thromboembolism is graded "limited, no conclusion".

4.7 Introduction fish intake and cause-specific and all-cause and mortality

This chapter is an introduction to the weight of evidence analysis chapters for the included mortality outcomes; cause specific and all-cause (Chapters 4.8-4.9).

Overview of studies summarized according to mortality outcomes

Chapter sections 4.8-4.9 summarizes the epidemiological evidence of fish intake and risk of death from specific disease causes (cause-specific mortality) and all-cause mortality.

In Western populations, non-communicable diseases, such as cancers and cardiovascular diseases (CVD) drive associations with all-cause mortality. In Norway, the trend in age-adjusted mortality rates has been a decline in cancer and CVD and increase in dementia (source: Norwegian Institute of Public Health, Cause of death registry, 2010-2019). However, due to changes in population structure and a growing number of elderly persons, age-adjusted trends may differ somewhat from trends in absolute death numbers. Deaths due to all other diseases (e.g., respiratory diseases, infections, organ failure) and non-disease causes (accidents and violent deaths) are also part of all-cause mortality. The contributions to cause mortality by different disease causes (non-communicable versus infectious) and non-disease causes, may vary between populations.

Cause-specific mortality from different disease is summarized before all-cause mortality. In this report, the summary of cause-specific mortality was limited to chronic disease studied for incidence that were also identified in studies of mortality; cardiovascular diseases (CVDs), diabetes type 2, and Alzheimer's disease (see Figure 4.7-1).

For CVD mortality the weight of evidence is summarized for CVD as a composite outcome, as well as for different subgroups of CVD (e.g., all cardiac mortality, coronary heart disease, and stroke). Although it may seem artificial to draw separate conclusions for outcomes nested within each other, the outcome classifications reflect those used in the literature. Conclusions on CVD overall may differ from those on CVD sub-groups, depending on the published evidence (or lack of evidence) for each outcome.

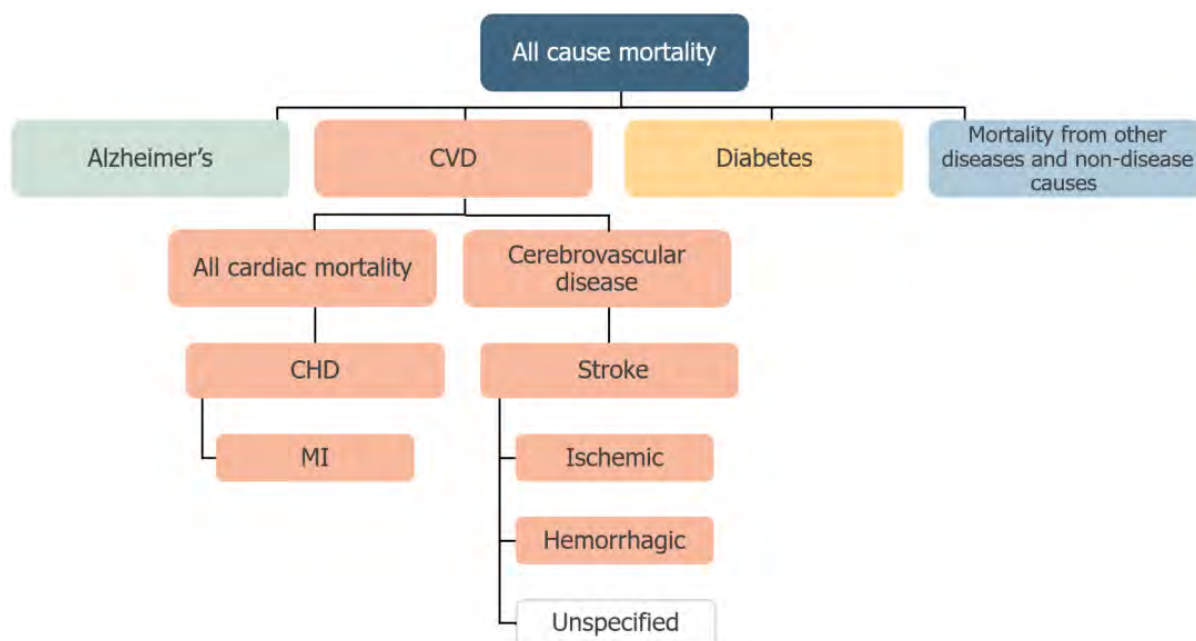


Figure 4.7-1 An overview of mortality outcomes in included primary studies.

Mechanisms mortality

For mechanisms of fish intake and cause-specific mortality outcomes, we refer to the sections on incidence of the outcome. Although mechanisms of the disease are largely thought to be the same, studies of mortality have been summarized separately from incidence. The reason is that results on mortality could be influenced by factors of importance to survival, either biological or other factors, such as access to and quality of health care services or competing risks from other causes of death. Of note, fish intake is not thought to influence all disease-causes included in studies of all-cause mortality. Nevertheless, all-cause mortality was considered a useful outcome as it may capture potential effects of fish on health outcomes in addition to those specified in the study protocol.

4.7.1 VKM's search for published systematic reviews, meta-analyses and primary studies on fish intake and mortality

4.7.1.1 Description of the identified systematic reviews and meta-analyses

VKM's search for systematic reviews and meta-analyses (see Chapter 3.1.2.2) identified 12 publications on the association between fish intake and mortality that fulfilled the inclusion criteria and were read as full papers. Four papers were excluded, see Table 4.7.1.1-1 for reason for exclusions.

Table 4.7.1.1-1 Reasons for exclusion of identified papers from the search of systematic reviews and meta-analysis of fish intake and mortality 2016-2021.

Included papers	Excluded paper and reasons for exclusions
Umbrella reviews Jayedi and Shab-Bidar, 2020 D'Alessandro et al., 2019 Kwok et al., 2019 Systematic reviews Jayedi et al., 2020: all-cause mortality in T2D patients Zhang et al., 2020 Jayedi et al., 2018: all-cause and CVD mortality Schwingshackl et al., 2017: all-cause mortality Wan et al., 2017: all-cause mortality Zhao et al., 2016: all-cause mortality	English et al., 2021 – no results on fish in paper, supplement unavailable. Micha et al., 2017: Umbrella review. Selection of papers were only done by one person. There was no information about any quality assessment for the included meta-analyses – graded C. Yamagishi et al., 2019: Only including mortality on outcomes which are not included in the VKM-assessment. Schwedhelm et al., 2016: Review among cancer survivors.

The meta-analyses are described in more detail below; first, main descriptions of the methods used and then main/selected results from each review.

Three of the identified nine studies were umbrella reviews (Jayedi and Shab-Bidar, 2020; D'Allesandro et al., 2019; Kwok et al., 2019). These umbrella reviews build on three relevant meta-analyses; two of which were also identified in VKM's search, Jayedi et al. (2018) and Schwingshackl et al. (2017), and one that was older, Zheng et al. (2012). Three additional meta-analyses, not included in any of the umbrella reviews, were identified in the VKM search; Zhao et al. (2016), Wan et al. (2017) and Zhang et al. (2020). Moreover, one relevant meta-analysis among T2D patients Jayedi et al. (2020) was included. Figure 4.7.1.1-1 shows a flow chart for the selection of meta-analyses.

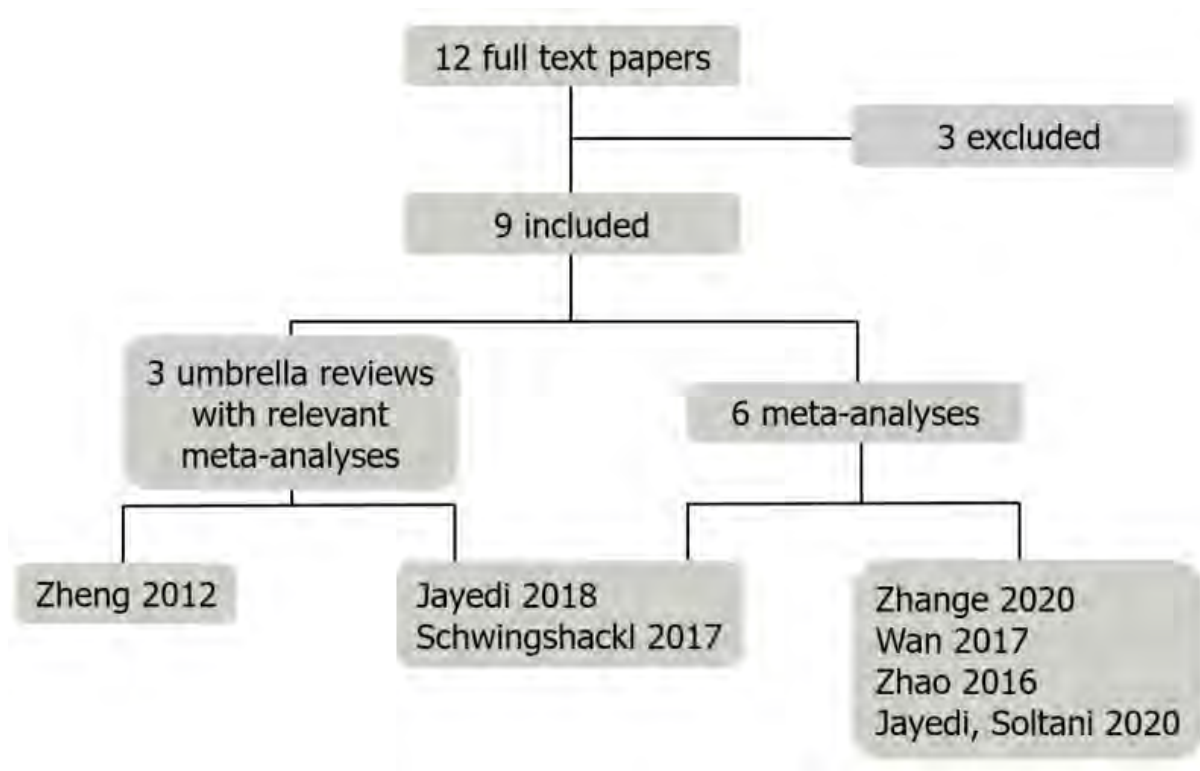


Figure 4.7.1.1-1 Flow-chart for selection of meta-analyses.

Umbrella reviews

Jayedi and Shab-Bidar (2020) is an umbrella review of meta-analyses of prospective studies investigating fish intake and different outcomes (CVD, type two diabetes (T2D), site-specific cancers, neurological disorders, all cause and cause-specific mortality, and any other diseases). The umbrella review is described in more detail elsewhere (CHD incidence Chapter 4.3.1). For fish intake and mortality Jayedi and Shab-Bidar (2020) included one meta-analysis for CVD mortality (Jayedi et al., 2018), one for CHD mortality (Zheng et al., 2012) and one for all-cause mortality (Schwingshackl et al., 2017). Jayedi et al. (2018) and Schwingshackl et al. (2017) were also identified in the VKM search. Zheng et al. (2012) was not identified in the VKM search due to publication date being outside the time period for the search. Jayedi et al. (2018) also included data on all-cause mortality.

The umbrella review by D'Alessandro et al. (2019) included Medline and Google Scholar searches for dose-response meta-analyses investigating the association between food groups and CVD, CHD, stroke, T2D, colorectal and breast cancer risk, up to December 2018. One of the inclusion criteria was that the meta-analysis should include linear and/or nonlinear dose-response meta-analyses of prospective studies (cohort studies, follow-up of RCTs, case-cohort studies, nested case-control studies). Nine meta-analyses were identified for fish intake, two of these had mortality as an outcome; CVD mortality (Jayedi et al., 2018), and CHD mortality (Zheng et al., 2012). More information about these two studies can be found below.

Kwok et al. (2019) is an umbrella review of meta-analyses of studies investigating fish intake and different outcomes (CVD and mortality). The authors did a systematic search in PubMed for meta-analyses published up to August 13, 2018. They chose to include the review with the highest number of studies, because the number of studies was part of the author's evidence grading criteria. For total fish and mortality, they identified one on all-cause mortality and CVD mortality (Jayedi et al., 2018). A description of Jayedi et al. (2018) is included below.

Meta-analyses of all-cause mortality

All-cause mortality in patient with type 2 diabetes: Jayedi et al. (2020) is a meta-analysis of prospective studies investigating the association of fish intake with the risk of all-cause mortality and risk of CVD including coronary heart disease (CHD), stroke, and myocardial infarction (MI) in patients with T2D. The authors did a systematic search in PubMed and Scopus databases up to June 2019. The quality of eligible studies was assessed with use of the 9-point Newcastle-Ottawa scale (Stang et al., 2010). Eight studies looking into fish intake and all-cause mortality were included. The quality of all the papers included in the meta-analysis were overall; 6 high-quality articles and 2 medium-quality articles. Methodological quality assessments of Jayedi et al. (2020) were made according to AMSTAR, and the study was found to have a moderate quality (AMSTAR assessment done by VKM assigned quality level B). The quality of meta-evidence was assessed by NutriGrade scoring system (Schwingshackl et al., 2016). The quality of the meta-evidence of fish intake and all-cause mortality was rated moderate by Jayedi et al. (NutriGrade score = 6).

All-cause and CVD mortality: Jayedi et al. (2018) conducted a literature search in PubMed and Scopus databases, from their inception up to August 2016, and then an updated search up to September 2016. The quality of eligible studies was assessed with use of the 9-point Newcastle-Ottawa scale (Stang et al., 2010). Eight prospective observational studies were included in the meta-analyses of fish intake and the CVD mortality, and they scored 7-9 by the Newcastle-Ottawa scale. Fourteen prospective observational studies were included in the meta-analyses of fish intake and all-cause mortality, and they scored 7-9 by the Newcastle-Ottawa scale, except one study scoring 6 (Engeset et al., 2015). The meta-analyses by Jayedi et al. (2018) had a good methodological quality (AMSTAR assessment done by Jayedi and Shab-Bidar (2020) assigned 9 out of 11 points). The quality of the meta-evidence of fish intake and CVD mortality was rated moderate based on NutriGrade scoring done by Jayedi and Shab-Bidar (2020). NutriGrade scoring was not available for all-cause mortality).

All-cause mortality: Schwingshackl et al. (2017) conducted a literature search PubMed, Embase, and Google Scholar through December 2016 and included 39 prospective observational studies from 37 publications. The study by Schwingshackl et al. (2017) had a good methodological quality (AMSTAR assessment done by Jayedi and Shab-Bidar (2020) assigned 10 out of 11 points). The quality of the meta-evidence of fish intake and over-all mortality was rated moderate based on the NutriGrade score (Schwingshackl et al., 2017).

All-cause mortality: The main aim of Wan et al. (2017) was to investigate the association between fish intake, omega-3 fatty acids and all-cause mortality through a meta-analysis of relevant prospective cohort studies. The literature search was performed in PubMed, Web of Science and Scopus through March 2017. The quality of eligible studies was assessed with the 9-point Newcastle-Ottawa scale (Stang et al., 2010). Twenty-two prospective cohort studies were included in the meta-analyses of fish intake and all-cause mortality, they scored 7-9 by the Newcastle-Ottawa scale, except for two studies scoring 5 and 6. The meta-analyses by Wan et al. (2017) had a good methodological quality (AMSTAR assessment done by VKM project group assigned quality level B).

All-cause mortality: Zhao et al. (2016) is a meta-analysis of prospective observational studies investigating the association between fish intake and all-cause mortality. The authors performed a systematic search in PubMed and Web of Science studies published before 31 December 2014. Twelve prospective cohort studies were included in the meta-analyses of fish intake and the all-cause mortality. The AMSTAR tool was used to assess the methodological quality of Zhao et al. (2016), and the study was found to have a moderate-low quality (AMSTAR assessment done by VKM assigned quality level B-C).

Meta-analyses of cause-specific mortality only

CVD mortality: Jiang et al. (2021) conducted a systematic literature review and meta-analysis of the association between fish, marine n-3 PUFA intake and CVD mortality risk in prospective cohort studies. The databases PubMed, Web of Science, Embase and MEDLINE were searched from inception to May 2021. The quality of eligible studies was assessed with use of the 9-point Newcastle-Ottawa scale (NOS) with score ranging from 0 (bad) to 9 (good). Eighteen studies, involving 1,267,951 participants and 51,628 CVD deaths, investigated the association between the fish intake and the CVD mortality, of which 10 cohorts met the requirements for dose-response analysis. NOS quality score ranged from 6–9 points among all included studies, and dose-response studies. There was no evidence of publication bias (funnel plot and Egger's test).

CHD mortality: Zhang et al. (2020) is a meta-analysis of prospective studies investigating the association between fish intake and CHD incidence and mortality. The authors performed a systematic literature search in Web of Science, Embase, and PubMed databases until October 2019. The quality of the eligible papers included in the meta-analysis was assessed by The Newcastle-Ottawa Scale criteria (Stang et al., 2010). Twenty-seven studies looking into fish intake and CHD mortality were included. The quality of all the papers included in the meta-analysis were overall 18 high-quality articles and 9 medium-quality articles. The AMSTAR tool was used to assess the methodological quality of Zhang et al. (2020), and the study was found to have a moderate quality (VKM assigned quality level B).

CHD mortality: Zheng et al. (2012) conducted literature search in PubMed and ISI Web of Science databases and included 17 prospective studies. The study by Zheng et al. (2012) had a good methodological quality. Jayedi and Shab-Bidar (2020) graded the quality of the

meta-evidence of fish intake and CHD mortality as low based on the NutriGrade scoring system.

4.7.1.2 Results from the meta-analyses

Below is a summary table for fish intake and all-cause mortality, CVD mortality and CHD mortality (Table 4.7.1.2-1) based on the identified meta-analyses.

Table 4.7.1.2-1 Summary of results from meta-analyses on total fish intake and mortality (all-cause and cause specific).

Author, year	Type of studies included	Total no studies	No of cases	Comparison	Summary RR (95% CI)	Heterogeneity	Overall conclusion
All-cause mortality							
Jayedi, 2020	Prospective observational studies in T2D patients	8	NA	Highest vs lowest	0.86, (0.76, 0.96)	$I^2=50\%$	Higher fish consumption associated with lower risks of all-cause mortality in patients with T2D
Jayedi, 2018	Prospective observational studies	14 (10 pub)	75451	20 g/d increment in fish intake	0.98 (0.97 to 1.00)	$I^2=81.9\%$	Inverse association between fish intake and risk of all-cause mortality
Schwingshackl, 2017	Prospective observational studies	39 (37 pub)	157688	Highest vs-lowest (intake range 0-250 g fish per day)	0.95 (0.92 to 0.98)	$I^2=51\%$	Higher fish consumption associated with lower risks of all-cause mortality
		19		100 g/day increment in fish intake	0.93 (0.88 to 0.98)	$I^2=53\%$	
Wan, 2017	Prospective observational studies	22	75150	Highest vs lowest	0.94 (0.90 to 0.98)	$I^2=50.2\%$	Higher fish consumption associated with lower risks of all-cause mortality
Zhao, 2016	Prospective observational studies	12	57641	Highest vs lowest	0.94, (0.90 to 0.98)	$I^2=39.1\%$	Higher fish consumption associated with lower risks of all-cause mortality
CVD mortality							
Jiang, 2021	Prospective observational studies	18	1 267 951, 51 628 CVD deaths	Highest vs lowest	0.91 (0.85, 0.98)	$I^2=70\%$	Higher fish consumption associated with lower risks of total CVD mortality
		10		20/g day increment in fish intake	0.96 (0.94, 0.99)	NA	Higher fish consumption associated with lower risks of total CVD mortality, sig linear trend (p trend = 0.002)

Author, year	Type of studies included	Total no studies	No of cases	Comparison	Summary RR (95 % CI)	Heterogeneity	Overall conclusion
Jayedi, 2018	Prospective observational studies	8 (7 papers)	11720	20 g/d increment in fish intake	0.96 (0.94 to 0.98)	$I^2=0\%$	Higher fish consumption associated with lower risks of CVD mortality
CHD mortality							
Zhang, 2020	Prospective observational studies	27 (25 papers)	10568	Highest vs lowest	0.85 (0.77 to 0.94)	$I^2=51.3\%$	Higher fish consumption associated with lower risks of CHD mortality
Zheng, 2012	Prospective observational studies	17 (14 papers)		16 studies on low intake (1 serv/wk); 1 serv/wk vs 1 serv/mo or 1-3 serv/mo	0.84 (0.75 to 0.95)	$I^2=20.1\%$	Fish intake of 1 or 2–4 serv/wk was associated with a significantly lower risk of CHD mortality. Fish intake of >5 serv/wk could marginally decrease CHD mortality (limited number of studies)
				13 studies on moderate fish intake; 2-4 serv/wk vs 1 serv/mo or 1-3 serv/mo	0.79 (0.67 to 0.92)	$I^2=56.7\%$	
				5 studies on High fish intake; 5 serv/wk vs 1 serv/mo or 1-3 serv/mo	0.83 (0.68 to 1.01)	$I^2 = 0\%$	
				15 g/day increment (dose-response)	0.94 (0.90 to 0.98)		

The four meta-analyses of the association between fish intake and all-cause mortality concluded that there was a significant inverse association. This was also concluded in a meta-analysis of studies among patients with T2D. An inverse association was also found between fish intake and mortality from both CVD and CHD.

4.7.1.3 Fish intake and all-cause and cause-specific mortality – primary studies

We evaluated 33 publications graded A or B with mortality from all causes as outcome. Most studies of all-cause mortality also included results on cause-specific mortality. Thus, among these studies were also many or all the studies (for some outcomes) that contributed results on mortality from Alzheimer's disease, CVD, CHD/MI, all heart disease, stroke, and T2D. Therefore, studies on all-cause mortality are described (study name, design, time period, size and age of the study population, and dietary assessment method) in Table 4.7.1.3-1 before the cause-specific mortality outcomes are summarized. Studies of cause-specific mortality only are not covered in table and are presented in supplementary tables under the different health outcomes.

Table 4.7.1.3-1 Overview of primary studies included in weight of evidence analysis of fish intake and all-cause mortality.

Author, year, country	Study name	Design	Inclusion year(s), end, follow-up time	Study population	Dietary assessment method	Dietary assessment period
Albert, 1998, USA	Physicians' Health Study (PHS)	Prospective cohort	1983 to 1995, 11 yrs follow-up	20 551 male physicians, 40-84 yrs	FFQ, semi quant, validated	Current, on average, at baseline
Bellavia, 2017, Sweden	Cohort of Swedish Men (COSM) and the Swedish Mammography Cohort (SMC)	Prospective cohort	1998 to 2014, 17 yrs of follow-up	72 522 (33 973 women and 38 549 men), 45-83 yrs, mean age around 60 yrs	FFQ, validated	Average frequency during the previous year, at baseline (1997)
Carballo-Casla, 2021, Spain	Seniors ENRICA-1 study	Prospective cohort	2008-2010 to 2020, follow-up 10.9 yrs (median)	3 165 (46% male), ≥60 yrs, mean age 70 yrs	Electronic diet history, repeated after 3 yrs	Usual intake, baseline and 3-year follow-up
Daviglus, 1997, USA	Chicago Western Electric Study	Prospective cohort	1957-1959, 30 yrs follow-up	1822 men, 40-55 yrs	Standardized interviews and questionnaires based on Burke's diet history method	Previous 28 days, at baseline and 1 year later, average
Engeset, 2015, Europe (10 countries)	EPIC (Spain, Greece, France, Italy, Germany, the Netherlands, the United Kingdom, Denmark, Sweden, and Norway)	Prospective cohort	1992–1999 to 2006-2010 (variations by study center)	480 535 (143,183 men and 337 352 women), Mostly 35–70 yrs, mean 51.5 (M) and 51.1 (W)	Dietary history or FFQ, country specific, validated and calibrated against 24h recall	Year before enrolment
Farvid, 2017, Iran	The Golestan Cohort Study	Prospective cohort	2004 to 2015, 8.1 yrs follow-up (median)	18 261 men and 24,142 women, 36–85 yrs	FFQ by face-to-face interview, validated	At baseline, frequency of food item consumption per day, week, month, or year
Folsom, 2004, USA	Iowa Women's Health Study (IWHHS)	Prospective cohort	1986 to 2000, 15 yrs follow-up	41 836 postmenopausal women, 55–69 yrs	FFQ, semi quant (from Willett), validated in other population	Usual intake, at baseline

Author, year, country	Study name	Design	Inclusion year(s), end, follow-up time	Study population	Dietary assessment method	Dietary assessment period
Gillum, 2000, USA	National Health and Nutrition Examination Survey (NHANES I) follow-up study	Prospective cohort	1971–1975 to 1992, 18.8 yrs follow-up (mean)	8825 (7421 white and 1404 black Americans). Oversampling of the elderly, women of childbearing age, and persons residing in poverty areas, 25–74 yrs	FFQ by interview	Usual intake, 3-month prior to interview
Mohan, 2021, global, 6 continents, 58 countries	PURE, ONTARGET, TRANSCEND, and ORIGIN, only data from PURE on general population	Prospective observational, multicenter	Follow-up to 2019 (PURE), Median follow-up (yrs) was 9.1 in PURE	191 558 (47.9% male), 51 731 with vascular disease and 139 827 generally healthy. PURE (n=147 541), Mean age PURE 51 (35-70) yrs	Country specific FFQs	Usual intake in previous year, at baseline
Nahab, 2016, USA	REasons for Geographic And Racial Differences in Stroke (REGARDS) study	Prospective cohort	2003-2007 to 2010, 5.1 yrs of follow up (median)	16 479 men and women (34% African Americans, 59% female, 74% were overweight or obese), 40-75 yrs	FFQ, Block98	Usual intake, past year, at baseline
Nakamura, 2005, Japan	National Integrated Project for Prospective Observation of Non-communicable Diseases and Its Trends in the Aged, 1980 (NIPPON DATA80)	Prospective cohort	1980 to 1999, 19 yrs follow-up	8 879 (3 945 men and 4 934 women), ≥30 yrs	Self-administered questionnaire	Usual average consumption, at baseline
Osler, 2003, Denmark	Copenhagen County Centre for Preventive Medicine (CPM) cohort (5 subcohorts incl MONICA I-III)	Prospective cohort	1982–1992 to 1997 (CHD incidence) or 2000 (mortality)	4 007 men and 3 533 women, incl a priori defined CHD high risk group (981 men and 622 women), 30–70 yrs	FFQ, validated	Average intake, at baseline

Author, year, country	Study name	Design	Inclusion year(s), end, follow-up time	Study population	Dietary assessment method	Dietary assessment period
Otsuka, 2019, Japan	National Institute for Longevity Sciences-Longitudinal Study of Aging (NILS-LSA)	Prospective cohort	1997-2000 to 2017, 11.7 yrs follow-up (mean)	1054 (49.3% male), 60-79 yrs, mean age 68.6 yrs	3-day (2 weekdays and 1 weekend day) dietary record without any supplements, weighing and photos of meals	3 days after baseline
Owen, 2016, Australia	Australian Diabetes, Obesity and Lifestyle Study (AusDiab)	Prospective cohort	1999-2000 to 2009 (CVD mortality, 9.7 yrs follow-up, median) or 2012 (all-cause mortality, 12.6 yrs follow-up, median)	11 247 (55% female), ≥25 yrs	FFQ, semi-quant, validated	Usual intake at baseline
Shao, 2021, China	Guangzhou Biobank Cohort Study	Prospective cohort	2003-2008 to 2017, follow-up 11.4 yrs (mean)	18 215 (71% women), ≥50 yrs, mean age 62.5 yrs	FFQ with portion picture book, validated for nutrients	Last 7 days
Takata, 2013, China	Shanghai Women's Health Study (SWHS) and the Shanghai Men's Health Study (SMHS)	Prospective cohort	1997-2000 (SWHS) or 2002-2006 (SMHS) to 2009, 11.2 yrs follow-up in women and 5.6 yrs in men (median values)	73 159 women and 61 137 men, 40-74 yrs, mean age 55 yrs (women) and 53 yrs (men)	FFQ, validated	Usual intake in previous year, at baseline
van den Brandt, 2019, the Netherlands	Netherlands Cohort Study (NLCS)	Case-cohort	1986 to 1996	8823 deaths (5797 in men and 3026 in women) and 3202 subcohort members, 55-69 yrs	FFQ, semi quant, validated	Habitual intake, year preceding baseline
Villegas, 2015, USA	Southern Community Cohort Study (SCCS)	Prospective cohort	2002-2009 to 2011, follow-up 5.5 yrs (mean)	77 604 (41% male, 64% blacks), 40-79 yrs	FFQ, validated, developed specifically for southeastern US	Usual intake in previous year, at baseline
Virtanen, 2019, Finland	Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD)	Prospective cohort	1984-1989 to 2014, 22.3 yrs follow-up (mean)	2641 men, 42-60 yrs	4-day dietary record with picture book for portion size	4 days at baseline, 1 of which was a weekend day

Author, year, country	Study name	Design	Inclusion year(s), end, follow-up time	Study population	Dietary assessment method	Dietary assessment period
Woo, 2002, Hong Kong	Hong Kong Old Age Allowance Scheme and Disability Allowance list	Prospective cohort	1991-1992 (baseline), follow-up 3 yrs	2032 (999 males and 1033 females) elderly Hong Kong Chinese, ≥ 70 yrs, mean age 80 yrs	Brief FFQ, interview with subject or main care giver	Dietary habits, weekly intake, at baseline
Yamagishi, 2008, Japan	Japan Collaborative Cohort Study for Evaluation of Cancer Risk (JACC) Study	Prospective cohort	1988-1990 to 1999 or 2003, 12.7 yrs follow-up	57 972 (22 881 men and 35 091 women), 40-79 yrs	FFQ, validated	Not specified
Yuan, 2001, China	Diet and cancer study, Shanghai	Prospective cohort	1986-1989 to 1998, 12 yrs of follow-up	18 244 men, 45-64 (mean age 55.8) yrs	FFQ by interview	Usual freq of consumption in previous year, at baseline
Zhang, 2018, USA	National Institutes of Health (NIH)-AARP (American Association of Retired Persons) Diet and Health Study	Prospective cohort	1995–1996 to 2011, follow-up 16 yrs	421 309 (57% male), 50–71 yrs, median age 62 yrs	FFQ (known as DHQ from the US National Cancer Institute), validated	Intake over past 12 months, at baseline

Author, year, country	Study name	Design	Inclusion year(s), end, follow-up time	Study population	Dietary assessment method	Dietary assessment period
Zhong, 2020, USA	Lifetime Risk Pooling Project: Atherosclerosis Risk in Communities study (ARIC), Coronary Artery Risk Development in Young Adults (CARDIA) study, Cardiovascular Health Study (CHS), Framingham Heart Study (FHS), Framingham Offspring Study (FOS), and Multi-Ethnic Study of Atherosclerosis (MESA)	Prospective cohort	1985-2002 (inclusion 1986-1990 for FHS, 1991-1995 for FOS, 1986-1989 for ARIC, 1985-1986 for CARDIA, 1989-1990 for CHS, 2000-2002 for MESA) to 2016, follow-up 19 yrs (median)	29 682 (44.4% male), mean age 53.7 yrs	FFQ, validated or diet history, depending on study	NA, probably usual intake at baseline
Zhuang, 2018, China, USA	China Health and Nutrition Survey (CHNS) and US National Health and Nutrition Examination Survey (NHANES III, and continuous 1999-2010)	Prospective cohort	1989 to 2011, follow-up 14 yrs (median) in CHNS; 1988–1994 to 2011, follow-up 9.8 yrs (median) in NHANES	14 117 (CHNS) and 33 221 (NHANES) men and women, ≥20 yrs, mean age 41 yrs (China) or 45 yrs (USA)	Three consecutive 24-h recalls in combination with weighing, 9 waves (1989 to 2011) in CHNS; 30-day consumption frequency combined with 24-h recall (NHANES)	Long-term diet (cumulative means) in CHNS, average intake in the past 30 days in NHANES
Excluded due to overlap						
Salonen, 1995, Finland	Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD)	Prospective cohort	1984-1989 to 1991 (acute MI) or 1992 (mortality), 5 yrs or 6 yrs of follow-up (mean values)	1833 men, 52.4 yrs	4-day dietary record	4 days at baseline
Patient populations with CVD/CHD/MI						

Author, year, country	Study name	Design	Inclusion year(s), end, follow-up time	Study population	Dietary assessment method	Dietary assessment period
Barzi, 2003, Italy	GISSI-Prevenzione clinical trial	Prospective cohort	1993-1995, 3.5 yrs (clinical) or 6.5 yrs (vital status) follow-up	11 246 male and female survivors of myocardial infarction, 19-90 yrs	Repeated short FFQ (baseline, 6, 18 and 42 months)	Long-term diet before event at baseline, current diet during follow-up
Burr, 1989, UK	Diet and Reinfarction trial (DART)	RCT- 2nd prevention	1983, 2 yrs follow-up	2033 men recovered from myocardial infarction, <70 yrs, (mean age 57 yrs)	Detailed dietary questionnaire at 6 months and 2 years after randomization	NA, baseline intake
Erkkila, 2003, Finland	Finnish sub-cohort of European Action on Secondary Prevention through Intervention to Reduce Events (EUROASPIRE)	Prospective cohort	1991-1994 (first hospitalization), baseline examination in 1995, 5 yrs of follow-up to to 2000 (hospitalization) or 2001 (deaths)	285 men and 130 women with coronary artery disease, 33-74 yrs, mean age 61 yrs	4-d food record (3 weekdays and 1 weekend day) completed at home. Portion size booklet.	Current intake at baseline ,4 days
Manger, 2010, Norway	Substudy of Western Norway B Vitamin Intervention Trial (WENBIT)	Prospective cohort	1999-2004 to 2006, 57 mo follow-up (median)	2412 patients (80.5% men) with well-characterized and treated coronary artery disease (90% statin users), ≥18 yrs, mean age 61.7 yrs	FFQ, semi quant, validated	Usual intake, previous year, at baseline
Mohan et al., 2021, Global, 6 continents, 58 countries	PURE, ONTARGET, TRANSCEND, and ORIGIN	Prospective observational, multicentre	Follow-up to 2019 (PURE), Median follow-up (yrs) was 9.1 in PURE, 4.5 in ONTARGET and TRANSCEND, and 6.2 in ORIGIN	191 558 (47.9% male), 51 731 with vascular disease and 139 827 generally healthy. PURE (n=147 541), ONTARGET and TRANSCEND (n = 31 491), ORIGIN (n=12 422). Mean age PURE 51 (35-70) yrs, ONTARGET and TRANSCEND 67 yrs, ORIGIN 64 yrs	Country specific FFQs (no amounts in ONTARGET and TRANSCEND), validated in some countries	Usual intake in previous year, at baseline
Diabetes populations						

Author, year, country	Study name	Design	Inclusion year(s), end, follow-up time	Study population	Dietary assessment method	Dietary assessment period
Deng, 2018, USA	Third National Health and Nutrition Examination Survey (NHANES III) follow-up study	Prospective cohort	1988-1994 to 2010, 17 yrs follow-up (mean)	1136 adult men and women with diabetes, non-institutionalized, ≥ 18 yrs, mean age 63.7 yrs	FFQ by interview	Usual intake, 3-month prior to interview
Hu, 2003, USA	Nurses' Health Study (NHS)	Prospective cohort	1980 to 1996, 16 yrs follow-up	5103 female nurses with diagnosed type 2 diabetes, 30-55 yrs	Repeated FFQ (1980, 1984, 1986, 1990, and 1994), semi-quant, validated	Average intake during the previous year
Wallin, 2018, Sweden	Cohort of Swedish Men (COSM) and the Swedish Mammography Cohort (SMC)	Prospective cohort	1998 to 2012, mean follow-up 11.8 yrs for incidence and 13.2 yrs for mortality	2225 (912 women and 1313 men) with type 2 diabetes, 45-84 yrs	FFQ, validated in men	Average frequency during the previous year, at baseline (1997)

4.7.2 VKM's systematic review of primary studies on fish intake and mortality from Alzheimer's disease

4.7.2.1 Included studies from search

Two publications presented results on mortality from Alzheimer's disease (Zhang et al., 2018, Zhuang et al., 2018). These publications were based on prospective studies from USA or China in both men and women. Both publications presented results on overall fish intake. One publication also included sub-types of fish (fried/non-fried, tuna only) but not fatty or lean fish. A description of the studies (study name, design, time period, size and age of the study population, and dietary assessment method) is included in an overview of studies on all-cause mortality (Table 4.7.1.3-1).

4.7.3 Results from the included primary studies on fish intake and mortality from Alzheimer's disease

We included two cohort studies with three estimates of the association between fish intake and risk of mortality from Alzheimer's disease. One study found a protective association (both men and women) whereas the other reported no association (Table 4.7.3-1).

Table 4.7.3-1. Results from prospective observational studies included in the weight of evidence analysis of fish intake and mortality from Alzheimer's disease.

Author, year, country	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low (95% CI)	Overall result
Zhang, 2018, USA	Fish, incl shellfish, M	g/d, quintiles	Quintile 5 vs 1, ≥ 30.03 vs ≤ 6.25 g/d	901	0.76 (0.61, 0.95)	Protective assoc. of intake in Q4-Q5 vs Q1, P -trend 0.0028
	Fish, incl shellfish, W	g/d, quintiles	Quintile 5 vs 1, ≥ 25.38 vs ≤ 4.61 g/d	706	0.62 (0.48, 0.80)	Protective assoc., P -trend < 0.0001
Zhuang, 2018, China, USA	Fish, M/W, NHANES study, USA only	g/d, 4 cat (null, tertiles among consumers)	Tertile 3 vs null, > 8.9 vs 0 g/d	115	1.10 (0.54, 2.25)	Protective assoc in second tertile vs null intake only, P -trend 0.83

4.7.3.1 Summary relative risks (RRs) based on VKM's inclusion of primary studies

The summary RR for highest versus lowest intake in the two studies was on the protective side (RR=0.76, 95% CI: 0.53, 1.09), but not statistically significant. Heterogeneity was not statistically significant ($P_{\text{heterogeneity}}=0.22$), but this may be due to few studies. The summary RR was dominated by Zhang et al. (2018) (80% relative weight).

Both publications were relatively recent (2018) and VKM's literature search did not identify any previous systematic reviews or dose-response analyses of fish and mortality from Alzheimer's disease for comparison.

4.7.4 Weight of evidence for fish intake and mortality from Alzheimer's

In this section, the evidence of the association between fish intake and mortality from Alzheimer's is weighed according to the WCRF criteria presented in Chapter 3.1.6 (Box 2).

Based on the two publications identified by VKM, and a summary RR that does not show a statistically significant association, the evidence for the association between fish intake and mortality from Alzheimer is graded "limited, no conclusion".

4.7.5 VKM's systematic review of primary studies on fish intake and mortality from cardiovascular diseases (CVD)

4.7.5.1 Included studies from search

We evaluated 21 publications graded A or B with mortality from CVD as a composite outcome: Albert et al., 1998; Bellavia et al., 2017; Daviglus et al., 1997; Deng et al., 2018; Farvid et al., 2017; Folsom et al., 2004; Gillum et al., 2000; Hengeveld et al., 2018; Kondo et al., 2019; Mohan et al. 2021; Morris et al., 1995; Nahab et al., 2016; Owen et al., 2016; Salonen et al., 1995; Shao et al. 2021; Takata et al., 2013; van den Brandt et al., 2019; Yamagishi et al., 2008; Zhang et al., 2018; Zhang et al. 2021; Zhuang et al., 2018.

There were multiple publications from the same studies, and one was excluded due to overlap (as described below), leaving 20 for further analysis. Of these publications, one (Deng et al., 2018) was conducted in a sub-population with type 2 diabetes, and one (Mohan et al., 2021) was conducted in patients with a history of CVD or at high risk of CVD, as well as in the general population (separate analyses). Most studies on CVD mortality are described (study name, design, time period, size and age of the study population, and dietary assessment method) in an overview of studies on all-cause mortality (Table 4.7.1.3-1). Three additional studies on CVD mortality (Hengeveld et al. 2018; Kondo et al 2019; Morris et al. 1995) are presented in Table 4.7.5.1-1.

Table 4.7.5.1-1 Overview of primary studies included in weight of evidence analysis of CVD mortality not described under all-cause mortality.

Author, year, country	Study name	Study design	Inclusion year(s), end, follow-up time	Study size, age	Dietary assessment method	Dietary assessment period
Hengeveld, 2018, the Netherlands	EPIC-Netherlands (Prospect and MORGEN sub-cohorts)	Prospective cohort	1993-1997 to 2011, 18 yrs follow-up (median 15.1 yrs)	34 033 (25% male), 20-70 yrs, mean age 48.7 yrs	FFQ, semi quant, validated	Usual intake, previous year, at baseline
Kondo, 2019, Japan	National Integrated Project for Prospective Observation of Non-communicable Diseases and Its Trends in the Aged, 1980 (NIPPON DATA80), including the National Nutrition Survey of Japan (NNSJ)	Prospective cohort	1980 to 2009, 29 yrs follow-up	9115 (4002 men and 5113 women), 30-79 yrs, mean age 49.9 (M) and 50.2 (W)	3-day weighed dietary record (recorded by household representative for any household member)	3 representative consecutive days, excluding weekends and holidays, at baseline
Morris, 1995, USA	Physicians' Health Study (PHS)	Prospective cohort	1982, 4 yrs of follow-up	21 185 male physicians, 40-84 yrs	FFQ, semi quant, validated	Average intake, previous year, at 12 mo follow-up

4.7.5.2 Overlapping publications

Both Morris et al. (1995) and Albert et al., (1998) reported on fish intake and CVD mortality in the US Physicians' Health Study. Albert 1998 had longer follow-up and more cases and was therefore kept in the main analysis according to the protocol, although Albert et al. (1998) included shellfish as part of fish intake, whereas Morris et al. (1995) did not.

Three publications reported on CVD mortality in the US National Health and Nutrition Examination Survey (NHANES) follow-up studies (Gillum et al., 2000, Deng et al., 2018, Zhuang et al., 2018). Both Deng et al. (2018) and Zhuang et al. (2018) used NHANES III, but Deng et al. (2018) was limited to participants with T2D and both studies were kept. Gillum et al. (2000) was based on NHANES I, which was understood to include a different sample of the US population than NHANES III. Therefore, all NHANES studies were kept (as for all-cause mortality).

4.7.5.3 Studies by design and geographic region

Among the 20 publications on CVD mortality overall (after excluding one overlapping publication), the majority were from the USA (7 studies) followed by Europe (5 studies) and Asia (5 studies). One study combined cohorts from China and the USA, and one study was from Australia. One study was a global multicenter study with data from 58 countries on 6 continents. All publications were based on studies with a prospective, observational design (cohort, case-cohort, cohort based on RCT, or health examination survey with follow-up).

Several publications were based on multiple data sets; the Cohort of Swedish Men (COSM) and the Swedish Mammography Cohort (SMC) (Bellavia et al., 2017), EPIC-Netherlands consisting of the Prospect and MORGEN sub-cohorts (Hengeveld 2018), Shanghai Women's Health Study (SWHS) and the Shanghai Men's Health Study (SMHS) (Takata et al., 2013) and the China Health and Nutrition Survey (CHNS) and US National Health and Nutrition Examination Survey III (Zhuang et al., 2018). Separate estimates were included for the CHNS (China) and NHANES III (USA) in Zhuang 2018. The global multi-center study by Mohan et al. 2021 presented separate analyses of one populationbased cohort (PURE) and cohorts based on drug trials (ONTARGET/TRANSCEND combined, and ORIGIN). For other studies, combined estimates were used if available.

4.7.5.4 Studies by sex, potential effect modification, and other sub-groups

Owen et al. (2016) reported a statistically significant sex interaction for the relationships of total fish consumption and non-fried fish consumption with CVD mortality ($P= 0.001$) and only sex-specific estimates were presented. Zhang et al. 2021 reported stronger associations with total fish in women than in men. Other studies that tested for effect modification by sex (Kondo et al., 2019, Takata et al., 2015, Yamagishi et al., 2008) found a non-significant test

of interaction, or no such effect, and pooled estimates for men and women combined were emphasized.

As previously described under studies of CVD incidence (Chapter 4.2.2.5) results in Mohan et al., 2021 were stratified by CVD history in the study participants (PURE study only), and results on CVD mortality are presented separately in this chapter for patient populations (Chapter 4.7.6.2). Zhang et al. 2021 stratified results by genetic CVD risk, defined as a family history of cardiovascular disease (CVD) or a CVD polygenic risk score (PRS). Results on CVD mortality did not differ by genetic risk and only the combined results for all participants are presented in this report.

4.7.5.5 Studies by fish exposure

All studies with estimates of CVD mortality, except Nahab et al. (2016), included a total fish exposure (sum of all fish, unspecified fish, or fish including shellfish and/or fish products). The most common classification was by preparation method (fried or non-fried). Only one study grouped fish intake by fat content (fatty or lean) and one used species (tuna only, and total fish excluding tuna) in addition to total fish. One Chinese study grouped fish intake by freshwater fish and saltwater fish. The studies of CVD mortality in the general population (n=19) were summarized for total fish (n=18), non-fried fish (n=3) and fried fish (n=2).

4.7.5.6 Studies assessing potential non-linearity

Takata et al. (2013) reported a test for non-linearity in the association of fish intake with CVD mortality. The test was statistically significant in women ($P=0.02$) and suggestive in men ($P=0.09$), but no figure was presented.

4.7.5.7 Studies with converted relative risk estimates

In two studies, the mid quintile (Bellavia et al., 2017) or the highest intake category (Kondo et al., 2019) were used as the reference. To facilitate comparisons with high-low meta-analyses, the relative risk estimates were converted to the value for the lowest category as reference (see Chapter 3, section 3.1.5. for methods description).

4.7.6 Results from the included primary studies on fish intake and mortality from CVD

4.7.6.1 Studies of total fish intake and mortality from CVD

We included 18 publications (all prospective, observational studies) on total fish intake with 20 estimates of the association with CVD mortality in the general population. The exposure levels and results (high-low relative risk, and overall) are included in Table 4.7.6.1-1. The estimates in Owen et al. (2016) were reported in a figure and extracted using WebPlotDigitizer.

Table 4.7.6.1-1 Results from studies included in the weight of evidence analysis of fish intake and CVD mortality.

Author, year, country	Study design*	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low (95 % CI)	Overall result
Albert, 1998, USA	Prospective observational	Fish, incl shellfish, M	Servings as main dish, 5 cat	≥5/wk vs <1/mo,	548	0.81 (0.49, 1.33)	No sig. assoc., <i>P</i> -trend 0.50
Bellavia, 2017, Sweden	Prospective observational	Fish, incl shellfish, M/W	g/d, quintiles	Quintile 5 vs 1 (Q3 as orig ref cat), 43.5–120 (median 53) vs 0–15.5 (median 11.5) g/d	4899	0.95 (0.81, 1.11), reported as 1.12 (1.00, 1.26) for Q5 vs Q3 and 1.18 (1.06, 1.32) for Q1 vs Q3	U-shape, sig. increased risk of intake in highest and lowest vs mid quintile
Daviglus, 1997, USA	Prospective observational	Fish, M	120-g units per 28 days, 4-point scale (0-3)	35 vs 0 g/d	573	0.74 (0.52, 1.06)	Protective trend, <i>P</i> = 0.01
Farvid, 2017, Iran	Prospective observational	Fish, M/W	Servings/d, 4 cat (tertiles of intake, null)	Cat 4 vs 1, 0.19 (median) vs 0 servings/d, standard serving size 85g	1467	0.96 (0.82, 1.13)	No sig. assoc., <i>P</i> -trend 0.88
Folsom, 2004, USA	Prospective observational	Fish, incl shellfish, W	Servings/wk, approx quintiles	≥2.5 vs <0.5 servings/wk	1589	0.95 (0.78, 1.15)	No sig. assoc., <i>P</i> -trend 0.11
Gillum, 2000, USA	Prospective observational	Fish, incl shellfish, M-white	Times/wk, 4 cat	>1/wk vs never	NA	0.95 (0.68, 1.33)	No sig. assoc.
		Fish, incl shellfish, M-black	Times/wk, 4 cat	>1/wk vs never	NA	1.08 (0.52, 2.21)	No sig. assoc.
		Fish, incl shellfish, W-white	Times/wk, 4 cat	>1/wk vs never	NA	1.06 (0.75, 1.50)	No sig. assoc.
		Fish, incl shellfish, W-black	Times/wk, 4 cat	>1/wk vs never	NA	0.99 (0.51, 1.93)	No sig. assoc.

Author, year, country	Study design*	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low (95 % CI)	Overall result
Hengeveld, 2018, the Netherlands	Prospective observational	Fish, M/W	Portions/wk, 3 cat	≥1/wk vs none, 28.7 g/wk fatty and 93.7 g/wk lean (median values)	540	0.96 (0.81, 1.13)	No sig. assoc.
Kondo, 2019, Japan	Prospective observational	Fish, incl shellfish, M/W	g/d, 3 cat (high as ref)	≥80 vs <40 g/d	1070	0.72 (0.57, 0.91), reported as 1.39 (1.10, 1.77) for low-high intake	Sig. protective assoc.: adverse assoc. of lowest (<40 g/d) vs highest (≥80 g/d) intake
Mohan, 2021, global, 6 continents, 58 countries	Prospective observational	Fish, incl shellfish, M/W, PURE only	g/mo or wk, 4 cat	≥350 g/wk vs <50 g/mo, 594 vs 0.1 g/wk (median values)		0.99 (0.84, 1.20)	No sig. assoc., <i>P</i> -trend 0.84
Owen, 2016, Australia	Prospective observational	Fish, M/W	Servings/mo or wk, 4 cat	≥2/wk vs <1/mo	277	0.74 (0.48, 1.14), extracted from fig. in paper	No sig. assoc. (based on fig. in paper)
Salonen, 1995, Finland	Prospective observational	Fish, M	g/d, binary	≥30 vs <30 g/d	24	2.08 (0.85, 5.11)	No sig. assoc.
Shao, 2021, China	Prospective observational	Fish, M/W	Servings/wk, 4 cat	≥11 vs 0-3 servings/wk	917	0.87 (0.72, 1.05)	Protective assoc. for 4-6 servings/wk but not higher, <i>P</i> -trend 0.40
Takata, 2013, China	Prospective observational	Fish, incl shellfish, M	g/d, quintiles, sex-specific	Quintile 5 vs 1, 107.2 vs 10.8 g/d	699	0.96 (0.74, 1.26)	No sig. assoc. (protective assoc. limited to quintile 4 vs 1), <i>P</i> -trend 0.57
		Fish, incl shellfish, W	g/d, quintiles, sex-specific	Quintile 5 vs 1, 105.2 vs 10.4 g/d	1090	0.78 (0.62, 0.98)	Protective assoc. of intake in quintile 5 vs 1, <i>P</i> -trend 0.04
van den Brandt, 2019, the Netherlands	Prospective observational, case-cohort	Fish, M/W	g/d, 4 cat	≥20 vs 0 g/day, 29.8 vs 0 g/day (median)	2985	1.45 (1.20, 1.74)	Sig. adverse assoc., <i>P</i> -trend 0.001

Author, year, country	Study design*	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low (95 % CI)	Overall result
Yamagishi, 2008, Japan	Prospective observational	Fish, M/W	g/d, quintiles, energy adjusted	Quintile 5 vs 1, range 72 to 229 (Q5) vs 0 to 27 (Q1) g/d	2045	0.82 (0.71, 0.95)	Sig. protective assoc. of intake in quintile 5 vs 1, <i>P</i> -trend 0.007
Zhang, 2018, USA	Prospective observational	Fish, incl shellfish, M	g/d, quintiles	Quintile 5 vs 1, ≥ 30.03 vs ≤ 6.25 g/d	14824	0.90 (0.85, 0.94)	Protective assoc., <i>P</i> -trend <0.0001
		Fish, incl shellfish, W	g/d, quintiles	Quintile 5 vs 1, ≥ 25.38 vs ≤ 4.61 g/d	7541	0.90 (0.83, 0.97)	Protective assoc., <i>P</i> -trend 0.0034
Zhang, 2021, UK	Prospective observational	Total fish, M/W	Times/wk, 4 cat	≥ 3 vs <1/wk	2455	0.88 (0.78, 0.99)	Protective assoc. in all cat above reference, <i>P</i> -trend 0.03
Zhuang, 2018, China, USA	Prospective observational	Fish, M/W, NHANES	g/d, 4 cat (null, tertiles among consumers)	Tertile 3 vs null, >8.9 vs 0 g/d	1495	0.81 (0.62, 1.06)	Protective trend, <i>P</i> -trend 0.04

4.7.6.2 Studies of fried and non-fried fish intake and CVD mortality in the general population

We included four publications (all prospective, observational studies) in the analysis of intake of fried fish (3 estimates) and non-fried fish (4 estimates) in relation to CVD mortality. The exposure levels and results (high-low relative risk, and overall) are included in Table 4.7.6.2-1. The estimates in Owen et al. (2016) for non-fried fish were reported in a figure and extracted using WebPlotDigitizer.

Table 4.7.6.2-1 Results from prospective observational studies included in the weight of evidence analysis of fried and non-fried fish intake and CVD mortality.

Author, year, country	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low (95 % CI)	Overall result
Fried fish						
Nahab 2016, USA	Fried fish, M/W	Servings/mo or wk, 4 cat	2/wk vs <1/mo	291	0.74 (0.35, 1.55)	No sig. assoc., <i>P</i> -trend 0.10

Author, year, country	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low (95 % CI)	Overall result
Zhang 2018, USA	Fried fish, W	g/d, quartiles	Quartile 4 vs 1, NA	7541	1.05 (0.99, 1.13)	Suggestive adverse trend, <i>P</i> -trend 0.019
	Fried fish, M	g/d, quartiles	Quartile 4 vs 1, NA	14824	1.02 (0.97, 1.07)	Protective assoc. of intake in quintile 2, but not higher vs Q1, <i>P</i> -trend 0.093
Non-fried fish						
Nahab 2016, USA	Non-fried fish, M/W	Servings/mo or wk, 4 cat	2/wk vs <1/mo	291	1.46 (0.87, 2.45)	No sig. assoc., <i>P</i> -trend 0.10
Owen 2016, Australia	Non-fried fish, M/W	Servings/mo or wk, 4 cat	≥2/wk vs <1/mo	277	0.70 (0.47, 1.02), extracted from fig. in paper	Suggestive threshold for protective effect of ≥1–3 servings vs <1 per month (based on fig. in paper)
Zhang 2018, USA	Non-fried fish, M	g/d, quintiles	Quintile 5 vs 1, NA	14824	0.84 (0.80, 0.89)	Protective assoc., <i>P</i> -trend <0.0001
	Non-fried fish, W	g/d, quintiles	Quintile 5 vs 1, NA	7541	0.87 (0.81, 0.94)	Protective assoc., <i>P</i> -trend <0.0001

4.7.6.3 Studies of total fish intake and CVD mortality in patient populations with prior CVD or high risk

We included two publications (four studies) with four estimates of the association between fish intake and CVD mortality in patients with prior CVD or at high risk of CVD from vascular disease, or with type 2 diabetes (Table 4.7.6.3-1). Three of four estimates, including in T2D diabetes patients only, were protective or suggestive protective, and one was statistically non-significant (men and women combined in all studies).

Table 4.7.6.3-1 Results from studies included in the weight of evidence analysis of fish intake and CVD mortality in patient populations.

Author, year, country	Study design	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low (95 % CI)	Overall result
Prior CVD or high risk							
Mohan, 2021, global, 6 continents, 58 countries	Prospective observational	Fish, incl shellfish, M/W, PURE	g/mo or wk, 4 cat	≥350 g/wk vs <50 g/mo, 594 vs 0.1 g/wk (median values)	NA	0.91 (0.71, 1.16)	No sig. assoc. P-trend 0.36
		Fish, incl shellfish, M/W, ONTARGET, TRANSCEND	g/mo or wk, 4 cat	≥350 g/wk vs <50 g/mo, 450 vs 2.8 g/wk (median values)	2265	0.81 (0.70, 0.92)	Protective or borderline protective assoc. in all categories, P-trend <0.001
		Fish, incl shellfish, M/W, ORIGIN	g/mo or wk, 4 cat	≥350 g/wk vs <50 g/mo, 568 vs 2.2 g/wk (median values)	1135	0.86 (0.74, 1.00)	Protective or borderline protective assoc. all categories, P-trend 0.01
Diabetes population							
Deng, 2018, USA	Prospective observational	Fish, incl shellfish, M/W, diabetics	Times/wk, 3 cat	>2 vs 1/wk	326	0.69 (0.50, 0.96)	Protective assoc. of intake >2 vs <1/wk, P-trend <0.001

4.7.6.4 Summary relative risks (RRs) based on VKM's inclusion of primary studies

The high-low summary relative risk (RR) for total fish and CVD mortality (based on 18 studies, Table 4.7.6.1-1) indicated a protective association for the highest versus lowest intake (RR=0.92, 95% CI: 0.86, 0.98). The estimate was statistically significant with significant heterogeneity ($P_{\text{heterogeneity}} < 0.001$). Among primary studies there was one report of statistically significant increased CVD mortality (van den Brandt et al., 2019, 6% relative weight). Heterogeneity was no longer significant ($P=0.55$) when this study was removed from the pooled RR in influence analysis.

The summary estimate in the recent meta-analysis by Jiang et al. 2021 based on 19 studies was almost identical (RR=0.91, 95%, 0.85, 0.98, 19 studies, $I^2=70\%$) to VKM's summary RR, also with substantial heterogeneity.

For intake of fried fish (two studies, Table 4.7.6.2-1), VKM's summary RR suggested a small, increased risk of CVD mortality (RR=1.03, 95% CI: 0.99, 1.07). The result was heavily dominated by Zhang et al. (2018) with over 400, 000 participants (weight of 99.7% in VKM's analysis) and heterogeneity was non-significant ($P_{\text{heterogeneity}}=0.38$). There was no previous meta-analysis to compare with.

For non-fried fish (three studies, Table 4.7.6.2-1), VKM's summary RR suggested no association with CVD mortality (RR= 0.89, 95% CI: 0.67, 1.19). Heterogeneity was borderline statistically significant ($P_{\text{heterogeneity}}=0.07$). This result was also dominated by Zhang et al. (2018) (53% weight), but less than for fried fish. There was no previous meta-analysis to compare with.

For total fish intake in patient populations, VKM's high-low summary RR for CVD mortality in patients with a CVD history or at high risk of CVD (one publication, three studies, Table 4.7.6.3-1) suggested statistically significant lower risk (RR=0.84, 95% CI: 0.77, 0.92) without significant heterogeneity ($P_{\text{heterogeneity}}=0.66$). One study in patients with T2D (Table 4.7.6.3-1) reported a protective association.

4.7.6.5 VKM's search compared to previous meta-analyses on CVD mortality

The meta-analysis by Jiang et al. (2021) included 18 publications on CVD mortality, of which three were not included by VKM. One study was excluded after quality assessment (Tomasallo et al. 2010), but two studies from Japan were not identified in the search. These studies focused on healthy lifestyle behaviors (Eguchi et al. 2014) or dietary patterns (Kobayashi et al. 2019) rather than fish specifically. However, VKM identified several publications not included in Jiang et al. (2021) (see Table 4.7.6.5-1 for overview of overlap), one older (Salonen et al. 1995) and some more recent (Farvid et al. 2017; Shao et al. 2021; Zhang et al. 2021; Zhuang 2018). The meta-analysis by Jayedi et al. (2018) included seven publications in their linear dose-response analysis. All were identified by VKM.

Table 4.7.6.5-1 Overview of prospective cohort studies included by VKM compared with two identified meta-analyses on cardiovascular disease (CVD) mortality overall.

	Included by VKM	Meta-analyses	
Publications		Jiang 2021	Jayedi 2018
Albert 1998	X	X	X
Bellavia 2017	X	X	X
Daviglus 1997	X	X	X
Farvid 2017	X		
Folsom 2004	X	X	X
Gillum 2000	X	X	
Hengeveld 2018	X	X	
Kondo 2019	X	X	
Mohan 2021	X	X	
Nahab 2016	X	X	
Owen 2016	X	X	X
Salonen 1995	X		
Shao 2021	X		
Takata 2013	X	X	X
van den Brandt 2019	X	X	
Yamagishi 2008	X	X	X
Zhang 2018	X	X	
Zhang 2021	X		
Zhuang 2018	X		
Overlapping			
Morris 1995	X		
Diabetes population			
Deng 2018	X	X	
Studies only in meta-analyses			
Eguchi 2014		X	
Kobayashi 2019		X	
Tomasallo 2010		X	
<i>Studies included</i>	<i>20</i>	<i>18</i>	<i>7</i>

4.7.7 Heterogeneity fish intake and CVD mortality

No evidence of heterogeneity was observed in the linear dose-response analysis by Jayedi et al. (2018) ($I^2 = 0\%$), but the recent meta-analysis by Jayedi et al (2021) reported substantial heterogeneity ($I^2 = 70\%$). The heterogeneity in the summary RR calculated by VKM for total fish and CVD mortality in the general population (18 studies) was explained by one study (van den Brandt et al., 2019).

4.7.8 Dose-response relationship fish intake and CVD mortality

Two previous meta dose-response analyses have reported an inverse, non-linear relationship between fish consumption and CVD mortality. Jayedi et al. (2018) found reduced risk of CVD mortality for fish intake from zero up to about 100 g/day, and Jiang et al. 2021 for intake up to about 90 g/day (judging from the confidence limits of the dose-response curves).

4.7.9 Weight of evidence for fish intake and CVD mortality

In this section the evidence of the association between fish intake and CVD mortality is weighed according to the WCRF criteria presented in Chapter 3.1.6, (Box 2).

Published evidence of fish intake and CVD mortality

Two previous meta-analyses (Jayedi et al. 2018, Jiang et al. 2021) indicated a protective association between fish intake and CVD mortality. The summary RR for primary studies included by VKM also indicated lower CVD mortality for the highest intakes of total fish (18 studies). The summary RRs suggested a potentially small, increased risk for intake of fried fish and a protective association for non-fried fish, but neither association was statistically significant. One primary study in patients with T2D reported a statistically significant protective association.

Heterogeneity

No heterogeneity was found in the meta-analyses by Jayedi et al. (2018). The significant heterogeneity observed between studies included by VKM on CVD mortality was explained by one primary study published in 2019 after Jayedi et al. 2018. The same primary study seems to be a source of heterogeneity in the most recent meta-analysis by Jiang et al. 2021.

Mechanism

There is evidence for several plausible mechanisms operating in humans (see Chapter 4.1 and 5.2).

Upgrading factors

There is evidence of an inverse dose-response relation from two independent meta-analyses.

4.7.9.1 Conclusion weight of evidence for fish intake and CVD mortality

There is evidence from more than two independent and good quality cohort studies on total fish intake (VKM included 18 studies and two independent meta-analyses including dose-response analyses). The published evidence suggests a protective association between fish intake and CVD mortality that is statistically significant.

VKM's summary RR for primary studies in the general population shows statistically significant lower risk of CVD mortality for the highest versus lowest intake of total fish and is supported by independent meta-analyses. The direction of the associations is generally consistent towards protective, but with some heterogeneity that seems to be explained by one primary study. There is evidence for biological plausibility. There is also a biological gradient in the association.

In conclusion, the evidence was graded "probable" for a protective effect of fish consumption on CVD mortality in the general population. VKM's summary RR for studies in patients with prior CVD or at high risk, is slightly stronger than for the general population. The effects of fatty and lean fish on CVD mortality could not be summarized as only study was identified.

4.7.10 VKM's systematic review of primary studies on fish intake and mortality from total heart disease

We evaluated 2 publications graded A (Zhang et al., 2018) or B (Deng et al., 2018) with mortality from all heart conditions as outcome. Heart disease is a broad categorization that in addition to ischemic heart disease, atherosclerosis, and heart failure, may include essential (primary) hypertension, hypertensive heart disease, hypertensive renal disease, hypertensive heart and renal disease, rheumatic heart diseases, aortic aneurysm and dissection (tear in the aortic lining), and other heart related conditions. Deng et al. (2018) was limited to a sub-population with T2D.

A description of both studies (study name, design, time period, size and age of the study population, and dietary assessment method) is included in an overview of studies on all-cause mortality (Table 4.7.1.3-1).

4.7.11 Results from the included primary studies on fish intake and total heart disease mortality

The exposure levels and results of the two included studies (both cohorts) with three estimates of the association between fish intake and risk of mortality from all heart disease are shown below (Table 4.7.11-1). Zhang et al. (2018) reported a protective association of similar magnitude in men and women. The association in type 2 diabetics (Deng et al., 2018) was on the protective side, but not statistically significant.

Table 4.7.11-1 Results from prospective observational studies included in the weight of evidence analysis of fish intake and total heart disease mortality (general population or patient population with type 2 diabetes).

Author, year, country	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low (95 % CI)	Overall result
Deng, 2018, USA	Fish, incl shellfish, M/W, diabetics	Times/wk, 3 cat	>2 vs 1/wk	275	0.79 (0.56, 1.12)	No sig. assoc.

Author, year, country	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low (95 % CI)	Overall result
Zhang, 2018, USA	Fish, incl shellfish, M	g/d, quintiles	Quintile 5 vs 1, ≥ 30.03 vs ≤ 6.25 g/d	12636	0.91 (0.86, 0.97)	Protective assoc., P -trend <0.0023
	Fish, incl shellfish, W	g/d: quintiles	Quintile 5 vs 1, ≥ 25.38 vs ≤ 4.61 g/d	5939	0.92 (0.84, 1.00)	Borderline protective estimate, P -trend 0.041

4.7.12 Weight of evidence for fish intake and cause-specific mortality from all heart disease

There is evidence of a protective association in one large US study. Another smaller US study in T2D diabetes population shows no statistically significant association. In conclusion, the evidence that fish intake is associated with mortality from all heart diseases is graded "limited, no conclusion".

4.7.13 VKM's systematic review of primary studies on fish intake and mortality from coronary heart disease (CHD)

4.7.13.1 Included studies from search

We evaluated 23 publications graded A or B with mortality from CHD as outcome: Albert et al., 1998; Ascherio et al., 1995; Daviglus et al., 1997; de Goede et al., 2010; Engeset et al., 2015; Erkkila et al., 2003; Farvid et al., 2017; Folsom et al., 2004; Hu et al., 2002; Iso et al., 2006; Jarvinen et al., 2006; Kromhout et al., 1985; Manger et al., 2010; Mozaffarian et al., 2003; Nakamura et al., 2005; Oomen et al., 2000; Osler et al., 2003; Salonen et al., 1995; Shao et al. 2021; Takata et al., 2013; Wallin et al., 2018; Yamagishi et al., 2008; Zhuang et al., 2018.

One publication was excluded due to overlap (as described below), leaving 22 for further analysis. Of these publications, two described patients surviving coronary heart disease (CHD) or myocardial infarction (Erkkila et al., 2003; Manger et al., 2010), and one described a sub-population with T2D (Wallin et al., 2018). These studies were summarized separately.

Of the 22 publications (excluding one overlapping), 13 are described (study name, design, time period, size and age of the study population) under all cause-mortality (Table 4.7.1.3-1). whereas eight additional studies on CHD mortality (Ascherio et al. 1995, de Goede et al. 2010, Hu et al 2002, Iso et al. 2006, Jarvinen et al. 2006, Kromhout et al. 1985, Mozaffarian et al. 2003, Oomen et al 2000; are presented in Table 4.7.13.1-1.

Table 4.7.13.1-1 Overview of primary studies included in weight of evidence analysis of CHD mortality not described under all-cause mortality.

Author, year, country	Study name	Study design	Inclusion year(s), end, follow-up time	Study size, age	Dietary assessment method	Dietary assessment period
Ascheri, 1995, USA	Health Professionals Follow-Up Study (HPFS)	Prospective cohort	1986, 6 yrs of follow-up	44 895 male health professionals, 40-75 yrs	FFQ, semi quant, validated	Average frequency during the previous year, at baseline
de Goede, 2010, the Netherlands	Monitoring Project on Risk Factors for Chronic Diseases (MORGEN)	Prospective cohort	1993-1997 to 2006 or 2007 (mortality only), 11.3 yrs follow-up (mean)	21 342 men and women, 45% male, 20-65 yrs (mean age 42 yrs)	FFQ, validated	Usual freq of consumption in previous year, at baseline
Hu, 2002, USA	Nurses' Health Study (NHS)	Prospective cohort	1980 to 1996, 16 yrs follow-up	84 688 female nurses, 34 -59 yrs	Repeated FFQ (1980, 1984, 1986, 1990, and 1994), semi-quant, validated	Average intake during the previous year
Iso, 2006, Japan	Japan Public Health Center-Based (JPHC) Study Cohort I	Prospective cohort	1990-1992 to 2001, 11 yrs follow-up	41 578 (19 985 men and 21 593 women), 40-59 yrs	Repeated FFQ (1990, 1995), validated	Average intake previous month (1990) or previous year (1995)
Jarvinen, 2006, Finland	Finnish Mobile Clinic	Prospective cohort	1966-1972, 21.5 yrs follow-up (mean)	2775 men and 2445 women, 30-79 yrs	Dietary history interview	Usual intake, previous year, at baseline
Kromhout, 1985, the Netherlands	Zutphen study	Prospective cohort	1960 to 1980, 20 yrs follow-up	872 men, 40-59 yrs	Cross-check dietary history method, adapted to Dutch situation, with wife present	Usual intake 6-12 months prior to interview, at baseline
Mozaffarian, 2003, USA	Cardiovascular Health Study	Prospective cohort	1989-90 to 2000, 9.3 yrs of follow-up (mean)	3910 men and women, ≥65, mean age 73 yrs	FFQ, picture sort version of the National Cancer Institute FFQ	Average intake during the previous year, at baseline
Oomen, 2000, Finland, Italy, the Netherlands	Seven Countries Study (Finland, Italy, the NL cohorts)	Prospective cohort	1969-1970 to 1990, 20 yrs follow-up	2738 men, 50-69 (mean 58) yrs	Cross-check dietary history method by interview, adapted to each country	Habitual intake 6-12 months preceding the interview

4.7.13.2 *Overlapping publications*

The overlapping publications were from the Zutphen study and described fish intake in relation to risk of CHD mortality in men from the Netherlands (Kromhout et al., 1985) or as part of a multi-center study in three countries (Finland, Italy, the Netherlands) from the Seven Countries Study (Oomen et al., 2000). In the most recent publication (Oomen et al., 2000) the Zutphen study contributed more cases during a later stage of follow-up when the study population was older, and the multi-center study was kept for further analysis.

4.7.13.3 *Studies in patient populations*

Three studies presented results on CHD mortality in patient populations, either in survivors of CHD or myocardial infarction (Erkkila et al., 2003; Manger et al., 2010 previously described under CHD incidence, Chapter 4.3.2.4) or in sub-populations with T2D in the Cohort of Swedish Men (COSM) and the Swedish Mammography Cohort (SMC) combined (Wallin et al. 2018).

4.7.13.4 *Studies by design and geographic region*

Among the 22 publications on CHD mortality (excluding one overlapping publication), the majority were from Europe (10 studies) followed by USA (6 studies) and Asia (5 studies).

All publications were based on studies with a prospective, observational design (cohort, cohort based on RCT, or health examination survey with follow-up). There were several multi-center studies. One study included cohorts from China and USA (Zhuang, 2018), but cause-specific mortality, including CHD mortality, was only available for USA. The EPIC study by Engeset et al. 2015 included cohorts from ten countries: Spain, Greece, France, Italy, Germany, the Netherlands, the United Kingdom, Denmark, Sweden, and Norway, and the study by Oomen et al. 2000 was based on three countries (Finland, Italy, the Netherlands) from the Seven Countries Study.

4.7.13.5 *Studies by sex, potential effect modification, and other sub-groups*

Among the 22 publications, most studies were conducted in both men and women (de Goede et al., 2010; Engeset et al., 2015; Farvid et al., 2017; Iso et al., 2006; Jarvinen et al., 2006; Mozaffarian et al., 2003; Nakamura et al., 2005; Osler et al., 2003; Shao et al. 2021; Takata et al., 2013; Wallin et al., 2018; Yamagishi et al., 2008; Zhuang et al., 2018), also studies of secondary prevention (Erkkila et al., 2003; Manger et al., 2010). Five studies were conducted in men only (Albert et al., 1998; Ascherio et al., 1995; Daviglus et al., 1997; Oomen et al., 2000; Salonen et al., 1995) and two in women only (Folsom et al., 2004; Hu et al., 2002), of which one in postmenopausal women (Folsom et al., 2004).

Several publications (also described under all-cause mortality) were based on multi-center studies or multiple cohorts, including Engeset et al. (2015) (EPIC study); Oomen et al.

(2000) (three countries from the Seven Countries Study); Takata et al. (2013) (the Shanghai Women's Health Study (SWHS) and the Shanghai Men's Health Study (SMHS)); Wallin et al. (2018) (the Cohort of Swedish Men (COSM) and the Swedish Mammography Cohort (SMC) limited to diabetes patients), and Zhuang et al. (2018) (comparison of the China Health and Nutrition Survey (CHNS) and US National Health and Nutrition Examination Survey III).

Pooled estimates (men and women, and/or cohorts combined) were extracted when available. Studies that stratified by sex or reported to have tested for effect modification by sex, reported a non-significant ($p \geq 0.05$) test of interaction, or no effect modification by sex (de Goede et al., 2010; Iso et al., 2006; Mozaffarian et al., 2003; Nakamura et al., 2005; Osler et al., 2003; Takata et al., 2013; Wallin et al., 2018; Yamagishi et al., 2008;). Country-specific estimates were presented and used for USA and China in Zhuang et al. (2018), and for Finland, Italy, and the Netherlands in Oomen et al. (2000) (lean fish only, for other types of fish only pooled estimates were presented).

4.7.13.6 *Studies by fish exposure*

All studies except one (Mozaffarian et al., 2003) with estimates of CHD mortality, included an overall fish exposure (sum of all fish, unspecified fish, or fish including shellfish and/or fish products). Other sub-classifications were fatty or lean, and by aquatic environment (saltwater/freshwater or seawater/lake). One study grouped fish intake by preparation method (fried or non-fried). The studies of CHD mortality in the general population ($n=19$, excluding patient populations) were summarized for total fish ($n=18$), and fatty/lean fish ($n=2$).

4.7.13.7 *Studies assessing potential non-linearity*

Albert et al. (1998) presented a figure (not shown in current report) of the relative risk of sudden cardiac death in relation to servings of fish per week in US men from the Physicians' Health Study. The statistical model (restricted cubic spline model with 4 knots) takes potential non-linearity into account.

4.7.13.8 *Studies with converted risk estimates*

Most studies presented relative risks of CHD mortality for categories of fish intake. Relative risk estimates were transformed to values with the lowest category as reference for three studies use other reference categories (Engeset et al. 2015; Nakamura et al. 2005; Osler et al. 2003). As the only study, Engeset et al. (2015) reported 99% confidence intervals (CIs), which were converted to 95% CIs for the high-low estimate before pooling with other studies to calculate summary RRs.

4.7.14 Results from the included primary studies on fish intake and CHD mortality

4.7.14.1 Studies of total fish intake and CHD mortality in the general population

We included 18 publications (all prospective, observational studies) with 20 estimates of the association between total fish intake and CHD mortality in the general population. The exposure levels and results (high-low relative risk, and overall) are included in Table 4.7.14.1-1.

Table 4.7.14.1-1 Results from prospective observational studies included in the weight of evidence analysis of fish intake and CHD mortality in the general population.

Author, year, country	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low (95% CI)	Overall result
Albert, 1998, USA	Fish, incl shellfish, M	Servings as main dish, 5 cat	≥5/wk vs <1/mo	308	0.81 (0.41, 1.61)	No sig. assoc., <i>P</i> -trend 0.49
Ascherio, 1995, USA	Fish, incl shellfish, M	Servings, 6 cat	≥6/wk vs 1/mo, 119 vs 0 g/d (mean)	264	0.77 (0.41, 1.44)	No sig. assoc., <i>P</i> -trend 0.14
Daviglus, 1997, USA	Fish, M	120-g units per 28 days, 4-point scale (0-3)	≥35 vs 0 g/d	430	0.62 (0.40, 0.94)	Protective assoc. of ≥35 vs 0 g, <i>P</i> -trend 0.04
de Goede, 2010, the Netherlands	Fish, incl shellfish, M/W	g/d, quartiles	Quartile 4 vs 1, >14 vs <3.3 g/d	82	0.52 (0.28, 0.95)	Protective assoc. of intake >7 g/d (quartiles 3,4), <i>P</i> -trend 0.02
Engeset, 2015, Europe (10 countries)	Fish, M	g/d, quintiles	Quintile 5 vs 1 (Q3 as orig ref cat), 76.2 vs 1.9 g/d	2215	1.19 (0.99, 1.45), reported as 1.23 (99% CI: 1.03, 1.47) for Q5 vs Q3 and 1.03 (99% CI: 0.85, 1.23) for Q1 vs Q3	Suggestive adverse assoc.
	Fish, W	g/d, quintiles	Quintile 5 vs 1 (Q3 as orig ref cat), 76.2 vs 1.9 g/d	1050	0.88 (0.65, 1.19), reported as 0.94 (99% CI: 0.71, 1.25) for Q5 vs Q3 and 1.07 (99% CI: 0.82, 1.41) for Q1 vs Q3	No sig. assoc. – pooled estimate
Farvid, 2017, Iran	Fish, M/W	Servings/d, 4 cat (tertiles of intake, null)	Cat 4 vs 1, 0.19 (median) vs 0 servings/d, standard serving size 85g	764	0.93 (0.75, 1.16)	No sig. assoc., <i>P</i> -trend 0.94
Folsom, 2004, USA	Fish, incl shellfish, W	Servings/wk, approx quintiles	≥2.5 vs <0.5 servings/wk	922	1.04 (0.80, 1.34)	No sig. assoc., <i>P</i> -trend 0.31
Hu, 2002, USA	Fish, incl shellfish, W	Servings/mo or wk, 5 cat, cumulative average	≥5/wk vs <1/mo	1029	0.55 (0.33, 0.91)	Protective assoc. of intake 1/wk or higher vs <1/mo, <i>P</i> -trend 0.01

Author, year, country	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low (95% CI)	Overall result
Iso, 2006, Japan	Fish, incl fish products, M/W	g/d, quintiles	Quintile 5 vs 1, 180 vs 23 g/d (median values)	62	1.08 (0.42, 2.76)	No sig. assoc., <i>P</i> -trend 0.31
Jarvinen, 2006, Finland	Fish, M	g/d, quintiles	Quintile 5 vs 1, 112.4 vs 5.8 g/d (mean values)	335	1.00 (0.70, 1.43)	No sig. assoc., <i>P</i> -trend 0.83
	Fish, W	g/d, quintiles	Quintile 5 vs 1, 70 vs 4.2 g/d (mean values)	163	0.59 (0.36, 0.99)	Protective assoc. of intake in quintile 5 vs 1, <i>P</i> -trend 0.02 - weaker in sensitivity analysis (<i>P</i> -trend=0.08)
Nakamura, 2005, Japan	Fish, M/W	Times/d or wk, 5 cat	≥2/d vs seldom (<1-2/wk as orig ref cat)	124	0.62 (0.17, 2.21), reported as 0.91 (0.35, 2.35) for ≥2/d vs <1-2/wk and 1.47 (0.63, 3.39) for seldom vs <1-2/wk	No sig. assoc., <i>P</i> -trend 0.54
Oomen, 2000, Finland, Italy, the Netherlands	Fish, M	g/d, 4 cat	≥40 vs 0 g/d	463	1.08 (0.76, 1.53)	No sig. assoc. - pooled estimate
Osler, 2003, Denmark	Fish, M/W	Times/mo or wk, 4 cat	≥2/wk vs ≤1/mo, NA	247	0.90 (0.51, 1.57) reported as 0.98 (0.62, 1.52) for ≥2/wk vs 1/wk (ref) and 1.09 (0.78, 1.52) for ≤1/mo vs 1/wk (ref)	No sig. assoc., <i>P</i> -trend 0.74
Salonen, 1995, Finland	Fish, M	g/d, binary	≥30 vs <30 g/d	18	2.38 (0.85, 6.71)	No sig. assoc.
Shao, 2021, China	Fish, M/W	Servings/wk, 4 cat	≥11 vs 0-3 servings/wk	397	0.99 (0.75, 1.32)	No sig. assoc., <i>P</i> -trend 0.79
Takata, 2013, China	Fish, incl shellfish, M/W	g/d, quintiles, sex-specific	Quintile 5 vs 1, 107.2 vs 10.8 g/d (men) and 105.2 vs 10.4 g/d (women)	476	1.02 (0.74, 1.41)	No sig. assoc., <i>P</i> -trend 0.90

Author, year, country	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low (95 % CI)	Overall result
Yamagishi, 2008, Japan	Fish, M/W	g/d, quintiles, energy adjusted	Quintile 5 vs 1, 72 to 229 (Q5) vs 0 to 27 (Q1) g/d, ranges	419	0.86 (0.62, 1.19)	No sig. assoc., <i>P</i> -trend 0.41
Zhuang, 2018, China, USA	Fish, M/W, NHANES/USA only	g/d, 4 cat (null, tertiles among consumers)	Tertile 3 vs null, >8.9 vs 0 g/d	1127	0.90 (0.66, 1.22)	No sig. assoc., <i>P</i> -trend 0.17

4.7.14.2 *Studies of fatty- and lean fish intake and CHD mortality in the general population*

The two publications with results on fatty and lean fish intake were both multicentre studies presenting pooled risk estimates from the EPIC study, and pooled and country-specific estimates from parts of the Seven Countries Study (Finland, Italy, the Netherlands), see Table 4.7.14.2-1.

Table 4.7.14.2-1 Results from prospective observational studies included in the weight of evidence analysis of fatty and lean fish intake and CHD mortality in the general population.

Author, year, country	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low (95 % CI)	Overall result
Fatty fish						
Engeset, 2015, Europe (10 countries)	Fatty fish, M	g/d, quintiles	Quintile 5 vs 1 (Q3 as orig ref cat), 35.6 vs 0.1 g/d	1994	0.95 (0.77, 1.16), reported as 0.92 (99% CI: 0.83, 1.12) for Q5 vs Q3 and 0.97 (99% CI: 0.81, 1.16) for Q1 vs Q3	No sig. assoc. - pooled estimate
	Fatty fish, W	g/d, quintiles	Quintile 5 vs 1 (Q3 as orig ref cat), 33.9 vs 0.2 g/d	944	0.97 (0.70, 1.33), reported as 1.14 (99% CI: 0.83, 1.57) for Q5 vs Q3 and 1.18 (99% CI: 0.91, 1.54) and Q1 vs Q3	No sig. assoc. - pooled estimate

Author, year, country	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low (95 % CI)	Overall result
Oomen, 2000, Finland, Italy, the Netherlands	Fatty fish, M	g/d, 3 cat	≥20 vs 0 g/d	463	0.87 (0.59, 1.27)	Suggestive protective. Sig. reduced risk for intake 1-19 g/d but not ≥20 g/d vs 0 - pooled estimate
Lean fish						
Engeset, 2015, Europe (10 countries)	Lean fish, M	g/d, quintiles	Quintile 5 vs 1 (Q3 as orig ref cat), 50.4 vs 0.1 g/d	1994	0.76 (0.61, 0.95), reported as 0.96 (99% CI: 0.79, 1.17) for Q5 vs Q3 and 1.26 (99% CI: 1.00, 1.57) for Q1 vs Q3	Protective assoc. – pooled estimate
	Lean fish, W	g/d, quintiles	Quintile 5 vs 1 (Q3 as orig ref cat), 52.0 vs 0.1 g/d	944	0.89 (0.65, 1.23) reported as 0.98 (99% CI: 0.72, 1.32) for Q5 vs Q3 and 1.10 (99% CI: 0.82, 1.48) for Q1 vs Q3	No sig. assoc. – pooled estimate
Oomen, 2000, Finland, Italy, the Netherlands	Lean fish, M	g/d, 4 cat or 3 cat	≥40 vs 0 g/d or ≥20 vs 0 g/d (the NL only)	463	Finland 1.08 (0.78, 1.50), Italy 0.80 (0.38, 1.66), the NL 1.29 (0.82, 2.03)	No sig. assoc. - no pooled estimate provided. <i>P</i> -trend Finland 0.63, Italy 0.57, the NL 0.27

None of the two pooled studies found a statistically significant association for the highest versus lowest intake of fatty fish intake with CHD mortality. The EPIC study reported a statistically significant protective association for the highest versus lowest intake of lean fish in men, but not in women. None of the country-specific estimates for lean fish in Oomen et al. (2000) were statistically significant (no overall estimate reported for all countries).

4.7.14.3 Studies of total fish intake and CHD mortality in patients

Two studies of secondary prevention included an estimate of the association between total fish intake and CHD mortality in patients with clinically established coronary artery disease. Both studies reported null findings with relative risks very close to 1 (null value) (Table 4.7.14.4-1). Almost all participants were treated for CHD, and 90% of the sample in Manger et al. (2010) were statin users. The only study of fish intake and CHD mortality in a population with type 2 diabetes (Cohort of Swedish Men and the Swedish Mammography Cohort combined), did not find statistically significant associations with total-, fatty- or lean fish (Table 4.7.14.3-1).

Table 4.7.14.3-1 Results from prospective observational studies included in the weight of evidence analysis of fish intake and CHD mortality in patients with coronary artery disease or type 2 diabetes.

Author, year, country	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low (95 % CI)	Overall result
Prior CHD/secondary prevention						
Erkkila, 2003, Finland	Fish, M/W	g/d, 3 cat (above/below median, null)	Cat 3 vs 1, >57 (above median) vs 0 g/d	16	1.04 (0.25, 4.31)	No sig. assoc., <i>P</i> -trend 0.73
Manger, 2010, Norway	Fish, incl fish products, M/W	g/d, quartiles	Quartile 4 vs 1, or Q2-4 vs 1, 200 vs 41.1 g/d (mean)	76	1.03 (0.54, 1.94)	No sig. assoc., <i>P</i> -trend 0.94
Diabetes populaion						
Wallin, 2018, Sweden	Fish, M/W	Servings/mo or wk, 4 cat	>3/wk vs ≤3/mo, 3.5 vs 0.5 servings/wk (median)	154	0.77 (0.43, 1.40)	No sig. assoc. (protective assoc. limited to cat 2 vs 1), <i>P</i> -trend 0.71
	Fatty fish (herring and mackerel) M/W	Servings/mo or wk, 3 cat	≥1/wk vs <1/mo, 1.5 vs 0 servings/wk (median)	154	1.00 (0.59, 1.67)	No sig. assoc., <i>P</i> -trend 0.58
	Fatty fish (salmon, whitefish, char), M/W	Servings/mo or wk, 3 cat	≥1/wk vs <1/mo, 1.5 vs 0 servings/wk (median)	154	0.80 (0.43, 1.48)	No sig. assoc., <i>P</i> -trend 0.42
	Lean fish, M/W	Servings/mo or wk, 3 cat	≥1/wk vs <1/mo, 1.5 vs 0 servings/wk (median)	154	0.74 (0.45, 1.20)	No sig. assoc. (protective assoc. limited to cat 2 vs 1), <i>P</i> -trend 0.24

4.7.14.4 Summary relative risks (RRs) based on VKM's inclusion of primary studies

For overall fish, VKM's high-low summary relative risk (RR) based on 18 studies (Table 4.7.14.1-1) indicated lower CHD mortality for high intakes (RR=0.91, 95% CI: 0.82, 1.01). The estimate was borderline statistically significant without significant heterogeneity ($P_{\text{heterogeneity}}=0.16$).

Compared with a previous meta-analysis, the high-low estimate in Zhang 2020 (based on a larger number and different selection of studies) was slightly stronger and statistically significant (RR=0.85, 95% CI: 0.77 to 0.94), but with some heterogeneity. The older meta-analysis by Zheng 2012 included a lower number of studies and reported estimates stratified by intake defined as (i) high (>5 servings/week), (ii) moderate (2–4 servings/week), (iii) low (1 serving/week) and (iv) very low (comparison group; >1 serving/month or 1–3 servings/month). Estimates (95% CI) were relatively similar to Zhang 2020 for all categories: RR=0.84 (0.75, 0.95) for low intake; RR=0.79 (0.67, 0.92) for moderate intake, and RR=0.83 (0.68, 1.01) for high intake.

VKM's high-low summary RR for fatty fish was 0.94 (95% CI: 0.81, 1.10) without significant heterogeneity ($P_{\text{heterogeneity}}=0.66$) and for lean fish 0.95 (95% CI: 0.75, 1.21), also without significant heterogeneity ($P_{\text{heterogeneity}}=0.15$), based on two pooled studies. The identified meta-analyses of CHD mortality (Zhang et al., 2020; Zheng et al., 2012) did not include summary RRs for fatty or lean fish.

VKM's high-low summary RR for CHD mortality in patients with coronary artery diseases was based on two studies of secondary prevention with few cases, and did not suggest an association with total fish intake (RR=1.03, 95% CI: 0.58, 1.84), $P_{\text{heterogeneity}}=0.99$).

4.7.14.5 VKM's search compared to previous meta-analyses on CHD mortality

Zhang et al. (2020) included 25 publications on CHD mortality, of which 9 were not included in VKM's pooled estimate. Of these, all except one publication (Kaushik et al., 2008) was identified in VKM's search but were excluded for different reasons, either after quality assessment (Mann et al., 1997, Tomasallo et al., 2010), or because the outcome was myocardial infarction (Kuhn et al., 2013, Gammelmark et al., 2016, Yuan et al., 2001), which was summarized as a separate outcome. Zhang et al. (2020) also included studies that VKM considered to be overlapping (Kromhout et al., 1985 covered by Oomen et al., 2000) or in selected populations such as smokers only (Rodriguez et al., 1996). The publication which was not identified (Kaushik et al., 2008) focused on retinal microvascular signs and vascular mortality rather than CHD mortality. VKM identified three publications, one older (Salonen et al., 1995) and two more recent (Zhuang et al. 2018; Shao et al. 2021) that were not included in Zhang et al. (2020).

The older meta-analysis by Zheng et al. (2012) included 14 publications, all included in Zhang et al. (2020) and identified by VKM, but two were excluded after quality assessment (Mann et al., 1997, Tomasallo et al., 2010) and one was included in the analysis of myocardial infarction (Yuan et al., 2001).

Table 4.7.14.5-1 Overview of prospective cohort studies included by VKM compared with two identified meta-analyses on coronary heart disease (CHD) mortality.

	Included by VKM	Meta-analyses	
Publications		Jayed 2020	Zheng 2012
Albert 1998	X	X	X
Ascherio 1995	X	X	X
Daviglus 1997	X	X	X
de Goede 2010	X	X	X
Engeset 2015	X	X	
Farvid 2017	X	X	
Folsom 2004	X	X	X
Hu 2002	X	X	X
Iso 2006	X	X	
Jarvinen 2006	X	X	X
Mozaffarian 2003	X	X	X
Nakamura 2005	X	X	
Oomen 2000	X	X	X
Osler 2003	X	X	
Salonen 1995	X		
Shao 2021	X		
Takata 2013	X	X	
Yamagishi 2008	X	X	X
Zhuang 2018	X		
Overlapping			
Kromhout 1985	X	X	X
Diabetes population			
Wallin 2018	X	X	
Secondary prevention			
Erkkila 2003	X		
Manger 2010	X		
Studies only in meta-analyses			
Gammelmark 2016		X	
Kaushik 2008		X	
Kuhn 2013		X	
Mann 1997		X	X
Rodriguez 1996		X	
Tomasallo 2010		X	X
Yuan 2001		X	X
<i>Studies evaluted</i>	<i>23</i>	<i>25</i>	<i>14</i>

4.7.15 Heterogeneity CHD mortality

Moderate heterogeneity was observed between studies in Zhang et al. (2020) ($I^2 = 51.2\%$). The heterogeneity was mainly in the magnitude, and not direction of association. There was no report of a statistically significant adverse association (evaluated from forest plot, not shown). Meta-regression to explore heterogeneity did not find significant covariates among publication year, continent, sex, follow-up period, method of evaluating fish consumption, adjustment (or not) for BMI, and adjustment (or not) for alcohol.

Zheng et al. (2012) reported low ($I^2 = 20.1\%$) and moderate ($I^2 = 56.7\%$) heterogeneity between studies for low and moderate fish intake, respectively. No heterogeneity was found for high intake of fish.

4.7.16 Dose-response relationship CHD mortality

Zhang et al. (2020) found that an increase in fish intake by 20 g/day was associated with a 4% reduction in CHD mortality. The non-linear dose-response curve (from restricted cubic spline model with 3 knots) suggested a threshold with no further reductions in risk for intakes higher than 60 g/day. Zheng et al. (2012) found that every 15 g/day increase of fish intake led to a significant reduction by 6% for CHD mortality.

4.7.17 Weight of evidence for fish intake and CHD mortality

In this section, the evidence of the association between fish intake and CHD mortality is weighed according to the WCRF criteria presented in Chapter 3.1.6 (Box 2).

Published evidence of fish intake and CHD mortality

The meta-analysis by Zhang et al. (2020) indicated a protective association of fish intake with CHD mortality (23 studies). The summary RR for primary studies included by VKM (18 studies) also indicated lower CHD mortality for high intakes, but the overall association was only borderline statistically significant. Three studies of CHD mortality in patient populations, two on secondary prevention and one in a sub-population with type 2 diabetes, showed no statistically significant associations.

VKM's summary RRs showed no statistically significant associations for intake of fatty fish or lean fish in relation to CHD mortality.

Heterogeneity

No significant heterogeneity was observed between primary studies included by VKM on total fish intake and CHD mortality. Moderate heterogeneity was found in the meta-analyses by Zhang et al. (2020).

Mechanism

There is evidence for several plausible mechanisms operating in humans (see Chapter 4.1).

Upgrading factors

Dose-response is an upgrading factor. An increase in fish intake by 20 g/day was associated with a 4% reduction in CHD mortality in a meta dose-response analyses by Zhang et al. (2020). The non-linear analysis suggested a threshold with no further reductions in risk for intakes higher than 60 g/day.

4.7.17.1 Conclusion weight of evidence fish intake and CHD mortality

There is evidence from more than two independent and good quality cohort studies on total fish intake (VKM included 18 studies in the general population, three in patients, and two previous meta-analyses, including a dose-response meta-analysis).

VKM's summary RR for primary studies in the general population is borderline statistically significant and suggests lower risk of CHD mortality for the highest versus lowest intake of total fish, which is supported by previous meta-analyses. The direction of association among studies included by VKM is generally consistent towards protective or null. There is evidence for biological plausibility and a dose-response relation.

In conclusion, the evidence is graded "probable" for a protective effect of fish intake on CHD mortality. VKM's summary RR for CHD mortality in patients with coronary artery disease is based on two studies of secondary prevention with few cases and do not suggest an association with total fish intake.

There were fewer studies of fatty fish and lean fish (two in total, both pooled analysis) than of total fish, and the evidence is graded "limited, no conclusion" for the effects of fatty and lean fish on CHD mortality.

4.7.18 VKM's systematic review of primary studies on fish intake and mortality from myocardial infarction (MI)

4.7.18.1 Included studies from search

We included five publications on mortality from MI: Daviglus et al. (1997); de Goede et al. (2010); Kuhn et al. (2013); Yamagishi et al. (2009); Yuan et al. (2001).

Three studies (Daviglus et al., 1997; Yamagishi et al., 2008; Yuan et al., 2001) are among the studies of all-cause mortality (Table 4.7.1.3-1) and two additional studies (de Goede et al., 2010; Kuhn et al. (2013) are described in Table 4.7.18.1-1.

Table 4.18.1-1 Overview of primary studies included in weight of evidence analysis of myocardial infarction mortality not described under all-cause mortality.

Author, year, country	Study name	Study design	Inclusion year(s), end, follow-up time	Study size, age	Dietary assessment method	Dietary assessment period
Kuhn, 2013, Germany	EPIC-Germany	Prospective cohort	1994-1998 to 2006, 8.1 yrs follow-up (mean)	48 315 (42% male), 35-65 yrs, mean age 50.5 yrs	FFQ, uncertain validity	Usual intake during the previous year, at baseline
de Goede, 2010, the Netherlands	Monitoring Project on Risk Factors for Chronic Diseases (MORGEN)	Prospective cohort	1993-1997 to 2006 or 2007 (mortality only), 11.3 yrs follow-up (mean)	21 342 men and women, 45% male, 20-65 yrs (mean age 42 yrs)	FFQ, validated	Usual freq of consumption in previous year, at baseline

4.7.18.2 *Studies by design and geographic region*

All five studies were based on data from prospective cohorts, one study (EPIC Germany) combined two independent cohorts (centers in Heidelberg and Potsdam). The study populations were distributed between Asia (2 studies), Europe (2 studies) and USA (one study). Mohan et al. 2021 was a global multicenter study.

4.7.18.3 *Studies by sex, potential effect modification, and other sub-groups*

Two studies included men only (Daviglus et al., 1997; Yuan et al., 2001). The remaining three studies reported estimates for men and women combined and reported no effect modification by gender (de Goede et al., 2010; Kuhn et al., 2013; Yamagishi et al., 2008). One study presented MI in men further stratified by sudden and non-sudden deaths (Daviglus et al., 1997), which was considered insufficient for a summary.

4.7.18.4 *Studies by fish exposure*

All studies included a total fish exposure (sum of all fish, unspecified fish, or fish including shellfish and/or fish products). Yuan et al., 2001 presented fish with and without shellfish. The result without shellfish was used in the current summary. Other sub-classifications of fish were not found among studies on MI mortality.

4.7.18.5 *Studies assessing potential non-linearity*

Kuhn et al. (2013) investigated potential non-linearity using restricted cubic-spline regression analyses and did not find significant departure from linearity for any of the CVD endpoints.

4.7.19 **Results from the included primary studies on myocardial infarction (MI) mortality**

4.7.19.1 *Studies of total fish intake and MI mortality in the general population*

The exposure levels (total fish) and results (high-low relative risk, and overall) in the five included publications with 5 estimates of the association of overall fish intake with MI mortality are included in Table 4.7.19.1-1. Three studies reported a statistically significant protective association for the highest intake, the other two reported null findings but on the protective side.

Table 4.7.19.1-1 Results from prospective observational studies included in the weight of evidence analysis of fish intake and all-cause mortality from myocardial infarction (MI) in the general population.

Author, year, country	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low (95 % CI)	Overall result
Daviglus, 1997, USA	Fish, M	120-g units per 28 days, 4-point scale (0-3)	8 vs 0 units, 35 vs 0	293	0.56 (0.33, 0.93)	Protective assoc. for ≥ 35 vs 0 g/d, P -trend 0.017
de Goede, 2010, the Netherlands	Fish, incl shellfish, M/W	g/d, quartiles	Quartile 4 vs 1, >14 vs <3.3 g/d	64	0.40 (0.19, 0.86)	Protective assoc. for intake >14 vs <3.3 g/d (quartile 4), P -trend <0.01
Yuan, 2001, China	Fish, M	g/week, 5 cat	≥ 150 vs <30 ,	113	0.35 (0.17, 0.72)	Protective assoc. of 30 – <60 and ≥ 150 vs <30 , P -trend=0.02
Kuhn, 2013, Germany	Fish, M/W	g/d, quintiles	Quintile 5 vs 1, >31.1 (median 40.4) vs <7.5 (median 2.7) g/d	117	0.84 (0.66, 1.08)	No sig. assoc., P -trend 0.21
Yamagishi, 2008, Japan	Fish, M/W	g/d, quintiles, energy adjusted	Quintile 5 vs 1, 72 to 229 (Q5) vs 0 to 27 (Q1) g/d, ranges	329	0.77 (0.53, 1.10)	No sig. assoc., P -trend 0.22

4.7.19.2 *Summary relative risk (RR) based on VKM's inclusion of primary studies*

For fish overall, the high-low summary relative risk (RR) based on five studies (Table 4.7.19.1-1) indicated significantly lower MI mortality for the highest vs. lowest intakes (RR=0.63, 95% CI: 0.46, 0.85). Heterogeneity was significant ($P_{\text{heterogeneity}}=0.01$), but all estimates were consistent in the direction of the association.

4.7.19.3 *VKM's search compared to previous meta-analyses MI mortality*

No previous meta-analysis of MI mortality only was found for a comparison of summary estimates and heterogeneity between studies, or for a dose-response relationship. The included meta-analyses on CVD or CHD mortality (Zhang et al., 2020; Zheng et al., 2012) did not make a clear distinction between results on CHD and MI (a more specific diagnosis). Thus, summary estimates were not presented for MI specifically.

The inclusion of results on MI from some primary studies may partly explain why the estimates for CHD in previous meta-analyses tended to be stronger (more protective) than VKM's summary RR, which included studies of CHD overall.

4.7.20 Weight of evidence for fish intake and MI mortality

In this section, the evidence of the association between fish intake and MI mortality is weighed according to the WCRF criteria presented in Chapter 3.1.6 (Box 2).

Published evidence of fish intake and MI mortality

The high-low summary RR for the five primary studies included by VKM indicates significantly lower mortality from MI with higher fish intake. There were no previous meta-analyses of MI limited to studies of mortality for comparison.

Heterogeneity

Borderline significant heterogeneity was observed between primary studies included in the summary RR calculated by VKM, but the heterogeneity reflected differences in the magnitude of the protective association, not direction.

Mechanism

There is evidence for several plausible mechanisms operating in humans (Chapter 5.2).

Upgrading factors

No substantial upgrading factors were evaluated or found.

4.7.20.1 *Conclusion weight of evidence fish intake and mortality from MI*

There is evidence from more than two independent and good quality prospective cohort studies (VKM included 5 studies in the general population, no previous meta-analysis). The published evidence indicates a protective association between fish intake and MI mortality that is consistent in direction. There is evidence for biological plausibility.

In conclusion, the evidence that consumption of fish reduces MI mortality is graded “probable”. The effects of fatty and lean fish on MI mortality could not be assessed as no studies were identified.

4.7.21 VKM’s systematic review of primary studies on fish intake and mortality from stroke and stroke sub-types

4.7.21.1 *Included studies from search*

We evaluated a total of 13 publications graded A or B with mortality from stroke as outcome, of which one (Deng et al., 2018) was limited to a sub-population with T2D. Total stroke (sum of ischemic, hemorrhagic, and unspecified strokes) was included in 11 publications (Deng et al., 2018; Farvid et al., 2017; Folsom et al., 2004; Kinjo et al., 1999; Nakamura et al., 2005; Oencia et al., 1996; Sauvaget et al., 2003; Shao et al., 2021, China; Yamagishi et al., 2008; Yuan et al., 2001; Zhuang et al., 2018), and all cerebrovascular disease in one publication (Zhang et al., 2018). One publication included sub-types of stroke (ischemic and hemorrhagic) without total stroke (Takata et al., 2013) and estimates were combined into total stroke by VKM. Thus, total stroke/cerebrovascular disease was assessed in all 12 studies in the general population.

Of the 13 publications, 10 are described (study name, design, time period, size and age of the study population, and dietary assessment method) among studies of all-cause mortality (Table 4.7.1.3-1). Studies of stroke mortality that did not contribute results on all-cause mortality (Kinjo et al., 1999; Oencia et al., 1996; Sauvaget et al., 2003) are described in Table 4.7.21.1-1.

Table 4.7.21.1-1 Overview of primary studies included in weight of evidence analysis of stroke mortality not described under all-cause mortality.

Author, year, country	Study name	Study design	Inclusion year(s), end, follow-up time	Study size, age	Dietary assessment method	Dietary assessment period
Kinjo, 1999, Japan	Hirayama Cohort Study	Prospective cohort	1966 to 1981	223 170 men and women, ≥ 40-69 yrs	Questionnaire	At baseline, no other specification
Orencia, 1996, USA	Chicago Western Electric Study	Prospective cohort	1957-1958, 30 yrs follow-up	1847 men, 40-55 yrs	Standardized interviews and questionnaires based on Burke's diet history method	Previous 28 days, at baseline and 1 year later, average
Sauvaget, 2003, Japan	Hiroshima/Nagasaki Life Span Study	Prospective cohort	1980 (men)-1981 (women) to 1996	37 130 (38% men), 34-103 yrs, mean age 56 yrs	FFQ, validated	Not specified

4.7.21.2 *Overlapping publications*

As described under all-cause mortality, both Deng et al. (2018) and Zhuang et al. (2018) used data from NHANES III, but because Deng et al. (2018) was limited to patients with type 2 diabetes, both publications were kept.

4.7.21.3 *Studies by sub-types of stroke mortality (ischemic or hemorrhagic)*

Six publications assessed fish intake in relation to risk of ischemic stroke and hemorrhagic stroke (Kinjo et al., 1999; Nakamura et al., 2005; Sauvaget et al., 2003; Shao et al. 2021, China; Takata et al., 2013; Yamagishi et al., 2008). These publications were studies of total stroke/cerebrovascular disease that also included results on stroke sub-types, except Takata et al. (2013), which included sub-types only. Yamagishi et al. (2008) presented hemorrhagic stroke by further sub-categorization (intraparenchymal or subarachnoid hemorrhagic stroke). The risk estimates were combined into one estimate (by fixed-effect meta-analysis) for total stroke (Takata et al. 2013) or hemorrhagic stroke (Yamagishi et al. 2008) before calculating VKM's summary estimates.

4.7.21.4 *Studies by design and geographic region*

Among the 13 included publications, the majority were based on study populations in Asia (8 studies; 4 from Japan, 3 from China and 1 from Iran) followed by USA (5 studies, including one in a type 2 diabetes sub-population). One study combined data from China and USA (Zhuang et al., 2018), but cause-specific mortality outcomes were unavailable in the Chinese data, so the study was counted among studies from USA. None of the studies were conducted in Europe. All studies had a prospective observational design (cohort, or health examination survey with follow-up).

4.7.21.5 *Studies by sex, potential effect modification, and other sub-groups*

Most studies (10 of 13) included both men and women (Deng et al., 2018; Farvid et al., 2017; Kinjo et al., 1999; Nakamura et al., 2005; Sauvaget et al., 2003; Shao et al. 2021; Takata et al., 2013; Yamagishi et al., 2008; Zhang et al., 2018; Zhuang et al., 2018). Pooled estimates for men and women combined were used when available. Few studies reported to have tested for effect modification by sex in analyses of stroke mortality, but these studies (Nakamura et al., 2005; Takata et al., 2013; Yamagishi et al., 2008), found a non-significant ($p \geq 0.05$) test of sex interaction, or no such effect. The test for sex interaction was borderline significant ($P_{\text{interaction}}=0.06$) for hemorrhagic stroke in Takata et al. (2013), but men and women were from different cohort studies (Shanghai Women's Health Study (SWHS) and the Shanghai Men's Health Study (SMHS)) that differed in aspects other than gender, including the length of follow-up. Two studies included only men (Orencia et al., 1996, Yuan et al., 2001) and one study included only post-menopausal women (Folsom et al., 2004).

4.7.21.6 *Studies by fish exposure*

All studies of mortality from total stroke/cerebrovascular disease or stroke sub-types included a total fish exposure (sum of all fish, unspecified fish, or fish including shellfish and/or fish products). The number of studies with sub-classifications of fish was too limited for further analyses; one Chinese study (Takata et al., 2013) grouped fish intake by aquatic environment (saltwater, freshwater) and one Japanese study (Sauvaget et al., 2013) presented broiled fish as a separate category. Thus, total fish was the only fish exposure assessed in relation to total stroke mortality/cerebrovascular disease (n=12), ischemic stroke (n=6), and hemorrhagic stroke (n=6) in the general population.

4.7.21.7 *Studies assessing potential non-linearity*

No dose-response analyses were found among the included studies on stroke mortality that took potential non-linearity into account.

4.7.21.8 *Studies with converted relative risk estimates*

To facilitate comparisons with high-low meta-analyses, relative risk estimates for stroke mortality were transformed to the value for the lowest category as reference in one study that used a different reference category (Nakamura et al., 2005).

4.7.22 Results from the included primary studies on fish intake and stroke and stroke sub-types

4.7.22.1 *Studies of total fish intake and mortality from total stroke*

We included 12 publications with 14 estimates of the association between total fish intake and total stroke mortality in the general population. The exposure levels and results (high-low relative risk, and overall) are included in Table 4.7.22.1-1. Large studies with 1400 cases or more (Kinjo et al., 1999; Sauvaget et al., 2003; Zhang et al., 2018) all found a protective effect of the highest vs. lowest intake. Estimates from other studies were generally on the protective side or close to null. The only estimate in a T2D population (Deng et al., 2018) was also protective.

Table 4.7.22.1-1 Results from prospective observation studies included in the weight of evidence analysis of fish intake and mortality from total stroke.

Author, year, country	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low (95% CI)	Overall result
Farvid, 2017, Iran	Fish, M/W	Servings/d, 4 cat (tertiles of intake, null)	Cat 4 vs 1, 0.19 (median) vs 0 servings/d, standard serving size 85g	507	1.03 (0.78, 1.36)	No sig. assoc., <i>P</i> -trend= 0.90
Folsom, 2004, USA	Fish, incl shellfish, W	Servings/wk, approximate quintiles	≥2.5 vs <0.5 servings/wk	313	1.06 (0.67, 1.67)	No sig. assoc., <i>P</i> -trend 0.65
Kinjo, 1999, Japan	Fish, M/W	Times/wk, 3 cat	≥4/wk vs <1/wk	11030	0.86 (0.79, 0.94)	Sig. protective assoc. of intake ≥4/wk vs <1/wk
Nakamura, 2005, Japan	Fish, M/W	Times/d or wk, 5 cat	≥2/d vs seldom (<1-2/wk as orig ref cat)	288	0.94 (0.48, 1.84) for ≥2/day vs seldom, reported as 1.26 (0.70, 2.29) for ≥2/d vs 1-2/wk and 1.34 (0.73, 2.44) for seldom vs 1-2/wk	No sig. assoc., <i>P</i> -trend 0.52
Orencia, 1996, USA	Fish, M	g/d, 4 cat	Cat 4 vs 1, ≥35 g/d vs none	76	1.34 (0.53, 3.41)	No sig. assoc.
Sauvaget, 2003, Japan	Fish, excl. dry fish, M/W	Composite score, 3 cat	High vs low score, 46 vs 18 g/d (median)	1462	0.85 (0.75, 0.98)	Sig protective assoc. of high and moderate vs low intake, <i>P</i> -trend=0.017
Shao 2021, China	Fish, M/W	Servings/wk: 4 cat	≥11 vs 0-3 servings/wk	374	0.79 (0.58, 1.06)	Borderline protective assoc., <i>p</i> -trend 0.29
Takata, 2013, China	Fish, incl shellfish, M/W	g/d, quintiles, sex-specific	Quintile 5 vs 1, 107.2 vs 10.8 g/day (men) and 105.2 vs 10.4 g/day (women)	864	0.76 (0.57, 1.02)	Borderline protective assoc., estimates for ischemic and hemorrhagic stroke combined by VKM using fixed effects meta-analysis

Author, year, country	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low (95 % CI)	Overall result
Yamagishi, 2008, Japan	Fish, M/W	g/d, quintiles, energy adjusted	Quintile 5 vs 1, 72 to 229 (Q5) vs 0 to 27 (Q1) g/d, ranges	972	0.91 (0.74, 1.13)	No sig. assoc., <i>P</i> -trend 0.40
Yuan, 2001, China	Fish, M	g/wk, 5 cat	≥150 vs <30	480	1.05 (0.77, 1.43)	No sig. assoc., <i>P</i> -trend 0.47
Zhang 2018, USA	Fish, incl shellfish, M	g/d, quintiles	Quintile 5 vs 1, ≥30.03 vs ≤6.25 g/d	2134	0.81 (0.70, 0.93)	Protective assoc. of intake in Q4-Q5 vs Q1, <i>P</i> -trend 0.0015
	Fish, incl shellfish, W	g/d, quintiles	Quintile 5 vs 1, ≥25.38 vs ≤4.61 g/d	1602	0.83 (0.71, 0.98)	Protective assoc., <i>P</i> -trend 0.014
Zhuang, 2018, China, USA	Fish, M/W, NHANES only	g/d, 4 cat (null, tertiles among consumers)	Tertile 3 vs null, >8.9 vs 0 g/d	368	0.58 (0.32, 1.06)	Protective trend, <i>P</i> -trend 0.05
T2D population						
Deng, 2018, USA	Fish, incl shellfish, M/W	Times/wk, 3 cat	>2 vs 1/wk	51	0.30 (0.11, 0.80)	Protective assoc. of intake >2 vs <1/wk, <i>P</i> -trend <0.001

NHANES=US National Health and Nutrition Examination Survey.

4.7.22.2 Studies of total fish intake and mortality from ischemic stroke

We included six publications with six estimates of the association between total fish intake and ischemic stroke mortality in the general population. The exposure levels and results (high-low relative risk, and overall) are included in Table 4.7.22.2-1.

Table 4.7.22.2-1 Results from prospective observation studies included in the weight of evidence analysis of fish intake and mortality from ischemic stroke.

Author, year, country	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low (95% CI)	Overall result
Kinjo, 1999, Japan	Fish, M/W	Times/wk, 3 cat	≥4/wk vs <1/wk	4084	0.99 (0.86, 1.14)	No sig. assoc.
Nakamura, 2005, Japan	Fish, M/W	Times/d or wk, 5 cat	≥2/d vs seldom (<1-2/wk as orig ref cat)	165	1.09 (0.22, 5.34) for ≥2/d vs seldom, reported as 1.09 (0.48, 2.43) for ≥2/d vs 1-2/wk and 1.00 (0.43, 3.23) for seldom vs 1-2/wk	No sig. assoc., <i>P</i> -trend 0.72
Sauvaget, 2003, Japan	Fish, excl. dry fish, M/W	Composite score: 3 cat	High vs low score, 46 vs. 18 g/d (median)	655	0.94 (0.77, 1.14)	No sig. assoc., <i>P</i> -trend=0.50
Shao 2021, China	Fish, M/W	Servings/wk: 4 cat	≥11 vs 0-3 servings/wk	111	0.70 (0.40, 1.22)	Null, <i>p</i> -trend 0.21
Takata, 2013, China	Fish, incl shellfish, M/W	g/d, quintiles, sex-specific	Quintile 5 vs 1, 107.2 vs 10.8 g/d (men) and 105.2 vs 10.4 g/d (women)	404	0.63 (0.41, 0.94)	Protective assoc. (quintiles 5 vs 1), <i>P</i> -trend 0.04
Yamagishi, 2008, Japan	Fish, M/W	g/d, quintiles, energy adjusted	Quintile 5 vs 1, 72 to 229 (Q5) vs 0 to 27 (Q1) g/d, ranges	319	0.93 (0.65, 1.34)	No sig. assoc., <i>P</i> -trend 0.78

NHANES=US National Health.

4.7.22.3 *Studies of total fish intake and mortality from hemorrhagic stroke*

The studies on ischemic stroke also presented results on hemorrhagic stroke. Thus, we included 6 publications with 7 estimates of the association between total fish intake and hemorrhagic stroke mortality in the general population. The exposure levels and results (high-low relative risk, and overall) are included in Table 4.7.22.3-1.

Table 4.7.22.3-1 Results from prospective observation studies included in the weight of evidence analysis of fish intake and mortality from hemorrhagic stroke.

Author, year, country	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low (95 % CI)	Overall result
Kinjo, 1999, Japan	Fish, M/W	Times/wk, 3 cat	≥ 4/wk vs <1/wk,	4773	0.87 (0.76, 0.98)	Sig. protective association of intake ≥ 4/wk vs <1/wk
Nakamura, 2005, Japan	Fish, M/W	Times/d or wk, 5 cat	≥2/d vs seldom (<1-2/wk as orig ref cat),	63	1.67 (0.18, 15.2) for ≥2/d vs seldom, reported as 0.92 (0.20, 4.23) for ≥2/d vs 1–2/wk and 0.55 (0.07, 4.37) for seldom vs 1–2/wk	No sig. assoc., <i>P</i> -trend 0.98
Sauvaget, 2003, Japan	Fish, excl. dry fish, M/W	Composite score, 3 cat	High vs low score, 46 vs. 18 g/d (median)	470	0.70 (0.54, 0.92)	Sig. protective assoc. of high and moderate vs low intake, <i>P</i> -trend=0.008
Shao 2021, China	Fish, M/W	Servings/wk: 4 cat	≥11 vs 0-3 servings/wk	97	1.04 (0.59, 1.81)	Null, p-trend 0.72
Takata, 2013, China	Fish, incl shellfish, M/W	g/d, quintiles, sex-specific	Quintile 5 vs 1, 107.2 vs 10.8 g/d (men) and 105.2 vs 10.4 g/d (women)	460	0.90 (0.43, 1.87)	No sig. assoc., <i>P</i> -trend 0.68
Yamagishi, 2008, Japan	Fish, M/W	g/d, quintiles, energy adjusted	Quintile 5 vs 1, 72 to 229 (Q5) vs 0 to 27 (Q1) g/d, ranges	223	Intraparenchymal hemorrhage: 0.95 (0.62, 1.47)	No sig. assoc., <i>P</i> -trend 0.58
	Fish, M/W	g/d, quintiles, energy adjusted	Quintile 5 vs 1, 72 to 229 (Q5) vs 0 to 27 (Q1) g/d, ranges	153	Subarachnoid hemorrhage: 0.96 (0.55, 1.68)	No sig. assoc., <i>P</i> -trend 0.84

Nakamura et al. (2005) had an extremely wide confidence interval for the re-calculated high-low risk estimate, due to very few events in the lowest category of fish intake.

4.7.22.4 *Summary relative risks (RRs) based on VKM's inclusion of primary studies*

For fish overall, the high-low summary relative risk (RR) based on 12 studies of total stroke mortality in the general population (Table 4.7.22.1-1) indicated lower mortality (RR=0.86, 95% CI: 0.81, 0.90) for the highest intake, without significant heterogeneity ($P_{\text{heterogeneity}}=0.64$). Stratified estimates for ischemic and hemorrhagic stroke in Takata et al. 2013 were entered as one pooled estimate for total stroke (fixed-effects meta-analysis). The three largest studies contributed 37% (Kinjo et al., 1999, Japan), 26% (Zhang et al., 2018, USA) and 15% (Sauvaget et al., 2003, Japan) relative weight. The summary RR only changed marginally when these studies were left out one by one (influence analysis). The summary RR for stroke mortality was slightly lower (more protective) than VKM's summary estimate for all CVD mortality (RR=0.92, 95% CI: 0.85, 1.00).

For fish overall in relation to sub-types of stroke the high-low summary RR based on 6 studies was statistically significant and indicated lower hemorrhagic stroke mortality (RR=0.86, 95% CI 0.78, 0.96, $P_{\text{heterogeneity}}=0.64$) for the highest intake. Stratified estimates for intraparenchymal and subarachnoid hemorrhagic stroke in Yamagishi 2008 were entered as one pooled estimate for hemorrhagic stroke (fixed-effects meta-analysis).

The high-low summary RR for ischemic stroke (based on the same 6 studies as hemorrhagic stroke) was on the protective side, but slightly smaller in magnitude compared with hemorrhagic stroke and not statistically significant (RR=0.92, 95% CI: 0.82, 1.03, $P_{\text{heterogeneity}}=0.36$). Heterogeneity between studies was not statistically significant for stroke sub-types.

4.7.22.5 *VKM's search compared to previous meta-analyses on stroke mortality*

VKM's literature search did not identify any previous systematic reviews and meta-analyses of fish intake and stroke mortality for comparison, only one continuous dose-response meta-analysis of cardiovascular (CVD) disease mortality as a composite outcome, including stroke (Jayedi et al., 2018).

4.7.23 Heterogeneity stroke mortality

VKM's summary RR of primary studies on stroke mortality did not suggest significant heterogeneity between studies. The primary studies were from Asia and USA, without any evidence from European studies, which could contribute to lower heterogeneity.

4.7.24 Dose-response relationship stroke mortality

In the absence of previous meta-analyses, the evidence of a dose-response relationships between fish intake and stroke, was largely limited to tests for linear trend across exposure categories in the included primary studies.

4.7.25 Weight of evidence for fish intake and stroke mortality

In this section, the evidence of the association between fish intake and stroke mortality is weighed according to the WCRF criteria presented in Chapter 3.1.6 (Box 2).

Published evidence of fish intake and stroke mortality

No previous meta-analyses of the association between fish intake and stroke mortality were found. The pooled estimate from the primary studies on fish intake and stroke mortality indicated significant lower mortality for high intakes. The same was observed for sub-types of stroke (ischemic and hemorrhagic stroke), but only borderline significant for ischemic stroke.

Heterogeneity

No significant heterogeneity was observed between studies on total fish intake and mortality from stroke or stroke sub-types included by VKM.

Mechanism

There is evidence for several plausible mechanisms operating in humans (see Chapters 4.1, and 5.2).

Upgrading factors

No upgrading factors were evaluated.

4.7.25.1 Conclusion weight of evidence fish intake and mortality from stroke

There is evidence from more than two independent and good quality cohort studies on total fish (VKM identified 12 studies in the general population, one in patients, but no previous meta-analysis). The published evidence indicates a protective association between fish intake and stroke mortality.

VKM's summary RR for primary studies in the general population shows statistically significant lower risk of stroke mortality for the highest versus lowest intake of total fish. The direction of the association is generally consistent (on the protective side or close to null). There is evidence for biological plausibility.

In conclusion, the evidence that consumption of fish reduces stroke mortality is graded “probable”. Current evidence was more limited for sub-types of stroke and the evidence is graded “limited, suggestive” for a protective effect of total fish on both ischemic stroke- and hemorrhagic stroke mortality.

The effect of fatty and lean fish on stroke mortality could not be assessed as no studies were identified.

4.7.26 VKM’s systematic review of primary studies on fish intake and mortality from type 2 diabetes

Four publications among those included in the summary of all-cause mortality (Table 4.7.1.3-1), presented results on cause-specific mortality from diabetes (Deng et al., 2018; Takata et al., 2013; Zhang et al., 2018; Zhuang et al., 2018). Deng et al. (2018) and Zhuang et al. (2018) both used data from NHANES with follow-up. Deng 2018 was limited to participants with T2D and both were kept.

The publications on diabetes mortality were based on studies with a prospective, observational design (cohort, or health examination survey with follow-up) from China (Takata et al., 2013), or USA (Deng et al., 2018, Zhang et al., 2018, Zhuang et al., 2018). Takata et al. (2013) combined data from the Shanghai Women’s Health Study (SWHS) and the Shanghai Men’s Health Study (SMHS). Zhuang et al. (2018) included data from both China and USA, but cause-specific mortality could only be analyzed using the US data (NHANES study). All studies included a total fish exposure (sum of all fish, unspecified fish, or fish including shellfish and/or fish products), and both men and women were included in the study samples. The number of studies with sub-classifications of fish was too limited for further analyses.

4.7.27 Results from the included primary studies on fish intake and T2D

The exposure levels and results (high-low relative risk, and overall) of the four included studies with five estimates of the association between fish intake and risk of mortality from diabetes are shown below. One study reported a statistically significant protective association. Results from other studies, including the study in type 2 diabetes patients, were not statistically significant.

Table 4.7.27-1 Results from prospective observational studies included in the weight of evidence analysis of fish intake and mortality from type 2 diabetes.

Author, year, country	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low (95% CI)	Overall result
Takata, 2013, China	Fish, incl shellfish, M/W	g/d, quintiles, sex-specific	Quintile 5 vs 1, 107.2 vs 10.8 g/d (men) and 105.2 vs 10.4 g/d (women)	333	0.61 (0.39, 0.95)	Protective assoc. (quintiles 4-5 vs 1), <i>P</i> -trend 0.005
Zhang, 2018, USA	Fish, incl shellfish, M	g/d, quintiles	Quintile 5 vs 1, ≥ 30.03 vs ≤ 6.25 g/d	1038	1.16 (0.96, 1.38)	No sig. assoc., <i>P</i> -trend 0.21
	Fish, incl shellfish, W	g/d, quintiles	Quintile 5 vs 1, ≥ 25.38 vs ≤ 4.61 g/d	701	0.90 (0.70, 1.15)	No sig. assoc., <i>P</i> -trend 0.56
Zhuang, 2018, China, USA	Fish, M/W, NHANES	g/d, 4 cat (null, tertiles among consumers)	Tertile 3 vs null, >8.9 vs 0 g/d	184	1.45 (0.56, 3.73)	No sig. assoc., <i>P</i> -trend 0.37
T2D population						
Deng, 2018, USA	Fish, incl shellfish, M/W, diabetics	Times/wk, 3 cat	>2 vs 1/wk	303	0.91 (0.66, 1.24)	No sig. assoc.

4.7.27.1 *Summary relative risk (RR) based on VKM's inclusion of primary studies*

The summary estimate for the highest versus lowest intake of total fish in the three studies in the general population (excluding Deng et al., 2018) was not statistically significant with a wide confidence interval (RR=0.92, 95% CI: 0.59, 1.43) and borderline significant heterogeneity ($P_{\text{heterogeneity}}=0.048$).

4.7.27.2 *VKM's search compared to previous meta-analyses on type 2 diabetes mortality*

Except for Takata et al. (2013), the publications were relatively recent (from 2018). VKM did not identify any previous systematic reviews or meta-analyses of fish intake and diabetes mortality for comparison.

4.7.28 **Weight of evidence for fish intake and type 2 diabetes mortality**

In this section, the evidence of the association between fish intake and T2D mortality is weighed according to the WCRF criteria presented in Chapter 3.1.6 (Box 2).

Published evidence of fish intake and T2D mortality

No previous meta-analysis of fish intake and T2D mortality was found. The summary RR calculated by VKM for fish intake and T2D mortality was based on few studies and was not statistically significant with a wide confidence interval.

Heterogeneity

Borderline statistically significant heterogeneity was observed between the three primary studies included by VKM on total fish intake and T2D mortality.

Mechanism

There is evidence for several plausible mechanisms operating in humans (see Chapters 4.1, 5.2).

Upgrading factors

No upgrading factors were evaluated.

4.7.28.1 *Conclusion weight of evidence for fish intake and mortality from T2D*

There is evidence from more than two independent and good quality cohort studies on total fish (VKM identified 3 studies in the general population, one in a population with T2D, no previous meta-analysis).

VKM's summary RR for primary studies in the general population has a wide confidence interval and shows no statistically significant association between total fish intake and T2D mortality. There is evidence for biological plausibility, but not a dose-response relation. In conclusion, the evidence was graded "limited, no conclusion" for an effect of total fish intake on T2D mortality.

4.8 Fish intake and all-cause mortality

4.8.1 VKM's search for published systematic review and meta-analyses and on fish intake and all-cause mortality

See Chapter 4.7.1.

4.8.2 VKM's systematic review of primary studies on fish intake and all-cause mortality

4.8.2.1 Included studies from search

We evaluated 33 publications graded A or B with mortality from all causes as outcome; Albert et al. (1998); Barzi et al. (2003); Bellavia et al. (2017); Burr et al. (1989); Carballo-Casla et al. (2021); Daviglus et al. (1997); Deng et al. (2018); Engeset et al. (2015); Erkkila et al. (2003); Farvid et al. (2017); Folsom et al. (2004); Gillum et al. (2000); Hu et al. (2003); Manger et al. (2010); Mohan et al. (2021); Nahab et al. (2016); Nakamura et al. (2005); Osler et al. (2003); Otsuka et al. (2019); Owen et al. (2016); Salonen et al. (1995); Shao et al. (2021); Takata et al. (2013); van den Brandt et al. (2019); Villegas et al. (2015); Virtanen et al. (2019); Wallin et al. (2018); Woo et al. (2002); Yamagishi et al. (2008); Yuan et al. (2001); Zhang et al. (2018); Zhong et al. (2020); Zhuang et al. (2018).

There were multiple publications from the same studies, but most publications contributed unique information to different analyses. Only one was excluded (as described below) leaving 32 for further analysis. Of the 32 publications eight were in patients; five were in patients with established CHD (Barzi et al., 2003; Burr et al., 1989; Erkkila et al., 2003; Manger et al., 2010) and one (four sub-cohorts) in patients with a history of CVD or at high-risk of CVD. Three publications were on T2D patients only (Deng et al., 2018; Hu et al., 2003; Wallin et al., 2018). In addition, two included studies stratified results by T2D status (Villegas et al. 2015; Zhang 2018). Patient studies or results were summarized separately.

A description of the studies (study name, design, time period, size and age of the study population, and dietary assessment method) can be found in Table 4.7.1.3-1).

4.8.2.2 Overlapping publications

There were two publications from the Kuopio Ischaemic Heart Disease Risk Factor Study (KIDH) (Salonen et al., 1995; Virtanen et al., 2019), two from the pooled analysis of the Cohort of Swedish Men (COSM) and the Swedish Mammography Cohort (SMC) (Bellavia et al., 2017; Wallin et al., 2018), and three from the US National Health and Nutrition Examination Survey (NHANES) follow-up studies (Gillum et al., 2000, Deng et al., 2018, Zhuang et al., 2018).

Within the KIDH (Finland), Virtanen et al. (2019) had longer follow-up and more cases than Salonen et al. (1995). Therefore, only Virtanen et al. (2019) was kept in the main weight of evidence analysis. Regarding the Swedish cohorts (COSM, SMC), Bellavia 2017 analysed the full cohorts, while Wallin et al. (2018) restricted to T2D patients in the cohorts. Both were kept (see section on studies in T2D groups).

Deng et al. (2018), Zhuang et al. (2018), and Gillum et al. (2000) all used data from the US National Health and Nutrition Examination Survey (NHANES) with follow-up. Deng et al. (2018) was based on NHANES III but limited to participants with T2D. Zhuang et al. 2018 was also based on NHANES III and included results stratified by history of T2D. The results in Deng et al. 2018 was used in VKM's summary of all cause mortality in T2D patients, whereas Zhuang et al. 2018 contributed results on the general population. Gillum 2000 was based on NHANES I, which was understood to include a different sample of the US population than NHANES III. Therefore, all NHANES studies were kept.

4.8.2.3 Studies by design and geographic region

The body of evidence (32 publications, excluding one overlapping study) on all-cause mortality had a relatively even geographic distribution between Asia (8 studies, of which 1 from Iran, the rest from Japan, China, or Hong Kong), Europe (11 studies), and USA (10 studies). One study combined cohorts from China and USA, one study was from Australia, and Mohan et al. 2021 was a global multicenter study with data from 58 countries on 6 continents. Mohan et al. (2021) presented data on the general population but also on patient populations with a history of CVD or at high risk of CVD. The remaining four studies in CVD patients (survivors of CHD or MI) were conducted in European populations (Finland, Italy, Norway, or the UK). The three studies in patients with T2D were conducted in US populations (two studies) or in Sweden (one study).

All studies had prospective, observational designs (cohort, case-cohort, or health examination survey with follow-up). There were several multi-center studies or studies that combined data from multiple cohorts (Table 4.7.1.3-1). Studies in patients were mainly follow-up studies of trials or interventions.

4.8.2.4 Studies of all-cause mortality in patients with type 2 diabetes (T2DM)

Three publications (Deng et al., 2018; Hu et al., 2003; Wallin et al., 2018) were limited to sub-populations with T2D in established cohort studies: the Nurses' Health Study (NHS), the National Health and Nutrition Examination Survey (NHANES III) follow-up study, and the pooled analysis of the Cohort of Swedish Men (COSM) and the Swedish Mammography Cohort (SMC). In addition, two studies presented estimates stratified by T2D; the Southern Community Cohort Study (Villegas et al., 2015) and the National Institutes of Health (NIH)-AARP (American Association of Retired Persons) Diet and Health Study (Zhang et al., 2018). Thus, five studies contributed evidence on all cause-mortality in study participants with T2D. All, except Wallin et al. (2018) (Sweden) were conducted in US populations.

4.8.2.5 Studies of all-cause mortality in patients with CVD or at high risk

As described above, five publications (one RCT, else cohorts or cohorts based on RCTs) had mortality from all causes as outcome in patients with established CHD or MI (Barzi et al., 2003; Burr et al., 1989; Erkkila et al., 2003; Manger et al., 2010). Three (Burr et al., 1989; Erkkila et al., 2003; Manger et al., 2010) were secondary prevention studies of CHD that contributed evidence on multiple outcomes. In brief, these studies were based on the Diet and Reinfarction trial (DART) (Burr et al., 1989); the Western Norway B Vitamin Intervention Trial (WENBIT) (Manger et al., 2010); and the Finnish sub-cohort of the European Action on Secondary Prevention through Intervention to Reduce Events (EUROASPIRE) (Erkkila et al., 2003). The fourth study was based on the GISSI-Prevenzione clinical trial and had mortality as the only outcome (Barzi et al., 2003). The interventions in the DART and GISSI-Prevenzione trials were in the form of dietary advice; to eat at least two weekly portions of fatty fish (DART), or to increase food components of the Mediterranean diet, including fish (GISSI-Prevenzione). In GISSI-Prevenzione, all participants were given dietary advice and the intake was monitored during follow-up. The other two studies assessed the effect of fish intake measured at baseline, and all were analyzed as cohort studies except DART, which was the only RCT with a fish intervention group and a control group. Therefore, only Burr 1989 is considered to contribute results from an RCT.

4.8.2.6 Studies by sex, potential effect modification, and other sub-groups

Most of the 32 studies were conducted in both men and women (Barzi et al., 2003; Bellavia et al., 2017; Deng et al., 2018; Engeset et al., 2015; Erkkila et al., 2003; Farvid et al., 2017; Gillum et al., 2000; Manger et al., 2010; Nahab et al., 2016; Nakamura et al., 2005; Osler et al., 2003; Otsuka et al., 2019; Owen et al., 2016; Shao et al. 2021; Takata et al., 2013; van den Brandt et al., 2019; Villegas et al., 2015; Wallin et al., 2018; Woo et al., 2002; Yamagishi et al., 2008; Zhang et al., 2018; Zhuang et al., 2018). Several studies combined male and female cohorts, such as the Shanghai Women's Health Study (SWHS) and the Shanghai Men's Health Study (SMHS) (Takata 2013); the Cohort of Swedish Men (COSM) and the Swedish Mammography Cohort (SMC) (Bellavia et al., 2017; Wallin et al., 2018); and parts of the EPIC study (some female cohorts only). Pooled estimates for men and women combined were used when available. Except for Owen et al. (2016), the studies that reported to have tested for effect modification by sex, found a non-significant ($p \geq 0.05$) test of interaction, or no such effect (Farvid et al., 2017; Zhuang et al., 2018, in Chinese and US cohorts, separately). Owen et al. (2016) reported a statistically significant sex interaction for the relationships of total fish consumption and non-fried fish consumption with all-cause mortality ($P=0.021$) and only sex-specific estimates were presented. One US study (Gillum et al., 2000) presented sex specific estimates by race (white or black Americans), and all estimates were included.

Five studies included only men (Albert et al., 1998; Burr et al., 1989; Daviglus et al., 1997; Virtanen et al., 2019; Yuan et al., 2001) and two studies only women (Folsom et al., 2004;

Hu et al., 2003). One was conducted in postmenopausal women (Folsom et al., 2004) and one in women with T2D (Hu et al., 2003).

Several studies were multi-center studies, including EPIC (Engeset et al., 2015), the Seven Countries Study (three of seven countries included, Oomen et al., 2000), and the comparative analysis of the China Health and Nutrition Survey (CHNS) and US National Health and Nutrition Examination Survey III (Zhuang et al., 2018). We emphasized pooled estimates, but separate estimates were included for the Chinese and US populations in Zhuang 2018. The EPIC study (Engeset et al., 2015) presented both pooled and country-specific estimates, and the results for Norway are commented under Results (Chapter 4.8.3.1). The Norwegian sub-cohort of EPIC consists of women from the Norwegian Women and Cancer study (NOWAC).

Two studies had very large sample sizes (>400 000 subjects); EPIC (10 European countries: Spain, Greece, France, Italy, Germany, the Netherlands, the United Kingdom, Denmark, Sweden, and Norway) and the US National Institutes of Health (NIH)-AARP (American Association of Retired Persons) Diet and Health Study. These studies included over 30,000 deaths from EPIC (Engeset et al., 2015), and over 80 000 deaths from NIH-AARP (Zhang et al., 2018) in the weight of evidence analysis of all-cause mortality.

4.8.2.7 Studies by fish exposure

All studies, except Nahab et al. (2016), included a total fish exposure (sum of all fish, unspecified fish, or fish including shellfish and/or fish products). Relatively few studies presented sub-classification of fish intake. Classifications used were by preparation method (fried or non-fried), fat content (fatty or lean) and species (tuna only, and total fish excluding tuna). One Chinese study grouped fish intake by freshwater and saltwater fish (Takata et al., 2013).

Apart from studies in patients (summarized separately), the remaining studies (n=24) were summarized for total fish (n=23), non-fried fish (n=4) and fried fish (n=3). Only one of the 24 studies grouped fish by fat content. Some studies presented results by several fish classifications and contributed to multiple summaries.

The trials and intervention studies in patients with CHD or CVD (previous or high risk) examined the effect of fatty fish in one study (dietary advice intervention), or usual intake of fish after the diagnosis. Studies in populations with T2D assessed the intake of fish (Deng et al., 2018; Hu et al., 2003; Wallin et al., 2018).

As the only study, Engeset et al. (2015) (EPIC study) presented effect estimates before and after calibration to correct for measurement error in fish intake as a continuous variable. VKM used the results for categories of fish intake (all uncalibrated) for comparisons with other studies and considered the calibrated results as a sensitivity analysis.

4.8.2.8 Studies assessing potential non-linearity

Two studies of fish intake and all-cause mortality performed a restricted cubic spline regression analysis that takes potential non-linearity into account (Bellavia et al., 2017; Zhang et al., 2018).

4.8.2.9 Studies with converted relative risk estimates

Four studies did not use the lowest intake category as reference (Bellavia et al., 2017, Engeset et al., 2015, Nakamura et al., 2005; Osler et al., 2003) and relative risks were recalculated for the highest versus lowest category (see Chapter 3, section 3.1.5 for methods description) to facilitate high-low meta-analysis and comparisons with previous meta-analyses. Both estimates (original and converted) are given in the result table.

As the only study, Engeset 2015 reported 99% confidence intervals (CIs) which were converted to 95% CIs for the high-low risk estimate used for pooling with other studies.

4.8.2.10 Studies with estimates of survival time

Most studies presented relative risks of mortality, but two studies provided additional estimates of differences in survival time or in the age at death, to give an indication of potential increases or decrease in longevity associated with fish intake (Bellavia et al., 2017; Deng et al., 2018). Results were adjusted for potential confounders in Bellavia 2017 using Laplace multivariable regression. The estimates in Deng et al. (2018) (limited to study participants with T2D) appeared to be unadjusted and should be interpreted with caution.

4.8.3 Results from the included primary studies on fish intake and all-cause mortality

4.8.3.1 Studies of total fish intake and all-cause mortality in general populations

We included 23 publications (all prospective, observational studies) with 30 estimates of the association between total fish intake and all-cause mortality in the weight of evidence analysis. One study that did not report estimates if not statistically significant, could not be included (Woo et al. 2002). The exposure levels and results (high-low relative risk, and overall) are included in Table 4.8.3.1-1.

Table 4.8.3.1-1 Results from studies included in the weight of evidence analysis of total fish intake and all-cause mortality.

Author, year, country	Study design	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low (95% CI)	Overall result
Albert, 1998, USA	Prospective observational	Fish, incl shellfish, M	Servings as main dish, 5 cat	≥5/wk vs <1/mo	1652	0.73 (0.55, 0.96)	Sig. protective assoc. for ≥1/wk vs <1/mo, <i>P</i> -trend 0.045
Bellavia, 2017, Sweden	Prospective observational	Fish, incl shellfish, M	g/d, quintiles	Quintile 5 vs 1, 47.1–120 (median 57) vs 0–17 (median 12) g/d	9562	0.90 (0.82, 0.99) for Q5 vs Q1, reported as 0.99 (0.91, 1.08) for Q5 vs Q3 and 1.10 (1.01, 1.20) for Q1 vs Q3	U-shape (fig in paper), null for intake in highest category but sig increased risk for intake in lowest vs mid category
		Fish, incl shellfish, W	g/d, quintiles	Quintile 5 vs 1, 38–120 (median 47) vs 0–15 (median 11.5) g/d	7168	1.03 (0.90, 1.17) for Q5 vs Q1, reported as 1.12 (1.02, 1.23) for Q5 vs Q3 and 1.09 (0.99, 1.19) for Q1 vs Q3	U-shape (fig in paper), increased risk for intake in highest (sig) and lowest (borderline sig.) vs mid category
Carballo-Casla, 2021, Spain	Prospective observational	Fish, M/W	Servings/wk	≥3 vs <3 servings/wk	646	0.91 (0.75, 1.10)	No sig. assoc.
Daviglus, 1997, USA	Prospective observational	Fish, M	120-g units per 28 days, 4 point scale (0–3)	≥35 vs. 0 g/d	1042	0.85 (0.64, 1.10)	No sig. assoc., <i>P</i> -trend 0.175
Engeset, 2015, Europe (10 countries)	Prospective observational	Fish, M/W	g/d, quintiles	Quintile 5 vs 1, 76.2 vs 1.9 g/d (same in men and women)	32587	1.03 (0.98, 1.08) for Q5 vs Q1, reported as 1.09 (99% CI: 1.04, 1.14) for Q5 vs Q3 and 1.06 (99% CI: 1.01, 1.12) for Q1 vs Q3	U-shape (fig in paper), sig. increased risk for intake in highest and lowest vs mid category
Farvid, 2017, Iran	Prospective observational	Fish, M/W	Servings/d, 4 cat (tertiles of intake, null)	Cat 4 vs 1, 0.19 (median) vs 0 servings/d, standard serving size 85g	3291	0.93 (0.83, 1.03)	No sig. assoc., <i>P</i> -trend 0.32

Author, year, country	Study design	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low (95 % CI)	Overall result
Folsom, 2004, USA	Prospective observational	Fish, incl shellfish, W	Servings/wk: approx quintiles	≥2.5 vs <0.5 servings/wk	4653	0.93 (0.83, 1.05)	No sig. assoc., <i>P</i> -trend 0.15
Gillum, 2000, USA	Prospective observational	Fish, incl shellfish, M-black	Times/wk, 4 cat	>1/wk vs never	277	1.11 (0.68, 1.81)	No sig. assoc.
	Prospective observational	Fish, incl shellfish, M-white	Times/wk, 4 cat	>1/wk vs never	1236	0.85 (0.68, 1.06)	Sig. protective assoc. of intake 1/wk but only borderline sig. for >1/wk vs never
	Prospective observational	Fish, incl shellfish, W-black	Times/wk, 4 cat	>1/wk vs never	285	0.82 (0.52, 1.28)	No sig. assoc.
	Prospective observational	Fish, incl shellfish, W-white	Times/wk, 4 cat	>1/wk vs never	1103	0.90 (0.71, 1.15)	No sig. assoc.
Mohan, 2021, global, 6 continents, 58 countries	Prospective observational	Fish, incl shellfish, M/W, PURE	g/mo or wk, 4 cat	≥350 g/wk vs <50 g/mo, 594 vs 0.1 g/wk (median values)		0.97 (0.88, 1.06)	No sig. assoc., <i>p</i> -trend 0.48
Nakamura, 2005, Japan	Prospective observational	Fish, M/W	Times/d or wk, 5 cat	≥2/d vs seldom	1745	0.88 (0.64, 1.22) for ≥2/d vs seldom, reported as 0.99 (0.77, 1.27) for ≥2/d vs 1-2/wk and 1.12 (0.87–1.44) for seldom vs 1-2/wk	No sig. assoc., <i>P</i> -trend 0.94
Osler, 2003, Denmark	Prospective observational	Fish, M/W	Times/mo or wk, 4 cat	≥2/wk vs ≤1/mo, NA	1329	1.20 (0.95, 1.53), reported as 1.06 (0.88,1.28) for ≥2/wk vs 1/wk (ref) and 0.88 (0.76,1.02) for ≤1/mo vs 1/wk (ref)	Lower risk with lower intakes, adverse trend (<i>P</i> =0.02)

Author, year, country	Study design	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low (95% CI)	Overall result
Otsuka, 2019, Japan	Prospective observational	Fish, M/W	g/d, tertiles	Tertile 3 vs 1, 141.4 vs 55.0 g/d (median values)	422	1.20 (0.89, 1.63)	No sig. assoc., <i>P</i> -trend 0.23
Owen, 2016, Australia	Prospective observational	Fish, M	Servings/mo or wk, 4 cat	≥2/wk vs <1/mo	686	0.96 (0.73, 1.26)	Sig. protective assoc. of 1–3 servings/mo vs <1/mo, but not higher. No sig. trend
		Fish, W	Servings/mo or wk, 4 cat	≥2/wk vs <1/mo	579	0.97 (0.72, 1.32)	No sig. assoc., no sig. trend
Shao, 2021, China	Prospective observational	Fish, M/W	Servings/wk, 4 cat	≥11 vs 0-3 servings/wk	2697	0.94 (0.84, 1.05)	Protective assoc. for 4-6 servings/wk but not higher, <i>p</i> -trend 0.56
Takata, 2013, China	Prospective observational	Fish, incl shellfish, M/W	g/d, quintiles, sex-specific	Quintile 5 vs 1, 107.2 vs 10.8 g/d (men) and 105.2 vs 10.4 g/d (women)	5836	0.84 (0.76, 0.92)	Protective assoc., <i>P</i> -trend <0.0001
van den Brandt, 2019, the Netherlands	Prospective observational, case-cohort	Fish, M/W	g/d, 4 cat	≥20 vs 0 g/d, 29.8 vs 0 g/d (median)	8823	1.34 (1.15, 1.56)	Sig. adverse assoc., <i>P</i> -trend 0.003
Villegas, 2015, USA	Prospective observational	Fish, M/W, ex chronic diseases	g/d, quintiles	Quintile 5 vs 1, 110 vs 5 g/d (median)	4566	0.93 (0.82, 1.04)	Borderline protective trend, <i>P</i> -trend 0.09
Virtanen, 2019, Finland	Prospective observational	Fish, M	g/d, quartiles	Quartile 4 vs quartile 1, median 102 vs. 0 g/d	1225	0.90 (0.77, 1.06)	No sig. assoc., <i>P</i> -trend 0.66
Yamagishi, 2008, Japan	Prospective observational	Fish, M/W	g/d, quintiles, energy adjusted	Quintile 5 vs 1, 72 to 229 (Q5) vs 0 to 27 (Q1) g/d	7008	0.92 (0.85, 1.00)	Borderline sig. protective effect of intake in quintile 5 vs 1, <i>P</i> -trend=0.08
Yuan, 2001, China	Prospective observational	Fish, incl shellfish, M	g/wk, 5 cat	≥200 vs <50 g/wk	2134	0.79 (0.69, 0.91)	Sig. protective assoc. of intake ≥50 vs <50 g/wk, <i>P</i> -trend 0.01

Author, year, country	Study design	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low (95% CI)	Overall result
Zhang, 2018, USA	Prospective observational	Fish, incl shellfish, M	g/d, quintiles	Quintile 5 vs. 1, ≥ 30.03 vs ≤ 6.25 g/d	54230	0.91 (0.89, 0.94)	Protective assoc., P -trend <0.0001
		Fish, incl shellfish, W	g/d, quintiles	Quintile 5 vs 1, ≥ 25.38 vs ≤ 4.61 g/d	30882	0.92 (0.88, 0.95)	Protective assoc., P -trend <0.0001
Zhong, 2020, USA	Prospective observational	Fish, incl shellfish, M/W	Servings/d, quintiles (cohort specific)	Quintile 5 vs 1, 0.47 vs 0.02 serving/d/1000 kcal	8875	0.95 (0.88, 1.02)	Null, P -trend 0.30
Zhuang, 2018, China, USA	Prospective observational	Fish, M/W, China Health and Nutrition Survey	g/d, 4 cat (null, tertiles among consumers)	Tertile 3 vs null, >68.0 vs 0 g/d	1007	0.70 (0.59, 0.85)	Protective assoc., P -trend <0.0001
		Fish, M/W, NHANES	g/d, 4 cat (null, tertiles among consumers)	Tertile 3 vs. null, >8.9 vs 0 g/d	5209	0.94 (0.80, 1.10)	No sig. assoc., P -trend 0.21

Of the three studies that investigated a potential U-shaped dose-relationship in the categorical analysis, two studies reported higher risks in both the lowest and highest categories (Bellavia et al., 2017; Engeset et al., 2015), whereas the third study (Nakamura et al., 2005) found no dose-response relationship. Bellavia et al. (2017) also estimated survival time adjusted for potential confounding variables and found that the higher risks of mortality at low and high levels of fish consumption corresponded to reductions in the age of death by a maximum of 8 months (95% CI: 2 to 14 months). This effect was observed for the highest intake in women (median 47 g/day, Table 4.8.3.1-1), for men the difference was not statistically significant.

As previously mentioned, Engeset et al. (2015) (EPIC study) presented country-specific estimates for total fish. For Norway (based on the Norwegian Women and Cancer study) there were no significant associations with all-cause mortality using country-specific quintiles or EPIC-wide quintiles of fish intake. In EPIC overall, the magnitude of the effect estimates for fish intake as a continuous variable were similar after

calibration. Statistical significance was lost in some cases as the confidence intervals became slightly wider. This is expected because the uncertainty in the calibration factors is also considered.

4.8.3.2 Studies of fried and non-fried fish intake and all-cause mortality in general populations

We included four publications (all prospective, observational studies) on all cause-mortality in the weight of evidence analysis for an association with intake of fried fish (four estimates) and non-fried fish (six estimates). The exposure levels and results (high-low relative risk, and overall) are included in Table 4.8.3.2-1.

Table 4.8.3.2-1 Results from prospective observational studies included in the weight of evidence analysis of fried and non-fried fish intake and all-cause mortality.

Author, year, country	Fish exposure, sex	Intake unit	High-low intake	Total cases	HR high-low (95 % CI)	Overall result
Fried fish						
Nahab, 2016, USA	Fried fish, M/W	Servings/mo or wk, 4 cat	2/wk vs <1/mo	1101	1.07 (0.75, 1.51)	No sig. assoc., <i>P</i> -trend 0.75
Villegas, 2015, USA	Fried fish, M/W, ex chronic diseases	g/d, quintiles	Quintile 5 vs 1, 56 vs 1 g/d (median)	4566	0.98 (0.86, 1.10)	No sig. assoc., <i>P</i> -trend 0.27
Zhang, 2018, USA	Fried fish, M	g/d, quartiles	Quartile 4 vs 1, NA	54230	1.01 (0.98, 1.03)	Protective assoc. of intake in quartile 2 but not higher vs Q1, <i>P</i> -trend 0.18
	Fried fish, W	g/d, quartiles	Quartile 4 vs 1, NA	30882	1.03 (0.99, 1.06)	Borderline adverse assoc., <i>P</i> -trend 0.011
Non-fried fish						
Nahab, 2016, USA	Non-fried fish, M/W	Servings/mo or wk, 4 cat	2/wk vs <1/mo	1101	1.18 (0.90, 1.55)	No sig. assoc., <i>P</i> -trend 0.17
Owen, 2016, Australia	Non-fried fish, M	Servings/mo or wk, 4 cat	≥2/wk vs <1/mo	686	1.11 (0.87, 1.42)	No sig. assoc., no sign trend
	Non-fried fish, W	Servings/mo or wk, 4 cat	≥2/wk vs <1/mo	579	0.91 (0.70, 1.17)	Sig. protective assoc. of 1–3 servings vs <1/month, but not higher, no sig. trend

Author, year, country	Fish exposure, sex	Intake unit	High-low intake	Total cases	HR high-low (95 % CI)	Overall result
Villegas, 2015, USA	Non-fried fish, M/W - ex chronic diseases	g/d, quintiles	Quintile 5 vs 1, 34 vs 0 g/d (median)	4566	0.89 (0.80, 1.00)	Protective assoc. (quintiles 4-5 vs 1, <i>P</i> -trend 0.03)
Zhang, 2018, USA	Non-fried fish, M	g/d, quintiles	Quintile 5 vs 1, NA	54230	0.90 (0.87, 0.92)	Protective assoc., <i>P</i> -trend <0.0001
	Non-fried fish, W	g/d, quintiles	Quintile 5 vs 1, NA	30882	0.91 (0.88, 0.94)	Protective assoc., <i>P</i> -trend <0.0001

For fried fish, there were no reports of a protective association with all-cause mortality, or the association was limited to a low intake level. For non-fried fish, the associations were either null or protective, where the two largest studies (Villegas 2015, Zhang 2018) found a protective effect of similar magnitude (10% lower risk for intake in the top vs bottom quintile) in both men and women.

4.8.3.3 Studies of total fish intake and all-cause mortality in patients with prior CVD or at high risk

We included five studies with seven estimates of the association of fish intake with risk of all-cause mortality in patients with prior CVD (including coronary heart disease or myocardial infarction), or patients at high risk of CVD from vascular disease (Table 4.8.3.3-1).

Table 4.8.3.3-1. Results from studies included in the weight of evidence analysis of fish intake and all-cause mortality in patients with prior CVD or at high risk.

Author, year, country	Study design*	Fish exposure, sex	Intake unit	High-low intake	Total cases	HR high-low (95 % CI)	Overall result
Barzi, 2003, Italy	Prospective observational	Fish, M/W	Times/wk, 4 cat, cumulative average	>2/wk vs never/almost never	1660	0.76 (0.62, 0.94)	Sig. protective assoc. of intake >2 and 2/wk vs never/almost never, <i>P</i> -trend 0.0003
Burr, 1989, UK	RCT-2nd prevention	Fatty fish, M	Portions/wk	Advice of two weekly portions (200-400 g) of fatty fish vs no advice	224	0.71 (0.54, 0.93)	Protective assoc. of intervention vs no intervention
Erkkila, 2003, Finland	Prospective observational	Fish, M/W	g/d, 3 cat (above/below median, null)	Cat 3 vs 1, >57 (above median) vs 0 g/d	34	0.37 (0.14, 1.00)	Suggestive protective assoc. of intake >57 g/d vs no consumption, <i>P</i> -trend= 0.059

Author, year, country	Study design*	Fish exposure, sex	Intake unit	High-low intake	Total cases	HR high-low (95% CI)	Overall result
Manger, 2010, Norway	Prospective observational	Fish, incl fish products, M/W	g/d, quartiles	Quartile 4 vs 1, or Q2-4 vs 2, 199 vs 41.1 g/d (mean)	137	0.95 (0.58, 1.55)	No sig. assoc., <i>P</i> -trend 0.98
Mohan, 2021, global, 6 continents, 58 countries	Prospective cohorts, multicenter	Fish, incl shellfish, M/W, PURE	g/mo or wk, 4 cat	≥350 g/wk vs <50 g/mo, 594 vs 0.1 g/wk (median values)	NA	0.91 (0.71, 1.16)	No sig. assoc. <i>P</i> -trend 0.36
		Fish, incl shellfish, M/W, ONTARGET, TRANSCEND	g/mo or wk, 4 cat	≥350 g/wk vs <50 g/mo, 450 vs 2.8 g/wk (median values)	3771	0.81 (0.70, 0.92)	Protective or borderline protective assoc. in all categories, <i>P</i> -trend <0.001
		Fish, incl shellfish, M/W, ORIGIN	g/mo or wk, 4 cat	≥350 g/wk vs <50 g/mo, 568 vs 2.2 g/wk (median values)	1877	0.86 (0.74, 1.00)	Protective or borderline protective assoc. all categories, <i>P</i> -trend 0.01

Among the four studies with total fish as the exposure, four or six estimates were protective or suggestive protective, and two were statistically non-significant (men and women combined). The intervention study (men only) found a protective effect of dietary advice to eat fatty fish on all-cause mortality.

4.8.3.4 Studies of total fish intake and all-cause mortality in patients with type 2 diabetes (T2D)

We included 5 publications with 6 estimates of the association between total fish intake and all-cause mortality in populations with risk of T2D (Table 4.8.3.4-1).

Table 4.8.3.4-1. Results from prospective observational studies included in the weight of evidence analysis of fish intake and all-cause mortality in sub-populations with T2D.

Author, year, country	Fish exposure, sex	Intake unit	High-low intake	Total cases	HR high-low (95 % CI)	Overall result
Hu, 2003, USA	Fish, incl shellfish, W	Servings/mo or wk, 5 cat, cumulative average	≥5/wk vs <1/mo	468	0.48 (0.29, 0.80)	Sig. protective assoc. of intake 1/wk or higher vs <1/mo, <i>P</i> -trend=0.005
Deng, 2018, USA	Fish, incl shellfish, M/W	Times/wk, 3 cat	>2 vs 1/wk	698	0.83 (0.67, 1.03)	Borderline protective assoc. of intake >2 vs <1/wk
Villegas, 2015, USA	Fish, M/W	g/d, quintiles	Quintile 5 vs 1, 110 vs 5 g/d (median)	1464	0.89 (0.77, 1.03)	Borderline protective assoc. in quintile 5 vs 1, <i>P</i> -trend 0.22
Wallin, 2018, Sweden	Fish, M/W	Servings/mo or wk, 4 cat	>3/wk vs ≤3/mo, 3.5 vs 0.5 servings/wk (median)	771	0.90 (0.69, 1.18)	Suggestive protective (borderline in cat 2-3 vs 1), <i>P</i> -trend 0.74
Zhang, 2018, USA	Fish, incl shellfish, M	g/d, quintiles	Quintile 5 vs 1, ≥30.03 vs ≤6.25 g/d	19499	0.93 (0.86, 1.00)	Borderline protective trend, <i>P</i> -trend 0.088
	Fish, incl shellfish, W	g/d, quintiles	Quintile 5 vs 1, ≥25.38 vs ≤4.61 g/d	10654	1.01 (0.91, 1.13)	No sig. assoc., <i>P</i> -trend 0.54

Associations were either on the protective side (statistically significant in one study), except for women in Zhang et al. (2018). In this study, results were stratified by reported type 2 diabetes and suggested weaker associations among diabetic participants. Significant effect modification was reported for women (*P*-interaction=0.017) but not in men (*P*-interaction=0.67). Cases numbers had to be estimated from the proportion with a history of diabetes (around 6% in women and 8% in men).

4.8.3.5 Summary relative risks (RRs) based on VKM's inclusion of primary studies

The high-low summary relative risk (RR) of all-cause mortality based on 23 publications of overall fish intake (Table 4.8.3.1-1) indicated a statistically significant protective association (RR=0.93, 95% CI: 0.90, 0.97) but with significant heterogeneity ($P_{\text{heterogeneity}} < 0.001$).

There was one report of increased mortality that was statistically significant (van den Brandt et al., 2019). Heterogeneity was reduced but remained significant ($P = 0.001$) when removing this study from influence analysis. VKM's high-low summary RR was very similar to results from previous high-low meta-analyses despite some differences in the selection of primary studies; Schwingshackl et al. (2017) reported a RR of 0.95 (95% CI: 0.92, 0.98) and Wan et al. (2017) and Zhao et al. (2016) both reported a RR of 0.94 (95% 0.90 to 0.98, both studies).

For intake of fried fish (high-low intake) in relation to all-cause mortality (3 studies, Table 4.8.3.2-1), VKM's high-low summary RR suggested a potentially small, increased risk (RR=1.02, 95% CI: 1.00, 1.03). Heterogeneity was non-significant ($P_{\text{heterogeneity}} = 0.74$). The very narrow CI was due to the inclusion of the study by Zhang et al. (2018) with over 400 000 participants, and this study dominated the result (weight of 98% in VKM's analysis). There was no previous meta-analysis to compare with.

For non-fried fish (4 studies, Table 4.8.3.2-1), VKM's high-low summary RR suggested a protective association that was borderline statistically significant (RR= 0.93, 95% CI: 0.86, 1.00), with non-significant heterogeneity ($P_{\text{heterogeneity}} = 0.16$). This result was also dominated by Zhang et al. (2018) (56% weight) and could not be compared with any previous meta-analysis.

For overall fish intake in patients with previous CVD or at high risk, VKM's high-low summary RR for all-cause mortality based on four prospective studies (six estimates, Table 4.8.3.3-1) suggested a statistically significant protective association (RR=0.83, 95% CI: 0.76, 0.90) without significant heterogeneity ($P_{\text{heterogeneity}} = 0.50$).

For overall fish intake in sub-populations with T2D, VKM's high-low summary RR for all-cause mortality (RR=0.95, 95% CI: 0.90, 1.01) based on 5 studies (Table 4.8.3.4-1) was similar to the protective association found in the general population. Heterogeneity was borderline significant ($P_{\text{heterogeneity}} = 0.07$, 5 studies), but there were no reports of adverse associations. The meta-analysis by Jayedi, Soltani 2020, found a protective association that was slightly stronger and statistically significant (RR=0.86, 95% CI: 0.76, 0.96) based on more studies.

4.8.3.6 VKM's search compared to previous meta-analyses on all-cause mortality

Jayedi et al. (2018) included 10 papers (14 prospective studies). VKM's search identified all papers except two (Bell 2014; Nagata 2002). Jayedi 2018 performed a linear dose-response analysis which partly explains the lower number of eligible studies compared with VKM's high-low analysis. Jayedi (2018) also excluded studies where the reference category was not

the lowest, instead of converting. The results suggested a 2% relative risk reduction (95% CI: 0 to 3%) per 20 g/day increase in fish intake.

Schwingshackl et al. (2017) included 37 papers (39 studies). Among these studies were all of those identified in VKM's search except for two studies published after 2017 (Zhang et al., 2018; Zhuang et al., 2018). Schwingshackl et al. (2017) included 21 publications not included by VKM (Atkins et al., 2014; Bell et al., 2014; Bongard et al., 2016; Fraser et al., 1997; Kappeler et al., 2013; Knoop et al., 2006; Lee et al., 2013; Limongi et al., 2016; Mann et al., 1997; Martinez Gonzalez et al., 2012; Nagata et al., 2002; Olsen et al., 2011; Prinelli et al., 2015; Roswall et al., 2015; Shi et al., 2015; Stefler et al., 2015; Tognon et al., 2011; Tognon et al., 2012; Tognon et al., 2014; Vormund et al., 2015; Whiteman et al., 1999). Schwingshackl et al. (2017) applied a wide search strategy for an extensive list of foods. This strategy may have captured studies where fish intake was not the main focus. Fourteen of the 21 publications focused on dietary patterns or indices, and results on fish were not captured if not mentioned in the title and abstract. Five studies were captured by VKM's search but excluded during screening because the abstract described omega-3 or fish oils. One study was graded C and did not pass VKM's quality assessment (Mann et al., 1997). Despite the large discrepancy in the number of studies, the summary RR differed little from VKM's estimate. This is because VKM also included many studies, and the weight of each study decreases as the number of studies increases.

Wan et al. (2017) included 22 papers. Two studies were not found by VKM (Kahn, 1984 and Keleman, 2005) because they focused on diet. One study was excluded due to lack of individual exposure data (Ness et al., 2005), and one did not pass VKM's quality assessment (Tomasallo et al., 2010). On the other hand, not all studies found by VKM were included, despite being published prior to 2017.

Zhao et al. (2016) included 12 papers of which three were not included in VKM SLR. One was not found in the search because it focused on diet and physical activity, not fish intake (Trichopolou et al., 2006), one was excluded in the title- and abstract screening for the same reason (focus on diet; Iimuro et al., 2012), and one was excluded because it only reported fish intake at the level of consumer/non-consumer.

Table 4.8.3.6-1 Overview of prospective cohort studies included by VKM compared with five identified meta-analyses on all-cause mortality.

Publications	Included by VKM	Meta-analyses				
		Jayedi 2020, Diabetes	Jayedi 2018	Schwingshackl 2017	Wan 2017	Zhao 2016
Albert 1998	X		X	X	X	X
Bellavia 2017	X		X	X		
Carballo-Casla 2021	X					
Daviglus 1997	X		X	X	X	X
Engeset 2015	X		X	X		
Farvid 2017	X					

	Included by VKM	Meta-analyses				
Folsom 2004	X		X	X	X	X
Gillum 2000	X			X	X	X
Mohan 2021	X					
Nahab 2016	X					
Nakamura 2005	X			X		
Osler 2003	X			X		
Otsuka 2019	X					
Owen 2016	X		X	X		
Shao 2021	X					
Takata 2013	X		X	X	X	X
van den Brandt 2019	X			X		
Villegas 2015	X			X	X	
Virtanen 2019	X					
Yamagishi 2008	X		X	X		X
Yuan 2001	X			X	X	
Zhang 2018	X					
Zhong 2020	X					
Zhuang 2018	X					
Woo 2002	X					
Salonen 1995	X			X		
Diabetes populations						
Deng 2018	X	X				
Hu 2003	X	X				
Wallin 2018	X	X				
Villegas 2015, stratified results	X	X		X	X	
Zhang 2018, stratified results	X	X				
Patients with CVD or CHD						
Barzi 2003	X					
Burr 1989	X					
Erkkila 2003	X					
Manger 2010	X					
Mohan 2021	X					
Only in meta-analyses						
Atkins 2014				X		
Bell 2014			X	X	X	X
Bongard 2016				X		
Feskens 1993		X				
Fraser 1997				X	X	
Iimuro 2012		X				
Kahn 1984					X	
Kappeler 2013				X	X	X
Kelemen 2005					X	
Knoops 2006				X	X	
Lee 2013				X	X	X
Limongi 2016				X		
Mann 1997				X	X	

	Included by VKM	Meta-analyses				
Martinez Gonzalez 2012				X		
Nagata 2002			X	X	X	X
Ness 2005					X	X
Olsen 2011				X	X	X
Prinelli 2015				X		
Roswall 2015				X		
Shi 2015				X		
Stefler 2015				X		
Tognon 2011				X	X	
Tognon 2012				X		
Tognon 2014				X		
Tomasallo 2010					X	
Trichopoulou 2006		X				
Trichopoulou 2009					X	
Vormund 2015				X		
Whiteman 1999				X		
<i>Publications evaluated</i>	<i>33</i>	<i>8</i>	<i>10</i>	<i>37</i>	<i>22</i>	<i>12</i>

4.8.4 Heterogeneity fish intake and all-cause mortality

Previous meta-analyses of fish intake and all-cause mortality have reported moderate to substantial between study heterogeneity (I^2 range 40% to 80%, Table 4.7.1.2-1) and therefore examined potential sources of heterogeneity in sub-group analyses.

Jayedi et al. (2018) performed sub-group analyses (continuous dose-response model) by sex; region (Europe and USA combined, or Asia); follow-up duration (cut-point 13 years); number of cases (cut-point 3000); study quality score; exclusion of participants with a history of CVD; adjustment (yes, no) for BMI, or physical activity, or intake of energy, or alcohol, or fruit and vegetables). None of the sub-group analyses fully accounted for the observed heterogeneity. Heterogeneity remained significant within men and within women, within studies from Europe and USA (but not Asia), within studies with a high-quality score, and within studies that controlled for potential confounders.

Schwingshackel et al. (2017) also performed sub-group analysis (continuous dose-response model) by sex; region (Europe, America, Asia, or Australia); follow-up duration (mean or median ≥ 10 compared with <10 years); number of cases (cut-point 1000); and dietary assessment method (validated compared with non-validated). The only statistically significant test for subgroup differences was found for region (association was weaker in studies from Europe). Studies with a low risk of bias (11 of 19) did not have a lower I^2 than all studies combined.

Wan et al. (2017) found that heterogeneity still existed in subgroup analysis (performed for high-low estimates) by sex, country, follow-up years (cut-point 12 years), and adjustment for confounding factors (yes, no), including BMI, or total energy intake, or education, or smoking status, or alcohol intake.

Zhao et al. (2016) performed sub-group analyses (high-low estimates) by gender; region (USA, Asia, or Europe); follow-up duration (cut-point 12 years); sample size (40 000 participants as the cut point); year of publication (before or after 2008); dietary assessment method (validated food frequency questionnaire or other); and adjustments (yes, no) for education, or BMI, or physical activity, or intake of fruit and vegetables, or red meat, or total energy. No association was found in studies from Europe (but only based on two studies), and the association was weaker in studies published prior to 2008.

In conclusion, the meta-analyses show an overall protective association, with most primary studies on the protective side or close to null. Within the range of associations (as illustrated in forest plots), there is still statistically significant heterogeneity. Sub-group analyses in previous meta-analyses cannot account for all between study variation when factors are examined one by one. Gender was not found to be an important source of heterogeneity in any of the studies. All studies noted a weaker association in studies from Europe. The reason for this remains unclear. Regional differences could potentially reflect differences in disease patterns, or healthcare, or other biological and/or methodological differences. Multivariable meta-regression in future studies is needed to examine to what extent regional differences are explained by other factors.

4.8.5 Dose-response relationship fish intake and all-cause mortality

Jayedi et al. (2018) performed a non-linear dose-response meta-analysis for fish intake and all-cause mortality that indicated a wave-shaped curve. For fish intake between 0 and 60 g/day there was a U-shaped association with a nadir (lowest point) at intake of about 20 g/day. For intakes above 60 g/day, risk decreased again but the relationship was not statistically significant for intakes above 40-50 g/day judging from the curve's 95% confidence limits. When stratified by region, the curves differed, which may explain the wave shape of the curve for regions combined. Also, the wave shape was much less pronounced for CVD mortality than for all-cause mortality, suggesting an influence of mortality from other diseases that could differ between world regions. For Asian studies the curve for all-cause mortality appeared to be linear (lower risk with higher intakes up to 120 g/day) but almost U-shaped for Western studies, with a nadir at intake of about 20 g/day. The curve's 95% confidence limits indicated non-significant associations of intakes higher than 50 g/day.

Schwingshackl et al. (2017) performed a non-linear dose-response analysis but found no departure from linearity in (n=19 studies, Table 4.7.1.2-1). All-cause mortality decreased by 7% (95% CI: 4%, 10%) for one serving of fish per day (100 g) vs none, and by 10% (95% CI: 4%, 16%) for two servings per day (200 g) vs none.

Wan et al. (2017) found a curvilinear association for fish intake with a potential threshold for all-cause mortality risk (n=10). They observed lower risk for intakes up to 40 g/day when the relative risk was reduced by 9% versus no intake. The curve's confidence limits did not show significantly lower all-cause mortality for intakes above 40 g/day.

Similar to Wan et al. (2017), Zhao et al. (2016) also found a significant non-linear relationship between fish intake and all-cause mortality (n=7) with a potential threshold, but the threshold was at a high intake level. The authors observed that with an increase in fish intake the risk estimates showed a sharp decline and then leveled off at about a daily intake of 60-80 gram of fish.

4.8.6 Weight of evidence for fish intake and all-cause mortality

In this section, the evidence of the association between fish intake and all-cause mortality is weighed according to the WCRF criteria presented in Chapter 3.1.6 (Box 2).

Published evidence of fish intake and all-cause mortality

All four meta-analyses of the association between fish intake and all-cause mortality found a significant protective association. The summary RR for the primary studies included by VKM on fish intake and all-cause mortality showed a significant protective association overall, similar in magnitude to the associations observed in Schwingshackle et al. (2017), Wan et al. (2017) and Zhao et al. (2016) despite some difference in selection of primary studies.

For intake of fried fish in relation to all-cause mortality, VKM's summary RR showed a potentially small, increased risk (RR=1.02, 95% CI: 1.00, 1.03). For non-fried fish, the summary RR suggested a protective association (RR= 0.93, 95% CI: 0.86, 1.00). Both RRs were only borderline statistically significant.

For overall fish intake in patients with T2D, VKM's summary RR (five studies) was similar to the protective association found for the general population, and to the association observed by Jayedi et al. (2020). For overall fish intake in patients with prior CVD or at high risk, VKM's summary RR (four studies) suggested a protective association that was statistically significant.

All this together supports that fish intake is associated with a decreased risk of all-cause mortality in the general population, and in patients with T2D and possibly with CVD. The evidence for an effect of cooking method is less strong but indicates that fried fish may increase risk, and non-fried fish reduce risk.

Heterogeneity

Significant heterogeneity was observed between studies in the included meta-analyses and VKM's summary RR based on the primary studies looking at total fish intake and all-cause mortality.

Mechanism / biological plausibility

There is evidence for several plausible mechanisms operating in humans (see Chapters 4.1, and 5.2).

Upgrading factors

There is evidence of a dose-response relationship from independent meta-analyses. No other upgrading factors have been evaluated.

4.8.6.1 Conclusion weight of evidence fish intake and all-cause mortality

There is evidence from more than two independent and good quality cohort studies on total fish (VKM included 23 studies in the general population, five in patients, and five meta-analyses including dose-response analyses). The published evidence indicates a significant protective association of fish intake with all-cause mortality.

VKM's summary RR for primary studies in the general population shows statistically significant lower all-cause mortality for the highest versus lowest intake of total fish and is supported by independent meta-analyses. The direction of association is generally consistent towards protective, but there is some heterogeneity between studies and one study showed increased mortality. There is evidence for biological plausibility and a dose-response relation.

In conclusion, the evidence is graded "probable" for a protective effect of fish consumption on all-cause mortality in the general population. VKM's summary RRs for studies in patients suggest a stronger effect for patients with prior CVD or at high risk, but for patients with T2D the effect is similar to that in the general population.

The evidence for effects of fatty and lean fish on all-cause mortality was graded "limited, no conclusion" based on one study.

4.9 Introduction fish intake and neurodevelopmental outcomes in children

This chapter is an introduction to the weight of evidence analysis chapters for neurodevelopmental outcomes in children related to maternal fish intake and fish intake in children (Chapters 4.10-4.11).

Neurodevelopment in children

The first years of life from gestation and onwards is a period of rapid brain growth and development with fluctuating growth spurts and increased susceptibility to influences (Walder et al., 2009; Cusick, 2016). Genetically driven, the development happens in close interaction with the environment where the brain depends upon biological and psychosocial influences for normal development (Fox et al., 2010; Nelson et al., 2007).

Timing is a key factor in determining the impact of exposures on the developing brain (Thompson and Nelson, 2001). The relationship between timing of exposures to the brain and the neurodevelopmental outcomes is complex, and various exposures may be linked to various sensitive periods (Anderson et al., 2001). With regard to nutrition for instance, the impact on neurodevelopment will vary according to the type of the nutritional influence (deficiency or excess) and to what extent these coincide with essential timeframes in the developing brain (Cusick et al., 2012). Some micronutrients exert their impact at very specific time points (e.g., folate for the folding of the neural tube early in pregnancy), whereas the significance of timing has not yet been firmly established for other key micronutrients. In the period from conception to approximately two years of age, the central nervous system is also in particular vulnerable to the adverse effects of neurotoxic exposure since the blood-brain barrier is not fully developed (Bellinger et al., 2018).

Child development in early childhood is challenging to measure. The rapid growth and the increased susceptibility of the developing brain leads to variability in developmental advances both within and between individuals, and to achieve reliable measures of child development in young children is therefore difficult (Brito et al., 2019). Moreover, due to limitations in abilities in young children (i.e., cognitive/language, motor, and socio-emotional abilities), the underlying skills in the different developmental domains overlap and mutually influence each other (Fernald et al., 2017). Thus, in this age group, tools are often less specific, and a test focusing on one domain also taps into abilities in other domains. Assessment tools in young children therefore tend to be blunt and less suitable for measuring specific skills (Brito et al., 2019).

Beyond the period of early childhood (i.e., approximately after 3 years of age), developmental advances lead to more specialized skills and there is a shift in the neurodevelopmental assessment tools introducing measures of specific cognitive functions such as attention, memory, and executive functioning, and from these, to generate measures of general abilities and IQ. The accuracy of the neurodevelopment outcomes increases, as well as the predictive ability of the tests (Brito et al., 2019).

Overview of studies summarized according to neurodevelopmental outcomes in children

VKM included primary studies of fish intake and neurodevelopmental outcomes in children below 18 years graded A or B in the quality assessment. These studies included both maternal and child fish consumption as exposure and a wide variety of neurodevelopmental outcomes. The majority were prospective cohort studies (birth and child cohorts), but also randomized controlled trials were identified.

Due to the qualitative differences in child development with age, the neurodevelopmental outcomes were divided in 3 main subcategories; *early child development* (outcome measures in children ≤ 3 years), *cognition* (cognitive outcome measures from 4-18 years) and *mental health* (birth-18 years, includes autism, ADHD and other mental health conditions). The findings were evaluated based on maternal and child fish consumption as exposure separately (Chapter 4.10 and 4.11). Figure 4.9-1 shows the categories of neurodevelopmental outcomes with maternal fish consumption and child fish consumption as exposure.

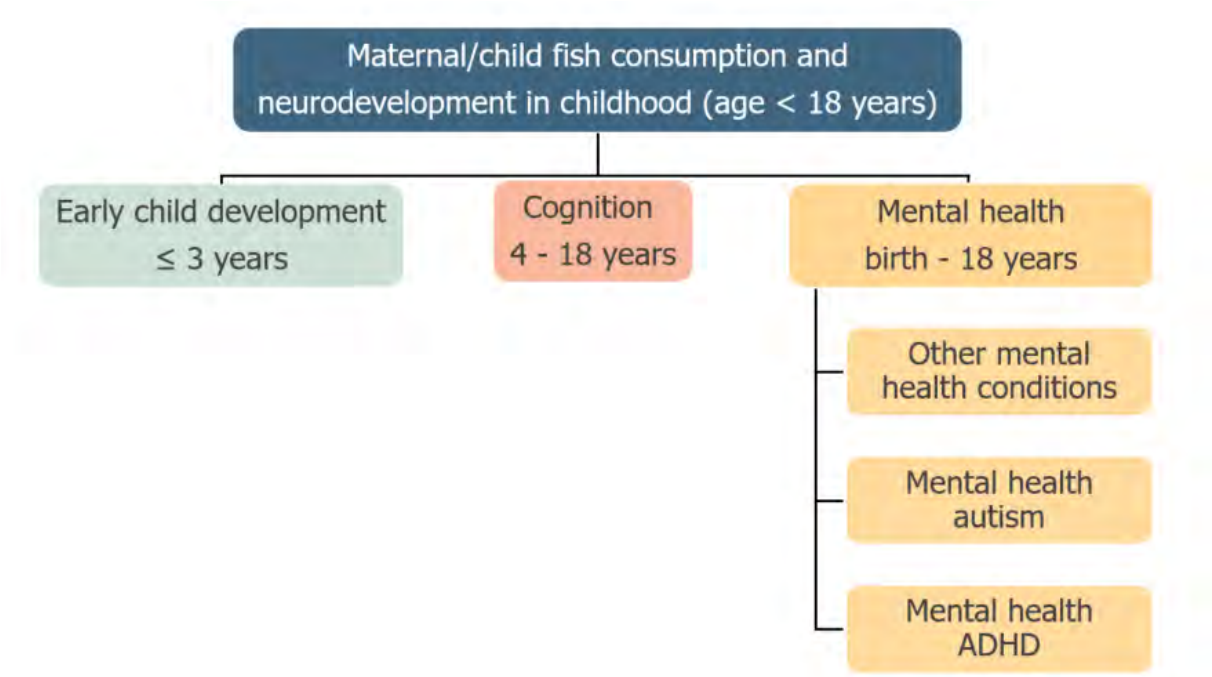


Figure 4.9-1 Overview over evaluated neurodevelopmental subcategories.

In general, the assessment tools used to assess neurodevelopment in the individual studies were standardized, validated and well-known, but there was great variation across studies in the included tools and how these were used in the comparisons. Studies included total/summary scores and multiple subscales/subtests or both, and there were variations to which the outcomes were presented on a continuous scale or dichotomized (high/low scorers) or both in the publications. As a result, many publications reported multiple outcomes (ranging from 1-25 in the same publication) and across several subcategories, and

hence also multiple comparisons. Only two of the studies stated a primary outcome and none of the papers reported statistical adjustment for multiple comparisons.

In the present VKM assessment, findings from the publications were reported across several subcategories. For instance, Hibbeln et al. (2007) and Julvez et al. (2016) reported findings from different ages during childhood and across several neurodevelopmental subcategories, and their findings were summarized under the sections for early child development, cognition, and mental health.

All estimates from the comparisons in the publications are described in the tables. In the “overall conclusion” column of these tables results are summarized to “Significant beneficial associations” if all comparisons are significant in the same direction, “No significant associations” if all comparisons are non-significant, and “Suggestive beneficial associations” if some of the comparisons are significant and consistent in a beneficial direction.

Mechanisms for neurodevelopment in children

Nutrients are important for structural and functional brain development (Georgieff et al., 2018). The impact of nutrients may be greater in the foetal and early postnatal period due to the high metabolic demands of the brain in this period.

Fish and seafood contains several nutrients that are essential for normal brain function and development such as selenium, vitamin D, vitamin B₁₂, and iodine (Avella-Garcia et al., 2014) and fatty fish is a rich source of long-chain n-3 polyunsaturated fatty acids (LC n-3 PUFA). The n-3 LC PUFA, such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are essential for cell membrane formation, the development of neurons, and their synaptic connections during the brain’s growth spurt, which mainly takes place from the last trimester of pregnancy up to 2 years of age (Lauritzen et al., 2016). The visual system and areas of the prefrontal cortex that mediate attention, inhibition and impulsivity are targets of early n-3 LC PUFA status in nonhuman primate models (Cusick et al., 2017). Other mechanisms of LC n-3 FAs, such as anti-inflammatory effects, that are also relevant to neuronal function in the brain are presented in Chapter 5.2.

Fish may also, however, be a dietary source of contaminants and environmental pollutants including well-established neurotoxins, such as persistent organic pollutants (POPs) and mercury. POPs have been suggested to affect biological processes critical for human brain development, such as synapse formation and neuronal differentiation. Early exposure (both pre- and/or perinatal) to mercury in the form of methylmercury (MeHg), even at moderate doses, have been linked to multiple deficits in neurons and glia, including abnormal migration, differentiation, and growth. In the fetal and early postnatal period, the brain may be particularly vulnerable to these neurotoxins since the blood-brain barrier is still under development (Bellinger et al., 2018).

4.9.1 VKM's search for previous systematic reviews and meta-analyses of maternal fish intake and neurodevelopment in children

4.9.1.1 Description of the identified publications

VKM's search for systematic reviews and meta-analyses (see Chapter 3.1.2.2) identified two publications on the association between maternal or child fish intake and neurodevelopment in children. Both papers were excluded; see Table 4.9.1.1-1 for reasons for exclusions. One meta-analysis of maternal seafood intake during pregnancy was identified by snowballing (Hibbeln et al., 2019) and included.

Table 4.9.1.1-1 Reasons for exclusion of identified papers from the search of systematic reviews and meta-analyses of maternal fish intake and child neurodevelopment 2016-2021.

Included papers	Excluded paper and reasons for exclusions
Hibbeln et al., 2019	Cohen et al., 2016 – graded C Khanna et al., 2009 – graded C

The meta-analysis is described in more details below; first a main description of the methods used and then main/selected results (see Table 4.9.1.2-1).

Hibbeln et al. (2019) did a systematic review focusing on two questions; 1) What is the relationship between maternal seafood consumption during pregnancy and lactation and the neurocognitive development of the infant, and 2) What is the relationship between seafood consumption during childhood and adolescence (up to 18 years of age) and neurocognitive development? The authors performed a systematic literature search and review following the methodology (<https://nesr.usda.gov>) of the USDA's NESR team (formerly known as the Nutrition Evidence Library), and the systematic review methodology as detailed by the 2020 Dietary Guidelines Advisory Committee (NESR website <https://nesr.usda>). Literature searches with date ranges of January 1980–April 2019 were conducted in three databases (Cochrane, EMBASE and PubMed). Eligible studies were required to have one of the following study designs: RCT, prospective cohort study or case control study. The quality of the eligible papers included in the systematic review were quality assessed using the revised Cochrane risk-of-bias tool for randomized trials (<https://www.riskofbias.info/welcome/rob-2-0-tool/current-version-of-rob-2>) or the risk of bias-nutritional observational scale (ROB-NOS) adapted for use in nutritional observational studies from ROBINS-1 (<https://nesr.usda.gov/2020-dietary-guidelines-advisory-committee-systematic-reviews>). The authors graded the total evidence for the two specific questions; strong, moderate, limited or grade not assignable. The grading included evaluation of internal validity, adequacy, and consistency of the evidence, as well as impact (including clinical impact) and generalizability (see table). The AMSTAR tool was used to assess the methodological quality of Hibbeln et al. (2019) by the VKM project group, and the study was found to have a high-moderate quality and assigned quality level B.

4.9.1.2 *Results from the systematic review*

Below is a summary table for maternal and child total fish intake and neurodevelopmental outcomes in children based on the identified systematic review.

Table 4.9.1.2-1 Summary of results from the systematic review on maternal or child total fish intake and neurodevelopmental outcomes in children.

Author, year	Type of studies included	No. studies	Sample size	Author's grading of meta-evidence	Author's conclusion
Maternal intake of fish					
Hibbeln, 2019	Prospective cohort studies which evaluated the relationship between maternal seafood consumption during pregnancy and lactation and the neurocognitive development of the infant	29	106 237 mother-child pairs	USDA Nutrition Evidence Systematic Review criteria for evaluation of evidence	"Moderate and consistent evidence indicates that consumption of a wide range of amounts and types of commercially available seafood during pregnancy is associated with improved neurocognitive development of offspring as compared to eating no seafood. This evidence does not meet the criteria for "strong" evidence only due to the absence of RCTs that may not be ethical or feasible to conduct." The authors found that the benefits to neurocognitive development began at the lowest amounts of seafood intake (~4 oz¹/wk) and continued into the highest intake categories in the included cohorts (>100 oz¹/wk). It seems as if benefits consistently increased from no seafood consumption upwards through approx. >12–30 oz¹/wk.
Child intake of fish					
Hibbeln, 2019	Prospective cohort studies, case-controls and RCTs which evaluated the relationship between seafood consumption during childhood and adolescence (up to 18 years of age) and neurocognitive development	6 RCTs 4 pros. 9 case-control	25 960 children	USDA Nutrition Evidence Systematic Review criteria for evaluation of evidence	"Moderate and consistent evidence indicates that consumption of >4 oz/wk and likely >12 oz/wk of a wide range of commercially available seafood during childhood through adolescence has beneficial associations to a wide spectrum of neurocognitive outcomes as compared to consuming no seafood. The evidence does not meet the criteria for "strong" evidence because of an insufficient number of randomized controlled trials." The authors could not conclude on amounts of seafood in the included studies that resulted in net adverse outcomes for any measures of neurocognitive development in children. However, they point out that upper ranges of consumption were not well described in the studies.

¹1 oz = 28.35 g.

4.10 Maternal fish intake and neurodevelopmental outcomes in children

4.10.1 VKM's search for previous systematic reviews and meta-analyses of maternal fish intake and neurodevelopment in children

See Chapter 4.9.1.

4.10.2 VKM's systematic review of primary studies on maternal fish intake and child neurodevelopment

4.10.2.1 *Included studies from the search*

A total of 22 publications graded A or B on maternal fish intake and child neurodevelopment were included in the evaluation (Daniels et al., 2004; Deroma et al., 2013; Gale et al., 2008; Golding et al., 2018; Hibbeln et al., 2007; Julvez et al., 2019; Julvez et al., 2016; Mendez et al., 2009; Mesirow et al., 2017; Oken et al., 2005; Oken et al., 2008a; Oken et al., 2008b, Oken et al., 2016; Sagiv et al., 2012; Steenweg-De Graff et al., 2016; Suzuki et al., 2010; Vejrup et al., 2018; Xu et al., 2016; Hamazaki et al., 2020; Vecchione et al., 2021; Markhus et al., 2020; Kvestad et al., 2021).

Selected study characteristics (study name, design, time, size and age of the study population and dietary assessment method) of the studies with maternal fish consumption and child neurodevelopment by subcategory are presented in Table 4.10.2.1-1.

Table 4.10.2.1-1 Overview of primary studies included in the weight of evidence analysis of maternal fish intake and neurodevelopmental outcomes in children.

Author, year, country	Study name	Design	Inclusion year(s), end, follow-up time (child age)	Study size, maternal age	Dietary assessment method
Early child development (age ≤3 years)					
Daniels, 2004, UK	ALSPAC	Birth cohort	1991-1992, 15 and 18 month follow up	7421, mean age 29 yrs	FFQ, 6- and 15-months post partum
Hamazaki, 2020, Japan	The Japan Environment and Children`s Study (JECS)	Birth cohort	2011 to 2014, 6 months and 1 year follow up	81697 and 77751, mean age ranging from 30.6-31.7 yrs	FFQ, mid-late pregnancy and second/third trimester
Hibbeln, 2007, UK	ALSPAC	Birth cohort	1991-1992, 6, 18, 30 and 42 months, 7- and 8-years follow-ups	5000-8801, mean age not reported	FFQ, 32 GW
Julvez, 2016, Spain	The Spanish Childhood and Environment project	Birth cohort	2004-2008, 14 months and 5 years follow-up	1892, mean age not reported	FFQ, 10-13 weeks and 28-32 GW
Kvestad, 2021, Norway	Mommy`s food	RCT	2016 to 2017, 1 year follow up	133, mean age 29.1 yrs and 29.7 in control og intervention group	NA
Markhus, 2020, Norway	Mommy`s food	RCT	2016 to 2017, 1 year follow up	133, mean age 29.1 yrs and 29.7 in control og intervention group	NA
Oken, 2005, US	Project Viva	Birth cohort	1999-2003, 6 months follow up	135, mean age not reported	FFQ, 26-28 GW
Oken, 2008a, Denmark	DNBC	Birth cohort	1997-2002, 6 and 18 months	101 042, mean age ranging from 28.2 yo 30.0 yrs	FFQ, 25 GW
Oken, 2008b, US	Project Viva	Birth cohort	1999-2003, 6 months follow up	341, mean age ranging from 31.7 to 32.8 yrs	FFQ, 26-28 GW
Suzuki, 2010, Japan	Tohoku study of child development, TSCD	Birth cohort	2001 and 2003, 3 days follow up	599, mean age 31.44 yrs	FFQ, 4 days after delivery
Vecchione, 2021, US	EARLI and HOME studies	Birth cohorts	2009, 3 years follow up and 2003-2006, 12 years	237 and 401, mean age 31.0 yrs	FFQ, 20 th and 36 th GW

Author, year, country	Study name	Design	Inclusion year(s), end, follow-up time (child age)	Study size, maternal age	Dietary assessment method
Xu, 2015, US	Health Outcomes and Measures of the Environment (HOME) Study,	Birth cohort	2003 to 2006, 5 weeks follow up	389, mean age 30 yrs	FFQ, 16 GW and 5 days after birth
Cognition (age 4-18 years)					
Julvez, 2016, Spain	The Spanish Childhood and Environment project	Birth cohort	2004-2008, 14 months and 5 years follow up	1892, mean age not reported	FFQ, 10-13 and 28-32 GW
Mendez, 2008, Spain	INMA	Birth cohort	1997-1998, 4-5 years follow up	482, mean age ranging from 28.442 to 30.445 yrs	FFQ, 3 mo post partum
Steenweg-De Graaff, 2015, Netherlands	Generation R study	Birth cohort	April 2002 to January 2006, 6 years follow up	8663, mean age not reported	FFQ, median 13.8 GW
Vejrup, 2018, Norway	MOBA	Birth cohort	1999-2008, 5 years follow up	38 581, mean age 30.7 yrs	FFQ, 22 GW
Deroma, 2013, Italy		Birth cohort	1999-2001, 7 to 9 years follow up	242, mean age 39.44 yrs	FFQ, 2-3 months post partum
Gale, 2008, UK		Birth cohort	1991-1992, 9 years follow up	226, mean age 27.0 (4.7)	FFQ, 15 and 32 GW
Hibbeln, 2007, UK	ALSPAC	Birth cohort	1991-1992, 6, 18, 30 and 42 months, 7- and 8-years follow-ups	5000-8801, mean age not reported	FFQ, 32 GW
Oken, 2016, US	Project Viva	Birth cohort	1999-2003, 7-8 years follow up	1068, mean age 32.2 yrs	FFQ, 26-28 GW and post partum
Mental health (from birth – 18 years) - mental health problems					
Gale, 2008, UK		Birth cohort	1991-1992, 9 years follow up	226, mean age 27.0 yrs	FFQ, 15 and 32 GW
Hibbeln, 2007, UK	ALSPAC	Birth cohort	1991-1992, 6, 18, 30 and 42 months, 7- and 8-years follow-ups	5000-8801, mean age not reported	FFQ, 32 GW
Mesirow, 2016, UK	ALSPAC	Birth cohort	1991–1992, 4-13 years follow up	7218, mean age not reported	FFQ, 32 GW

Author, year, country	Study name	Design	Inclusion year(s), end, follow-up time (child age)	Study size, maternal age	Dietary assessment method
Mental health (from birth – 18 years) – autism					
Golding, 2019, UK	ALSPAC	Birth cohort	1991–1992, 3, 5, 7, 9 and 11 years follow up	3840, mean age not reported	FFQ, 32 GW
Julvez, 2019, Spain	The Spanish Childhood and Environment project	Birth cohort	2004-2008, 8 years follow- up	1641, maternal age not reported	FFQ, 10-13 and 28-32 GW
Steenweg-De Graaff, 2015, Netherlands	Generation R study	Birth cohort	April 2002 to January 2006, 6 years follow up	8663, mean age not reported	FFQ, median 13.8 GW
Vecchione, 2021, US	EARLI and HOME studies	Birth cohorts, pooled	2009, 3 years follow up and 2003-2006, 12 years	237 and 401, mean age 31.0 yrs	FFQ, 20 th and 36 th GW
Mental health (from birth – 18 years) – ADHD					
Julvez, 2019, Spain	The Spanish Childhood and Environment project	Birth cohort	2004-2008, 8 years follow-up	1641, mean age not reported	FFQ, 10-13 and 28-32 GW
Sagiv, 2012, US		Birth cohort	1993-1998, 8 years follow up	515, mean age not reported	FFQ, shortly after birth

GW=gestation week.

4.10.2.2 *Overlapping publications*

There were no overlapping publications.

4.10.2.3 *Studies by design and geographic region*

Among the 22 included studies, two reported findings from a randomized controlled trial (RCT) involving maternal lean fish consumption with early child development outcomes. The remaining 20 studies reported results from prospective birth cohort designs.

Most of the included studies are from Europe (i.e., UK, Norway, Spain, Sweden, Denmark, Italy, and Netherlands), five studies are from the US, while one study report findings from Japan. Studies include large cohorts, such as the Avon Longitudinal Study of Parents and children (ALSPAC) study, the Spanish Childhood and Environment project, Project Viva, Generation R, the Norwegian Mother, Father and Child Cohort Study (MOBA) and the Danish National Birth Cohort (DNBC), in addition to smaller studies with smaller sample size.

4.10.2.4 *Studies by sex, potential effect modification and other sub-groups*

All studies included both boys and girls, and two studies did the analyses stratified by sex (Xu et al., 2015; Vejrup et al., 2018). One study stratified by seven single nucleotide polymorphisms (SNPs) (Julvez et al., 2019), one by maternal mercury concentration (Oken et al., 2005) and one by breastfeeding status (Mendez et al., 2008).

4.10.2.5 *Studies by fish exposure*

Most studies included total fish intake (sum of all fish, unspecified fish or fish including shellfish and/or fish products). In studies that presented fish intake with and without the inclusion of shellfish, the results without shellfish were considered the main result in line with VKM's protocol for the current analysis.

The most common sub-classification of fish intake was by fat content (fatty or lean). A more infrequent sub-classification was by flesh color (e.g., white, or dark/oily fish). One study also a priori divided the fatty fish into large and small fatty fish (Julvez et al., 2016) based on categories in the FFQ. Large fatty fish was "baked or steamed larger fatty fish such as tuna, swordfish and albacore" and smaller fatty fish was "mackerel, sardines, anchovies and salmon and tinned sardines/mackerel". One study included timing of the exposure during pregnancy: fatty fish early or late in pregnancy (Gale et al., 2008) and one study also included intake of canned fish (Deroma et al, 2013). For the studies dividing into large and small fatty fish (Julvez et al, 2016) and early or late in pregnancy (Gale et al, 2008), there were no information on whether these analytical strategies were pre-specified.

In the analyses, fish consumption was used both as continuous and categorized variables (quintiles, quartiles, tertiles). In the presentation of results, we present the estimates for high vs. low intake when the exposure is used as a categorical variable.

4.10.2.6 *Studies with converted risk estimates*

Of note, Hibbeln et al. (2007) presents estimates for low vs. high intake (none vs. >340 g/wk) and not high vs low as in other studies, but the estimates were close to unity (no association) and not converted as no summary RR was calculated by VKM.

4.10.3 Results from the included primary studies on maternal fish intake and child neurodevelopment

The results from the publications are presented separately for each neurodevelopmental subcategory, early child development, cognition, and mental health.

4.10.3.1 *Studies of maternal total fish intake and early child development*

We identified ten studies, all prospective birth cohorts, with maternal total fish intake as exposure and early child development as outcome (Daniels et al., 2004; Oken et al., 2005; Hibbeln et al., 2007; Julvez et al., 2016; Oken et al., 2008a; Oken et al., 2008b; Suzuki et al., 2010; Xu et al., 2015; Hamazaki et al., 2020; Vecchione et al., 2021) (Table 4.10.3.1-1).

Table 4.10.3.1-1 Results from studies included in the weight of evidence analysis of maternal total fish intake and early child development ≤3 years.

Author, year, country, timing	Intake unit	High-low intake	N	Child age	Development outcome measure	Estimates high low or continuous, (95 % CI)	Overall results	Overall conclusion, domain
Daniels, 2004, UK, 32 GW	Meals/wk, 4 cat	>4 vs rarely/never	7421	15 mo	MCDI ¹ , low test scores, vocabulary comprehension	OR 0.9 (0.07, 1.2)	No sig. assoc., <i>P</i> -trend=0.9	Suggestive beneficial assoc. (sig. assoc. in 5 out of 10 comparisons for dichotomized outcomes, and in 4 out of 5 comparisons for continuous scale outcomes)
	Meals/wk, 4 cat	>4 vs rarely/never	7421	15 mo	MCDI ¹ , low test scores, social activity	OR 0.7 (0.5, 0.9)	Sig. assoc., <i>P</i> -trend=0.02	
	Meals/wk, 4 cat	>4 vs rarely/never	7421	18 mo	DDST ¹ , low test scores, total	OR 0.8 (0.6, 1.1)	No sig. assoc., <i>P</i> -trend=0.04	
	Meals/wk, 4 cat	>4 vs rarely/never	7421	18 mo	DDST ¹ , low test scores, language	OR 0.7 (0.5, 0.9)	Sig. assoc., <i>P</i> -trend=0.04	
	Meals/wk, 4 cat	>4 vs rarely/never	7421	18 mo	DDST ¹ , low test scores, social	OR 1.1 (0.7, 1.5)	No sig. assoc., <i>P</i> -trend=0.7	
	Meals/wk, 4 cat	>4 vs rarely/never	7421	15 mo	MCDI ¹ , high test scores, vocabulary comprehension	OR 1.5 (1.1, 2.0)	Sig. assoc., <i>P</i> -trend=0.05	
	Meals/wk, 4 cat	>4 vs rarely/never	7421	15 mo	MCDI ¹ , high test scores, social activity	OR 1.8 (1.4, 2.4)	Sig. assoc., <i>P</i> -trend=0.02	
	Meals/wk, 4 cat	>4 vs rarely/never	7421	18 mo	DDST ¹ , high test scores, total	OR 1.0 (0.8, 1.6)	No sig. assoc., <i>P</i> -trend=0.07	
	Meals/wk, 4 cat	>4 vs rarely/never	7421	18 mo	DDST ¹ , high test scores, language	OR 1.3 (1.0, 1.8)	Sig. assoc., <i>P</i> -trend=0.03	
	Meals/wk, 4 cat	>4 vs rarely/never	7421	18 mo	DDST ¹ , high test scores, social	OR 1.0 (0.8, 1.3)	No sig. assoc., <i>P</i> -trend=0.09	
	Meals/wk, 4 cat	>4 vs rarely/never	7421	15 mo	MCDI ¹ , mean score, vocabulary comprehension	β 71.9 (70.5, 73.8) vs 68.2 (66.3, 70.5)	Sig. assoc., <i>P</i> -trend=0.03	
	Meals/wk, 4 cat	>4 vs rarely/never	7421	15 mo	MCDI ¹ , mean score, social activity	β 17.2 (16.9, 17.5) vs 16.4 (16.0, 16.7)	Sig. assoc., <i>P</i> -trend=0.002	
	Meals/wk, 4 cat	>4 vs rarely/never	7421	18 mo	DDST ¹ , mean score, total	β 37.8 (37.5, 38.1) vs 37.2 (36.9, 37.6)	Sig. assoc., <i>P</i> -trend=0.004	
	Meals/wk, 4 cat	>4 vs rarely/never	7421	18 mo	DDST ¹ , continuous score, language	β 7.4 (7.3, 7.6) vs 7.1 (6.9, 7.3)	Sig. assoc., <i>P</i> -trend=0.004	

Author, year, country, timing	Intake unit	High-low intake	N	Child age	Development outcome measure	Estimates high low or continuous, (95 %CI)	Overall results	Overall conclusion, domain
	Meals/wk, 4 cat	>4 vs rarely/never	7421	18 mo	DDST ¹ , continuous score, social	β 8.2 (8.0, 8.3) vs 8.1 (7.9, 8.2)	No sig. assoc., <i>P</i> -trend=0.5	
Hibbeln, 2007, UK 32 GW	g/wk, 3 cat	>340 g/wk (ref) vs none	8801	6 mo	DDST ² , sub-optimum scores, gross motor	OR 1.10 (0.90, 1.34)	No sig. assoc., <i>P</i> -trend=0.326	Suggestive beneficial assoc. (sig. protective assoc. in 6 out of 14 outcomes)
	g/wk, 3 cat	>340 g/wk (ref) vs none	8801	18 mo	DDST ² , sub-optimum scores, gross motor	OR 1.02 (0.85, 1.22)	No sig. assoc., <i>P</i> -trend=0.842	
	g/wk, 3 cat	>340 g/wk (ref) vs none	8801	30 mo	DDST ² , sub-optimum scores, gross motor	OR 0.97 (0.80, 1.18)	No sig. assoc., <i>P</i> -trend=0.940	
	g/wk, 3 cat	>340 g/wk (ref) vs none	8801	42 mo	DDST ² , sub-optimum scores, gross motor	OR 0.96 (0.78, 1.18)	No sig. assoc., <i>P</i> -trend=0.716	
	g/wk, 3 cat	>340 g/wk (ref) vs none	8801	6 mo	DDST ² , sub-optimum scores, fine motor	OR 1.01 (0.83, 1.23)	No sig. assoc., <i>P</i> -trend=0.529	
	g/wk, 3 cat	>340 g/wk (ref) vs none	8801	18 mo	DDST ² , sub-optimum scores, fine motor	OR 1.25 (1.04, 1.51)	Sig. protective assoc., <i>P</i> -trend=0.022	
	g/wk, 3 cat	>340 g/wk (ref) vs none	8801	30 mo	DDST ² , sub-optimum scores, fine motor	OR 1.04 (0.85, 1.27)	No sig. assoc., <i>P</i> -trend=0.616	
	g/wk, 3 cat	>340 g/wk (ref) vs none	8801	42 mo	DDST ² , sub-optimum scores, fine motor	OR 1.35 (1.09, 1.66)	Sig. protective assoc., <i>P</i> -trend=0.005	
	g/wk, 3 cat	>340 g/wk (ref) vs none	8801	6 mo	DDST ² , sub-optimum scores, social development	OR 1.15 (0.95, 1.40)	No sig. assoc., <i>P</i> -trend=0.217	
	g/wk, 3 cat	>340 g/wk (ref) vs none	8801	18 mo	DDST ² , sub-optimum scores, social development	OR 1.01 (0.83, 1.24)	No sig. assoc., <i>P</i> -trend=0.894	
	g/wk, 3 cat	>340 g/wk (ref) vs none	8801	30 mo	DDST ² , sub-optimum scores, social development	OR 1.24 (1.01, 1.53)	Sig. protective assoc., <i>P</i> -trend=0.033	
	g/wk, 3 cat	>340 g/wk (ref) vs none	8801	42 mo	DDST ² , sub-optimum scores, social development	OR 1.21 (0.98, 1.50)	Borderline sig. protective assoc., <i>P</i> -trend=0.038	
	g/wk, 3 cat	>340 g/wk (ref) vs none	8801	6 mo	DDST ² , sub-optimum scores, communication	OR 1.30 (1.04, 1.63)	Sig. protective assoc., <i>P</i> -trend=0.018	

Author, year, country, timing	Intake unit	High-low intake	N	Child age	Development outcome measure	Estimates high low or continuous, (95 %CI)	Overall results	Overall conclusion, domain
	g/wk, 3 cat	>340 g/wk (ref) vs none	8801	18 mo	DDST ² , sub-optimum scores, communication	OR 1.26 (1.03, 1.53)	Sig. protective assoc., <i>P</i> -trend=0.049	
Oken, 2008a, Denmark, 25 GW	g/d, 5 cat	Q5 vs Q1 (58.6 vs 5.4 g/d)	28958	6 mo	Developmental milestones ³ , motor	OR 1.17 (1.09, 1.25)	Sig. beneficial assoc.	Sig. beneficial assoc.
	g/d, 5 cat	(58.6 vs 5.4 g/d)	25446	18 mo	Developmental milestones ³ , motor	OR 1.24 (1.15, 1.33)	Sig. beneficial assoc.	
	g/d, 5 cat	(58.6 vs 5.4 g/d)	28958	6 mo	Developmental milestones ³ , social and cognitive	OR 1.33 (1.23, 1.44)	Sig. beneficial assoc.	
	g/d, 5 cat	(58.6 vs 5.4 g/d)	25446	18 mo	Developmental milestones ³ , social and cognitive	OR 1.28 (1.19, 1.37)	Sig. beneficial assoc.	
	g/d, 5 cat	(58.6 vs 5.4 g/d)	28958	6 mo	Developmental milestones ³ , total	OR 1.25 (1.17, 1.34)	Sig. beneficial assoc.	
	g/d, 5 cat	(58.6 vs 5.4 g/d)	25446	18 mo	Developmental milestones ³ , total	OR 1.29 (1.20, 1.38)	Sig. beneficial assoc.	
Hamazaki, 2020, Japan; mid-late pregnancy	g/d, 5 cat	Q5 vs Q1 (median 69.3 vs 5.4 g/d)	81697	6 mo	ASQ-3 ⁴ , low scores, communication	OR 0.96 (0.79, 1.09)	No sig. assoc., <i>P</i> -trend=0.2	Suggestive beneficial assoc. (sig. protective assoc. in 3 out of 10 comparisons)
	g/d, 5 cat	Q5 vs Q1 (median 69.3 vs 5.4 g/d)	81697	12 mo	ASQ-3 ⁴ , low scores, communication	OR 1.00 (0.88, 1.14)	No sig. assoc., <i>P</i> -trend=0.6	
	g/d, 5 cat	Q5 vs Q1 (median 69.3 vs 5.4 g/d)	81697	6 mo	ASQ-3 ⁴ , low scores, gross motor	OR 0.96 (0.81, 1.13)	No sig. assoc., <i>P</i> -trend=0.2	
	g/d, 5 cat	Q5 vs Q1 (median 69.3 vs 5.4 g/d)	81697	12 mo	ASQ-3 ⁴ , low scores, gross motor	OR 0.98 (0.89, 1.08)	No sig. assoc., <i>P</i> -trend=0.8	
	g/d, 5 cat	Q5 vs Q1 (median 69.3 vs 5.4 g/d)	81697	6 mo	ASQ-3 ⁴ , low scores, fine motor	OR 1.02 (0.89, 1.16)	No sig. assoc., <i>P</i> -trend=0.2	

Author, year, country, timing	Intake unit	High-low intake	N	Child age	Development outcome measure	Estimates high low or continuous, (95 %CI)	Overall results	Overall conclusion, domain
	g/d, 5 cat	Q5 vs Q1 (median 69.3 vs 5.4 g/d)	81697	12 mo	ASQ-3 ⁴ , low scores, fine motor	OR 0.90 (0.81, 0.99)	Sig. protective assoc., <i>P</i> -trend=0.02	
	g/d, 5 cat	Q5 vs Q1 (median 69.3 vs 5.4 g/d)	81697	6 mo	ASQ-3 ⁴ , low scores, problem-solving	OR 0.88 (0.79, 0.99)	Sig. protective assoc., <i>P</i> -trend=0.01	
	g/d, 5 cat	Q5 vs Q1 (median 69.3 vs 5.4 g/d)	81697	12 mo	ASQ-3 ⁴ , low scores, problem-solving	OR 0.90 (0.81, 0.99)	Sig. protective assoc., <i>P</i> -trend=0.005	
	g/d, 5 cat	Q5 vs Q1 (median 69.3 vs 5.4 g/d)	81697	6 mo	ASQ-3 ⁴ , low scores, personal-social	OR 1.01 (0.84, 1.22)	No sig. assoc., <i>P</i> -trend=0.08	
	g/d, 5 cat	Q5 vs Q1 (median 69.3 vs 5.4 g/d)	81697	12 mo	ASQ-3 ⁴ , low scores, personal-social	OR 0.99 (0.87, 1.12)	No sig. assoc., <i>P</i> -trend=0.6	
Vecchione, 2021, US, GW 20 and 36	Times/wk, 4 cat	Daily or more vs none	146	36 mo	MSEL, early learning composite ⁵	β 6.55 (-1.94, 15.04)	No sig. assoc.	No sig. assoc.
			270	36 mo	Bayley-II ⁶ , mental development	β -0.78 (-5.86, 4.31)	No sig. assoc.	
Julvez, 2016, Spain, 10-13 and 28-32 GW	g/wk, 5 cat	Q5 (854 g/wk) vs Q1 (195 g/wk)	1892	14 mo	Bayley-II ⁷ mental development	β 2.06 (-0.13, 4.26)	No sig assoc., <i>P</i> -trend=0.08	No sig. assoc.
Oken, 2008b, US, 26-28 GW	Servings/wk, 3 cat	>2 vs never	341	3 yrs	Language and visual motor ability, PPVT, vocabulary ⁸	β 1.2 (-3.5, 6.0)	No sig. assoc.	Suggestive beneficial assoc. (sig. beneficial association in 2 out of 5 outcomes)
	Servings/wk, 3 cat	>2 vs never	341	3 yrs	Language and visual motor ability, WRAVMA ⁹ , drawing	β 6.0 (1.8, 10.2)	Sig. beneficial assoc.	

Author, year, country, timing	Intake unit	High-low intake	N	Child age	Development outcome measure	Estimates high low or continuous, (95 % CI)	Overall results	Overall conclusion, domain
	Servings/wk, 3 cat	>2 vs never	341	3 yrs	Language and visual motor ability, WRAVMA ⁹ , pegboard	β 2.9 (-1.4, 7.1)	No sig. assoc.	
	Servings/wk, 3 cat	>2 vs never	341	3 yrs	Language and visual motor ability, WRAVMA ⁹ , matching	β 2.8 (-3.1, 8.6)	No sig. assoc.	
	Servings/wk, 3 cat	>2 vs never	341	3 yrs	Language and visual motor ability, WRAVMA ⁹ , total score	β 5.3 (0.9, 9.6)	Sig. beneficial assoc.	
Oken, 2005, US, 26-28 GW	Serving/wk continuous	Mean 1.2 β (range 0-5)	135	6 mo	Visual recognition memory ¹⁰	β 2.8 (0.2, 5.4)	Sig. beneficial assoc.	Sig. beneficial assoc.
Suzuki, 2010, Japan, 4 days after birth	g/year, continuous		498	3 days	Neonatal behavioral assessment, NBAS 7 subscales ¹¹ , motor score	β 0.078, $P>0.10$ Adjusted estimates for the other 6 outcomes are not presented	No sig. assoc.	No sig. assoc.
Xu, 2015, US, 16 GW and 5 days after birth	Meals/pregnancy, continuous		389	5 wk	Neobehavioral assessment, NNAS 3 ¹² , special handling	β -0.0027, SE 0.0009, $P=0.002$	Sig. beneficial assoc.	Suggestive beneficial assoc. (sig. beneficial assoc. in 2 out of 5 comparisons. Non-sig. results (3 outcomes) are not reported in study)
	Meals/pregnancy, continuous		389	5 wk	Neobehavioral assessment, NNAS ¹² , higher asymmetry, girls	β 0.007, SE=0.003, $P=0.02$	Sig. beneficial assoc.	

Total fish consumption is the overall fish intake (sum of all fish, unspecified fish or fish including shellfish and/or fish products). GW=gestation week.

¹MacArthur Communicative Development Inventory (MCDI), vocabulary comprehension, social activity and Denver Developmental Screening Test (DDST), language, social and total score, ²Denver Developmental Screening Test, gross and fine motor (all ages), social and communicative skills (6 and 18 mo only), sub-optimum scores, ³Developmental milestones, motor, social/cognition and total, continuous, ⁴Ages and Stages Questionnaire, 3rd edition, scores $>-2SD$, ⁵Mullen Scales of Early Learning, ⁶Bayley Scales of Infant and Toddler Development, 2nd edition, mental development index, continuous, betas express standard scores (100 (15)), ⁷Bayley Scales of Infant and Toddler Development, 2nd edition, mental development index, continuous, betas express standard scores (100 (15)), ⁸Peabody Picture Vocabulary Test (PPVT), continuous, betas expressed as standard scores (mean (SD) 100 (15)), ⁹Wide Range Assessment of Visual Motor Abilities (WRAVMA), drawing, pegboard, matching and total score, continuous, betas expressed as standard scores (mean (SD) 100 (15)),

¹⁰Visual Recognition Memory, continuous, betas express the VRM score (percent novelty preference), mean (range) score in the study 59.8 (10.8, 92.5),

¹¹Neonatal Behavioural Assessment Scale (NBAS), habituation, orientation, motor, range of state, regulation of state, autonomic stability and reflex, betas express the NBAS score, mean (SD) motor score in the study was 4.69 (0.64), ¹²NICU Network Neurobehavioral Scale (NNAS), attention, handling, asymmetry (boys/girls separately), betas express the NNAS scores, mean (SD) scores not known.

In the ten identified studies, the age of the children at outcome assessment varied from three days to 42 months. Two studies used the Denver Developmental Screening Test (Daniels et al., 2004; Hibbeln et al., 2007), while six used different assessment tools assessing language, communication, social, cognitive, and motor skills (Oken et al., 2005; Oken et al., 2008a; Oken et al., 2008b; Julvez et al., 2016; Hamazaki et al., 2020; Vecchione et al., 2021). Two studies assessed neonatal behavior in very young infants (Suzuki et al., 2010; Xu et al., 2015).

In the eight studies where the children were within the range of six to 42 months leaving out Suzuki et al. (2010) and Xu et al. (2015), two studies reported significant associations between maternal fish intake with all included outcomes on developmental milestones (Oken et al., 2008a) and visual recognition memory (VRM) (Oken et al., 2005). While the first study estimates are reported as ORs for attaining developmental milestones in the highest vs. lowest quintile of intake (ORs ranging from 1.17 to 1.29), the latter, using betas expressing the VRM score, is more challenging to interpret in terms of the strength of the findings.

In the remaining six studies, one study included five developmental tests and examined associations using the outcomes dichotomized on the lowest and highest score, as well as on a continuous scale (15 comparisons in total) (Daniels et al., 2004). Generally, findings in this study suggest higher maternal intake (more than four meals weekly compared to none) was associated with higher developmental scores, with nine of 15 comparisons significant. Hibbeln et al. (2007) reported higher odds for sub-optimum developmental scores (fine motor, social development, and communication) with no fish intake at 6, 18, 30 and 42 months, with six out of 14 comparisons significant. Hamazaki et al. (2020) examined the odds for lower child development scores at 6 and 12 months with higher maternal fish intake, reporting significant association in 3 out of 10 comparisons (fine motor and problem-solving skills). A study in fewer children measuring the associations between maternal fish consumption and vocabulary and visual motor abilities (total five tests) reported significant higher standardized scores with higher intake in two out of five outcomes, namely on the total score of visual-motor abilities (5.3, 95%CI 0.9, 9.6), and on the drawing subtest (6.0, 95%CI 1.8, 10.2) (Oken et al., 2008b).

Finally, Julvez et al. (2016) reported no significant associations between maternal total fish intake and infant mental development, and Vecchione et al. (2021) reported no significant association between maternal total fish intake and development scores.

For the two studies in young infants at three days (Suzuki et al., 2010) and five weeks (Xu et al., 2015) with neonatal behavior as an outcome, one study reported no significant associations between the neonatal behavior subscales and maternal total fish intake (Suzuki et al., 2010), while the second study reported protective associations in two out of five comparisons with maternal fish intake, namely need for special handling and asymmetry for girls. Notably, for both studies scores are not standardized and the reported β values are therefore hard to interpret.

4.10.3.2 *Studies of maternal fatty and lean fish intake and early child development*

The two studies reporting findings from a RCTs on maternal lean fish consumption and early child development outcomes the first year of life (Kvestad et al., 2021; Markhus et al., 2020) report conflicting findings. Markhus et al. (2020) reported a negative effect of the lean fish intake on the cognitive subscale score of the Bayley scales of infant and toddler development, 3rd edition, but no significant effect on the motor and language subscales. Kvestad et al. (2021) reported a positive effect of the maternal lean fish intake on socio-emotional development, but no significant effect on scores of general development (Table 4.10.3.2-1).

The study that also reported findings on the associations between maternal fatty (large and small species) and lean fish intake and the early child development outcomes reported no associations with large fatty species, but a significant trend (P -trend=0.03) of higher mental development scores with higher maternal intake of small fatty fish species (Developmental quotient of 2.45 (95%CI 0.54, 4.36) in high vs. low intake) (Julvez et al., 2016). In this study there were no significant association between maternal lean fish consumption and the early child development outcome (Julvez et al., 2016).

Table 4.10.3.2-1 Results from studies included in the weight of evidence analysis for maternal fatty or lean fish intake and early child development ≤3 years.

Author, year, country, timing	Intake unit	High-low intake, or intervention	N	Child age	Development outcome measure	Effect measure intervention or high low (95 % CI or SD)	Overall results	Overall conclusion, domain
RCT, lean fish								
Kvestad, 2021, Norway, 20-36 GW	Times/wk	200 g cod 2 times/wk vs habitual diet	133	3 mo	ASQ-2 ² , general	Mean (95% CI) 225.2 (215.1, 235.3) vs 227.2 (217.0, 237.3)	No sig. effect	No sig. effect on general development (<i>P</i> =0.633, <i>F</i> -test). Sig. positive effect of maternal lean fish intake on socio-emotional development (<i>P</i> =0.020, <i>F</i> -test)
	Times/wk	200 g cod 2 times/wk vs habitual diet	133	9 mo	ASQ-2 ² , general	Mean (95% CI) 247.7 (240.4, 255.0) vs 247.5 (239.7, 255.3)	No sig. effect	
	Times/wk	200 g cod 2 times/wk vs habitual diet	133	11 mo	ASQ-2 ² , general	Mean (95% CI) 213.5 (202.2, 224.9) vs 213.5 (202.2, 224.9)	No sig. effect	
	Times/wk	200 g cod 2 times/wk vs habitual diet	133	3 mo	ASQ ³ , socio-emotional	Mean (95% CI) 20.9 (17.3, 24.4) vs 26.1 (22.5, 29.6)	Sig. positive effect	
	Times/wk	200 g cod 2 times/wk vs habitual diet	133	9 mo	ASQ ³ , socio-emotional	Mean (95% CI) 20.5 (14.3, 26.7) vs 26.8 (21.2, 32.4)	Sig. positive effect	
Markhus, 2020, Norway, 20-36 GW	Times/wk	200 g cod 2 times/wk vs habitual diet	133	11 mo	Bayley III ¹ , cognitive subscale	Mean (SD) 95 (9) vs 99 (10), <i>P</i> =0.045	Sig. negative effect	Suggestive effect.
	Times/wk	200 g cod 2 times/wk vs habitual diet	133	11 mo	Bayley III ¹ , language subscale	Mean (SD) 96 (8) vs 95 (8), <i>P</i> =0.67	No sig. effect	Sig. negative effect of maternal lean fish intake on cognitive score.
	Times/wk	200 g cod 2 times/wk vs habitual diet	133	11 mo	Bayley III ¹ , motor subscale	Mean (SD) 92 (7) vs 94 (8), <i>P</i> =0.24	No sig. effect	No sig. effects on language or motor score
Birth cohort, fatty fish								

Author, year, country, timing	Intake unit	High-low intake, or intervention	N	Child age	Development outcome measure	Effect measure intervention or high low (95 % CI or SD)	Overall results	Overall conclusion, domain
Julvez, 2016, Spain, 10–13 and 28–32 GW	g/wk, quartiles, large fish	Q4 vs Q1 (median 238g/wk vs none)	1892	14 mo	Bayley-II ⁴ , mental development	β 0.51 (–1.43, 2.46)	No sig. assoc., <i>P</i> -trend=0.62	Suggestive beneficial assoc. (sig. assoc. for small, but not large fatty fish)
	g/wk, quartiles, small fish	Q4 vs Q1 (median 147 g/wk vs none)	1892	14 mo	Bayley-II ⁴ , mental development	β 2.45 (0.54, 4.36)	Sig. protective assoc., <i>P</i> -trend=0.03	
Birth cohort, lean fish								
Julvez, 2016, Spain, 10–13 and 28–32 GW	g/wk, quintiles	Q5 vs Q1 (median 557 vs 90 g/wk)	1892	14 mo	Bayley-II ⁴ , mental development	β 1.77 (-0.46, 3.99)	No sig. assoc., <i>P</i> -trend=0.21	No sig. assoc.

¹Bayley Scales of Infant and Toddler Development, 3rd edition, cognitive, language and motor composite score, ²Ages and Stages Questionnaire, 2nd edition, ³Ages and Stages Questionnaire socio-emotional, 2nd edition, ⁴Bayley Scales of Infant and Toddler Development 2nd edition, mental development index, continuous, betas express standard scores (mean 100, SD 15).

4.10.3.3 *Maternal total fish consumption and cognition (age 4-18 years)*

We identified eight studies that reported findings of maternal total fish consumption on cognitive outcomes in children aged four years and above (Julvez et al., 2016; Mendez et al., 2008; Steenweg-De Graaff et al., 2015; Vejrup et al., 2018; Deroma et al., 2013; Gale et al., 2008; Hibbeln et al., 2007; Oken et al., 2016) (Table 4.10.3.3-1).

The age for the cognitive assessments in the included studies varied from four to ten years. Most studies used widely known tools of general abilities such as the Wechsler, McCarthy, and Kaufmann tests (Deroma et al., 2013; Gale et al., 2008; Hibbeln et al., 2007; Oken et al., 2016; Julvez et al., 2016), while one study reported non-verbal cognitive abilities (SON-R 21/2-7 test) (Steenweg-De Graaff et al., 2015) and one language impairments by various tools (Vejrup et al., 2018).

Table 4.10.3.3-1 Results from studies included in the weight of evidence analysis for maternal total fish intake and cognition in children 4-18 years.

Author, year, country, timing	Intake unit	High-low intake	N	Child age	General ability / cognition / language	Estimates high low or continuous, (95 % CI)	Overall results	Overall conclusion, domain
Deroma, 2013, Italy, 2-3 months after birth	Servings/wk, continuous		242	7 yrs	WISC ¹ , full IQ	β 1.16, $P=0.43$	No sig. assoc.	No sig. assoc.
	Servings/wk, continuous		242	7 yrs	WISC ¹ , verbal IQ	β -0.07, $P=0.96$	No sig. assoc.	
	Servings/wk, continuous		242	7 yrs	WISC ¹ , performance IQ	β 2.12, $P=0.15$	No sig. assoc.	
Gale, 2008, UK, early pregnancy (15 GW)	Meals/wk, 4 cat	≥ 3 meals/wk vs never	217	9 yrs	WASI ² , full IQ	β 1.19 (-1.55, 13.3)	No sig. assoc.	No sig. assoc.
Gale, 2008, UK, late pregnancy (32 GW)	Meals/wk, 4 cat	≥ 3 meals/wk vs never		9 yrs	WASI ² , full IQ	β 5.86 (-6.24, 8.61)	No sig. assoc.	
Hibbeln, 2007, UK, 32 GW	g/wk, 3 cat	>340 g/wk (ref) vs none	5407	8 yrs	WISC ³ , full IQ, sub-optimum score	OR 1.29 (0.99, 1.69)	Borderline sig. assoc. (low-high), sig. trend (P -trend=0.038)	Suggestive beneficial assoc. (sig. assoc. in verbal IQ, borderline sig. assoc. for total score and no sig. assoc. for performance IQ)
	g/wk, 3 cat	>340 g/wk (ref) vs none	5407	8 yrs	WISC ³ , verbal IQ, sub-optimum score	OR 1.48 (1.16, 1.90)	Sig. assoc., (P -trend=0.004)	
	g/wk, 3 cat	>340 g/wk (ref) vs none	5407	8 yrs	WISC ³ , performance IQ, sub-optimum score	OR 0.98 (0.76, 1.27)	No sig. assoc., P -trend=0.902	
Julvez, 2016, Spain, 10-13 and 28.32 GW	g/wk, quintiles	Q5 vs Q1 (854 vs 195 g/wk)	1589	5 yr	McCarthy ⁴ , total score	β 2.08 (-0.04, 4.21)	Borderline sig. beneficial assoc., P -trend=0.049	Suggestive beneficial assoc. (borderline sig. assoc. with total score, no sig. assoc. with 6 subscale scores)
	g/wk, quintiles	Q5 vs Q1 (854 vs 195 g/wk)	1589	5 yr	McCarthy ⁴ , verbal	β 1.57 (-0.67, 3.81)	No sig. assoc.	
	g/wk, quintiles	Q5 vs Q1 (854 vs 195 g/wk)	1589	5 yr	McCarthy ⁴ , perceptual performance	β 1.74 (-0.44, 3.91)	No sig. assoc.	

Author, year, country, timing	Intake unit	High-low intake	N	Child age	General ability / cognition / language	Estimates high low or continuous, (95 % CI)	Overall results	Overall conclusion, domain
	g/wk, quintiles	Q5 vs Q1 (854 vs 195 g/wk)	1589	5 yr	McCarthy ⁴ , memory	β 1.94 (−0.34, 4.22)	No sig. assoc.	
	g/wk, quintiles	Q5 vs Q1 (854 vs 195 g/wk)	1589	5 yr	McCarthy ⁴ , quantitative	β 1.56 (−0.69, 3.81)	No sig. assoc.	
	g/wk, quintiles	Q5 vs Q1 (854 vs 195 g/wk)	1589	5 yr	McCarthy ⁴ , motor	β 1.61 (−0.62, 3.85)	No sig. assoc.	
	g/wk, quintiles	Q5 vs Q1 (854 vs 195 g/wk)	1589	5 yr	McCarthy ⁴ , executive function	β 1.93 (−0.24, 4.09)	No sig. assoc.	
Mendez, 2008, Spain, during pregnancy	Times/wk, 4 cat	>3 times/wk vs <1 time/wk	392	4 yrs	McCarthy ⁵ , total score	β 4.68 ($P < 0.05$)	Sig. beneficial assoc.	Sig. beneficial assoc.
Oken, 2016, US, 26-28 GW	Servings/wk, 3 cat	≥ 3 serv/wk vs none	1068	6-10 yrs	Kaufman ⁶ , verbal	β 0.48 (−2.76, 3.72)	No sig. assoc.	No sig. assoc.
	Servings/wk, 3 cat	≥ 3 serv/wk vs none	1068	6-10 yrs	Kaufman ⁶ , non-verbal	β −1.32 (−5.49, 2.85)	No sig. assoc.	
	Servings/wk, 3 cat	≥ 3 serv/wk vs none	1068	6-10 yrs	WRAVMA ⁷ , drawing	β −0.26 (−4.48, 3.96)	No sig. assoc.	
	Servings/wk, 3 cat	≥ 3 serv/wk vs none	1068	6-10 yrs	WRAML ⁸ , design memory	β −0.67 (−1.36, 0.03)	No sig. assoc.	
	Servings/wk, 3 cat	≥ 3 serv/wk vs none	1068	6-10 yrs	WRAML ⁸ , picture memory	β −0.36 (−1.13, 0.40)	No sig. assoc.	
	Servings/wk, 3 cat	≥ 3 serv/wk vs none	1068	6-10 yrs	WRAML ⁸ , summary memory	β −0.99 (−2.11, 0.13)	No sig. assoc.	

Author, year, country, timing	Intake unit	High-low intake	N	Child age	General ability / cognition / language	Estimates high low or continuous, (95 % CI)	Overall results	Overall conclusion, domain
Steenweg-De Graaff, 2015, Netherlands, median GW 13.8	g/d, 2 cat	13.6 g/day vs none	3162	6 yrs	Non-verbal IQ SON-R 21/2-7 ⁹	β 1.45 (–0.33, 3.22)	No sig. assoc.	No sig. assoc.
Vejrup, 2018, Denmark, 22 GW	g/wk, 3 cat	>400 g/wk vs 0-100 g/wk	38397	5 yrs	ASQ ¹⁰ , language subscale	β –0.06 (–0.1, –0.01)	Sig. protective assoc.	Sig. protective assoc.
	g/wk, 3 cat	>400 g/wk vs 0-100 g/wk			Language 20 ¹¹	β –0.05 (–0.1, –0.01)	Sig. protective assoc.	
	g/wk, 3 cat	>400 g/wk vs 0-100 g/wk			SLAS ¹²	β –0.07 (–0.1, –0.03)	Sig. protective assoc.	

¹Wechsler intelligence scale for children, full, verbal and performance IQ, continuous, standardized scores with mean (SD) of 100 (15); ²Wechsler Abbreviated Scale of Intelligence, full scale IQ, continuous, standardized scores with mean (SD) of 100 (15); ³Wechsler intelligence scale for children (WISC-III UK), total, verbal and performance standardized scores with mean (SD) of 100 (15), sub-optimum scores categorized on lowest quartile; ⁴McCarthy Scales of Children's Abilities; total, verbal, perceptual-performance, memory, quantitative, motor, executive function, continuous, standardized scores mean (SD) of 100 (15); ⁵McCarthy Scales of Children's Abilities, total score, continuous, standardized scores with mean (SD) of 100 (15); ⁶Kaufman Brief Intelligence Test, verbal and non-verbal, continuous, standardized scores mean (SD) of 100 (15); ⁷Wide Range Assessment of Visual Motor Abilities (WRAVMA), drawing, continuous, standardized scores mean (SD) of 100 (15) and; ⁸Wide Range Assessment of Memory and Learning (WRAML) design memory, picture memory and summary memory, standardized scores mean (SD) of 10 (3); ⁹SON-R 21/2-7; sum of Mosaics (visualization abilities), Categories (reasoning abilities), continuous, standardized scores with mean (SD) of 100 (15); ¹⁰Ages and Stages Questionnaire - language subscale, continuous, standardized z-score; ¹¹Language 20 – Twenty statements about Language related difficulties list, standardized z-score; ¹²Speech and Language Assessment Scale, standardized z-score.

Of the eight studies, three used the Wechsler Intelligence Scale for Children (WISC) in children 7-9 years old (Table 4.10.3.3-1) (Deroma et al., 2013; Gale et al., 2008; Hibbeln et al., 2007), reporting findings on full IQ (all studies) and for verbal and performance IQ (Deroma et al., 2013; Hibbeln et al., 2007) (mean (SD) score of 100 (15)). While two of these studies reported no significant associations between maternal fish intake and the WISC scores (Deroma et al., 2013; Gale et al., 2008), the final study reported a trend of increased risk for sub-optimum WISC scores with lower maternal intake for the full and the verbal subscale IQ but not for the performance IQ (Hibbeln et al., 2007).

Two studies, using the McCarthy scales of children's abilities in 4- and 5-year-olds, reported marginally significant improvement in the total score with increased maternal fish intake (Julvez et al., 2016; Mendez et al., 2008), but not with the subtest scores (Julvez et al., 2016). Additionally, one study reported less language impairments with increased maternal fish intake in five-year-old children (Vejrup et al., 2018).

The remaining two studies reported non-significant associations of maternal fish consumption with the six included cognitive outcomes in 6–10-year-olds (Oken et al., 2016), and no significant associations between maternal intake and non-verbal IQ in 6-year-old children (Steenweg-De Graaff et al., 2015).

4.10.3.4 *Maternal fatty and lean fish consumption and cognition*

For maternal fatty fish consumption, there was one report of a protective association between maternal intake of large fatty species and total IQ score, but not with small fatty species (Julvez et al., 2016), while the second identified study reported no significant associations (Gale et al., 2008). In Julvez et al. (2016), there were no significant association between maternal lean fish intake and the cognitive score (Table 4.10.3.4-1).

Table 4.10.3.4-1 Results from studies (birth cohorts) included in the weight of evidence analysis for maternal fatty or lean fish intake and cognition in children 4-18 years.

Author, year, country	Fish type, timing	Intake unit	High-low intake	N	Child age	General ability measure	Estimates high low, β (95 % CI)	Overall results
Gale, 2008, UK	Fatty, early pregnancy, 15 GW	Meals/wk, 4 cat	≥ 3 meals/wk vs never	217	9 yr	WASI ² , full IQ	$\beta = -0.99$ (-6.01, 4.02)	No sig. assoc.
	Fatty, late pregnancy, 32 GW	Meals/wk, 4 cat	≥ 3 meals/wk vs never	217	9 yr	WASI ² , full IQ	$\beta = -0.29$ (-5.34, 4.76)	No sig. assoc.
Julvez, 2016, Spain	Fatty fish, large, 10–13 and 28–32 GW	g/wk, quartile	Q4 (238 g/wk) vs Q1 (none)	1589	5 yr	McCarthy ¹ , total score	$\beta = 2.29$ (0.42, 4.16)	Sig. beneficial assoc., P -trend=0.02
	Fatty fish, small, 10–13 and 28–32 GW	g/wk, quartile	Q4 (147 g/wk) vs Q1 (none)	1589	5 yr	McCarthy ¹ , total score	$\beta = 0.91$ (-0.93, 2.76)	No sig. assoc., P -trend=0.25
	Lean, 10–13 and 28–32 GW	g/wk, quintile	Q5 (557 g/wk) vs Q1 (90 g/wk)	1589	5 yr	McCarthy ¹ , total score	$\beta = 1.89$ (-0.25, 4.03)	No sig. assoc., P -trend=0.11

GW=gestational week.

¹McCarthy Scales of Children's Abilities; ²Wechsler Abbreviated Scale of Intelligence, full, verbal and performance IQ.

4.10.3.5 *Maternal fish intake and mental health*

We identified eight studies on maternal total fish consumption and mental health in children up to 18 years (Gale et al., 2008; Hibbeln et al., 2007; Sagiv et al., 2012; Steenweg-De Graaff et al., 2015; Julvez et al., 2016; Mesirow et al., 2016; Julvez et al., 2019; Vecchione et al., 2021). Of these, four studies were on autism, two on ADHD, and three studies were on other mental health conditions.

On maternal fish consumption and child autistic symptoms, one study reported associations with total, fatty and lean fish consumption (Julvez et al., 2016), one with fatty and lean fish (Golding et al., 2018) and the remaining with total fish intake only (Steenweg-De Graaff et al., 2015; Vecchione et al., 2021) (Tables 4.10.3.5-1 and 4.10.3.5-2).

The three identified studies on maternal total fish consumption and child autistic symptoms (Julvez et al., 2016; Steenweg-De Graaff et al., 2015; Vecchione et al., 2021) reported contrasting findings. While Julvez et al. (2016) reported a reduction in autistic symptoms with higher maternal total fish intake, Steenweg-De Graaff et al. (2015) and Vecchione et al. (2021) reported no significant association.

Julvez et al. (2016) divided fatty fish into small and large species and found a significant reduction in autistic symptoms with higher maternal intake for large, but not for small species. In the second study on maternal fatty fish consumption, the associations were examined on autistic traits and on diagnosed autism at different ages in childhood, reporting a significant trend of poor social cognition, coherent speech, and sociability with lower intake, but not on repetitive behavior and diagnosed autism (Golding, et al. 2018).

For lean fish and autistic symptoms, Golding et al. (2018) reported the same associations as with fatty fish, while Julvez et al. (2016) reported no significant association.

We identified two studies reporting findings on the associations between maternal fish consumption and childhood ADHD symptomatology. One of these studies reported on maternal total, fatty and lean fish intake (Julvez et al., 2019) and one on total fish intake only (Sagiv et al., 2012). These studies included different sets of tests of attention function and ADHD symptoms, the assessment batteries in the two studies differed, however. Both identified studies reported protective associations between maternal fish intake and the outcomes. One study reported protective associations in three out of nine included outcomes (Sagiv et al., 2012). In this study there were also adverse associations in one outcome, namely a higher risk of error of commission (incorrect response). For the second study, there were protective associations in two out of three outcomes (Julvez et al., 2019). Notably, this was comparing high intake to low intake, in which median intake in the higher category was more than 800 grams/week and low intake was no fish intake.

For maternal large fatty fish and lean fish intake and ADHD, Julvez et al. (2019) reported significant associations between intake and tests of attention function, but not for small fatty fish.

Of the three studies on the associations between maternal fish consumption and other mental health problems in childhood, all reported findings on the association between total fish intake and mental health problems (Gale et al., 2008; Hibbeln et al., 2007; Mesirow et al., 2016), while one also examined association with total and fatty fish intake (Gale et al., 2008). The latter study divided further into early and late pregnancy exposure. We did not identify studies on maternal lean fish consumption and childhood mental health problems. All studies used the Strength and Difficulties questionnaire (SDQ) as a measure of mental health problems. The SDQ is a widely used and validated questionnaire in research on mental health in children and includes a total score and five subscale scores (emotional symptoms, prosocial behavior, conduct problems, hyperactivity, and peer problems).

One study reported no significant association between total maternal fish intake in early and late pregnancy and scores on SDQ (Gale et al., 2008), while results from the second study showed a significantly smaller chance for sub-optimum pro-social behavior scores with higher intake, but no associations in the other five subscales (Hibbeln et al., 2007). The third study by Mesirow et al. (2016) investigated maternal fish consumption and conduct problem trajectories (early onset vs. low conduct problem) and reported lower maternal total fish intake in the group of children with early onset problems compared to low conduct problem children.

For maternal fatty fish intake, Gale et al. (2008) reported a significant lower risk for higher hyperactivity scores with maternal fish intake less than once a week compared to never, but no significant associations for the other 4 subscales.

Table 4.10.3.5-1 Results from birth cohort studies included in the weight of evidence analysis for maternal total fish intake and mental health in children.

Author, year, country, timing	Intake unit	High-low intake	N	Child age	Outcome measure	Estimates high-low or continuous, (95 %CI)	Overall results	Overall conclusion
Autistic symptoms								
Julvez, 2016, Spain, 10–13 and 28–32 GW	g/wk, quintiles	Q5 vs Q1 (854 vs 195 g/wk)	1589	5 yrs	Autistic symptoms; CAST ²	β -0.55 (-1.06, -0.04)	Sig. assoc., <i>P</i> -trend=0.037	Sig. protective assoc.
Steenweg-De Graaff, 2015, Netherlands, median GW 13.8	g/d, 2 cat	13.6 g/day, use vs none	3802	6 yrs	Autistic traits; SRS ³	β -0.022 (-0.055, 0.010)	No sig. assoc	No sig. assoc.
Vecchione, 2021, US, GW 20 and 36	Times/wk, 4 cat	Daily or more) vs none	426	3 to 8 yrs	Autism spectrum disorder traits, SRS ¹	β 0.68 (-5.97, 7.34)	No sig. assoc	No sig. assoc.
ADHD								
Julvez, 2019, Spain, 10–13 and 28–32 GW	g/wk, quintiles	Q5 vs Q1	1644	8 yrs	ANT ⁷ , HRT-SE (ms)	β -11.8 (-24.2, 0.7)	No sig. assoc., <i>P</i> -trend=0.118	Suggestive beneficial assoc. (sig. protective assoc. in 2 out of 3 outcomes)
	g/wk, quintiles	Q5 vs Q1	1644	8 yrs	ANT ⁷ , omissions	HR 0.76 (0.61, 0.94)	Sig. assoc., <i>P</i> -trend=0.002	
	g/wk, quintiles	Q5 vs Q1	1644	8 yrs	CPRS-R:S ⁸	HR 0.84 (0.73, 0.97)	Sig. assoc., <i>P</i> -trend=0.004	
Sagiv, 2012, US, shortly after birth	Serving/wk, 2 cat	>2 vs ≤ 2	457	8 yrs	CRS-T ⁴ , inattentive behavior	RR 0.6 (0.4, 0.9)	Sig. assoc	Suggestive beneficial assoc. (sig. protective assoc. in 3 out of 9 outcomes)
	Serving/wk, 2 cat	>2 vs ≤ 2	457	8 yrs	CRS-T ⁴ , impulsive behavior/hyperactivity	RR 0.4 (0.2, 0.6)	Sig. assoc	
	Serving/wk, 2 cat	>2 vs ≤ 2	457	8 yrs	CRS-T ⁴ , total	RR 0.6 (0.4, 0.9)	Sig. assoc.	
	Serving/wk, 2 cat	>2 vs ≤ 2	457	8 yrs	NES2 – CPT ⁵ , mean reaction time (milli sec.)	β 10.1 (-3.9, 24.1)	No sig. assoc.	
	Serving/wk, 2 cat	>2 vs ≤ 2	457	8 yrs	NES2 – CPT ⁵ , reaction time variability (milli sec.)	β -0.5 (-6.3, 5.4)	No sig. assoc.	

Author, year, country, timing	Intake unit	High-low intake	N	Child age	Outcome measure	Estimates high-low or continuous, (95 % CI)	Overall results	Overall conclusion
	Serving/wk, 2 cat	>2 vs ≤ 2	457	8 yrs	NES2 – CPT ⁵ , errors of omission	RR 0.9 (0.7, 1.2)	No sig. assoc.	
	Serving/wk, 2 cat	>2 vs ≤ 2	457	8 yrs	NES2 – CPT ⁵ , errors of commission	RR 1.1 (0.9, 1.3)	No sig. assoc.	
	Serving/wk, 2 cat	>2 vs ≤ 2	457	8 yrs	WISC-III ⁶ , processing speed	β 2.0 (-0.8, 4.8)	No sig. assoc.	
	Serving/wk, 2 cat	>2 vs ≤ 2	457	8 yrs	WISC-III ⁶ , freedom from distractibility	β 1.5 (-1.1, 4.0)	No sig. assoc.	
Other mental health conditions/mental health problems								
Gale, 2008, UK, early pregnancy, 15 GW	Meals/wk, 3 cat	≥1 meals/wk vs never	217	9 yrs	SDQ ⁹ , high scores, total difficulties	OR 0.23 (0.04 to 1.24).	No sig. assoc.	No sig. assoc.
Hibbeln, 2007, UK, 32 GW	g/wk, 3 cat	>340 g/wk (ref) vs none	6582	7 yrs	SDQ ¹¹ , low scores, total score	OR 1.17 (0.86, 1.60)	No sig. assoc., <i>P</i> -trend=0.3832	Suggestive beneficial assoc. (sig. assoc. in 1 of 6 outcomes)
	g/wk, 3 cat	>340 g/wk (ref) vs none	6582	7 yrs	SDQ ¹¹ , low scores, prosocial	OR 1.44 (1.05, 1.97)	Sig. assoc., <i>P</i> -trend=0.0249	
	g/wk, 3 cat	>340 g/wk (ref) vs none	6582	7 yrs	SDQ ¹¹ , low scores, hyperactivity	OR 1.13 (0.84, 1.53)	No sig. assoc., <i>P</i> -trend=0.6293	
	g/wk, 3 cat	>340 g/wk (ref) vs none	6582	7 yrs	SDQ ¹¹ , low scores, emotional	OR 1.09 (0.83, 1.44)	No sig. assoc., <i>P</i> -trend=0.6810	
	g/wk, 3 cat	>340 g/wk (ref) vs none	6582	7 yrs	SDQ ¹¹ , low scores, conduct	OR 1.21 (0.89, 1.64)	Sig. assoc., <i>P</i> -trend=0.2869	
	g/wk, 3 cat	>340 g/wk (ref) vs none	6582	7 yrs	SDQ ¹¹ , low scores, peer problems	OR 1.25 (0.96, 1.62)	No sig. assoc., <i>P</i> -trend=0.1753	
Mesirow, 2016, UK, months after delivery	Serving/wk, continuous		5348	4-13 yrs	Conduct problems ¹² (CP) trajectories ³ EOP vs low CP	F-value=11.49, <i>P</i> =0.001	Sig. protective assoc	Sig. protective assoc.

GW=gestational week. 1Social Responsiveness Scale (SRS), total raw scores, 2Childhood Asperger Syndrome Test (CAST), 3Social Responsiveness Scale (SRS), 4Connors Rating Scale – Teacher (CRS-T) dichotomized 86th percentile, 5NES2 Continuous Performance Test (NES2-CPT), 6Wechsler Intelligence for Children, 3rd edition (WISC-III), coding, symbol search (processing speed), digit span and arithmetic (freedom from distractibility), 7The Attention Network

Test (ANT), 8Conners' Parent Rating Scale Short Form Revised (CPRS-R:S), 9Strength and Difficulties Questionnaire (SDQ), emotional problems, conduct problems, hyperactivity/inattention, peer problems, total problems, high scores (upper 10-20%), 9Strength and Difficulties Questionnaire (SDQ), emotional problems, conduct problems, hyperactivity/inattention, peer problems, total problems, high scores (upper 10-20%), 11SDQ, emotional problems, conduct problems, hyperactivity/inattention, peer problems, total problems, sub-optimum scores (10% lowest tail), 12Strength and difficulties questionnaire, conduct problem subscale, early onset conduct problem (EOP) vs. low conduct problem (CP) trajectories 3-14 yrs).

Table 4.10.3.5-2 Results from birth cohort studies included in the weight of evidence analysis for maternal fatty or lean fish intake and mental health in children.

Author, year, country	Fish intake, timing	Intake unit	High-low intake	N	Child age	Description	Estimates high-low or continuous, (95 % CI)	Overall results	Overall conclusion
Autism									
Golding, 2018, UK	Fatty fish, 32 GW	Times/wk, 3 cat	>1 vs never	2800	7 yrs	Poor social cognition ¹		<i>P</i> -trend=0.017	Suggestive beneficial assoc. (sig. trend for a higher proportion with the autistic traits with lower maternal intake in 3 out of 4 outcomes, no sig. assoc. with diagnosed autism)
		Times/wk, 3 cat	>1 vs never	2800	9 yrs	Poor coherent speech ²		<i>P</i> -trend=0.044	
		Times/wk, 3 cat	>1 vs never	2800	3 yrs	Poor sociability ³		<i>P</i> -trend=0.010	
		Times/wk, 3 cat	>1 vs never	2800	5 yrs	Repetitive behavior ⁴		<i>P</i> -trend=0.971	
		Times/wk, 3 cat	>1 vs never	2800	11 yrs	Diagnosed autism		<i>P</i> -trend=0.672	
	Lean fish, 32 GW	Times/wk, 3 cat	>1 vs never	2800	7 yrs	Poor social cognition ¹		<i>P</i> -trend<0.001	Suggestive beneficial assoc. (sig. trend for a higher proportion with autistic traits with lower maternal intake in 3 out of 4 outcomes, no sig.
		Times/wk, 3 cat	>1 vs never	2800	9 yrs	Poor coherent speech ²		<i>P</i> -trend=0.026	
		Times/wk, 3 cat	>1 vs never	2800	3 yrs	Poor sociability ³		<i>P</i> -trend=0.029	

Author, year, country	Fish intake, timing	Intake unit	High-low intake	N	Child age	Description	Estimates high-low or continuous, (95 %CI)	Overall results	Overall conclusion
		Times/wk, 3 cat	>1 vs never	2800	5 yrs	Repetitive behavior ⁴		<i>P</i> -trend=0.481	assoc. with diagnosed autism)
		Times/wk, 3 cat	>1 vs never	2800	11 yrs	Diagnosed autism		<i>P</i> -trend=0.640	
Julvez, 2016, Spain	Fatty fish, large, 10–13 and 28–32 GW	g/wk, continuous and 4 cat	Q4 (238 g/wk) vs Q1 (none)	1589	5 yrs	Autistic symptoms; CAST ⁵	β -0.57 (-1.01, -0.13)	Sig. assoc., <i>P</i> -trend=0.013	Sig. protective assoc.
	Fatty fish, small, 10–13 and 28–32 GW	g/wk, continuous and 4 cat	Q4 (147 g/wk) vs Q1 (none)	1589	5 yrs	Autistic symptoms; CAST ⁵	β -0.37 (-0.81, 0.07)	No sig. assoc., <i>P</i> -trend=0.11	No sig. assoc.
	Lean fish, 10–13 and 28–32 GW	g/wk, 5 cat	Q5 (557 g/wk) vs Q1 (90 g/wk)	1589	5 yrs	Autistic symptoms; CAST ⁵	β -0.70 (-1.22, -0.19)	Sig. assoc., <i>P</i> -trend=0.10	Sig. assoc.
ADHD									
Julvez, 2019, Spain	Fatty fish, large, 10–13 and 28–32 GW	g/wk, 4 cat	Q4 vs Q1	1644	8 yrs	ANT ⁶ , HRT-SE (ms)	β -9.4 (-20.3, 1.5)	No sig. assoc., <i>P</i> -trend=0.094	Suggestive beneficial assoc. (sig. assoc. in 2 out of 3 outcomes)
		g/wk, 4 cat	Q4 vs Q1	1644	8 yrs	ANT ⁶ , Omission errors	HR 0.76 (0.63, 0.95)	Sig. assoc., <i>P</i> -trend=0.004	
		g/wk, 4 cat	Q4 vs Q1	1644	8 yrs	CPRS-R:S ⁷	HR 0.86 (0.76, 0.97)	Sig. assoc., <i>P</i> -trend=0.02	
	Fatty fish small, 10–13 and 28–32 GW	g/wk, 4 cat	Q4 vs Q1	1644	8 yrs	ANT ⁶ , HRT-SE (ms)	β -10.3 (-21.2, 0.5)	No sig. assoc., <i>P</i> -trend=0.078	Suggestive beneficial assoc. (sig. assoc. in 2 out of 3 outcomes)
		g/wk, 4 cat	Q4 vs Q1	1644	8 yrs	ANT ⁶ , Omission errors	HR 0.79 (0.65, 0.95)	Sig. assoc., <i>P</i> -trend=0.005	
		g/wk, 4 cat	Q4 vs Q1	1644	8 yrs	CPRS-R:S ⁷	HR 0.94 (0.83, 1.07)	No sig. assoc., <i>P</i> -trend=0.326	
		g/wk, 4 cat	Q4 vs Q1	1644	8 yrs	ANT ⁶ , HRT-SE (ms)	β -3.4 (-14.4, 7.7)	No sig. assoc., <i>P</i> -trend=0.118	Suggestive beneficial assoc. (sig. protective

Author, year, country	Fish intake, timing	Intake unit	High-low intake	N	Child age	Description	Estimates high-low or continuous, (95 % CI)	Overall results	Overall conclusion
	Lean fish, 10–13 and 28–32 GW	g/wk, 4 cat	Q4 vs Q1	1644	8 yrs	ANT ⁶ , Omission errors	HR 0.77 (0.64, 0.93),	Sig assoc., <i>P</i> -trend=0.007	assoc. in 2 out of 3 outcomes)
		g/wk, 4 cat	Q4 vs Q1	1644	8 yrs	CPRS-R:S ⁷	HR 0.89 (0.78, 1.05)	Sig. assoc., <i>P</i> -trend=0.034	
Mental health problems									
Gale, 2008, UK	Fatty fish, early pregnancy, 15 GW	Meals/wk, 3 cat	≥3 vs never	217	9 yrs	SDQ ⁸ , high score, total difficulties	OR 0.83 (0.22, 3.04)	No sig. assoc.	No sig. assoc.
		Meals/wk, 3 cat	≥3 vs never	217	9 yrs	SDQ ⁸ , high score, emotional symptoms	OR 0.79 (0.20, 3.08)	No sig. assoc.	
		Meals/wk, 3 cat	≥3 vs never	217	9 yrs	SDQ ⁸ , high score, conduct problems	OR 0.36 (0.11, 1.21)	No sig. assoc.	
		Meals/wk, 3 cat	≥3 vs never	217	9 yrs	SDQ ⁸ , high score, hyperactivity	OR 0.41 (0.15, 1.12)	No sig. assoc.	
		Meals/wk, 3 cat	≥3 vs never	217	9 yrs	SDQ ⁸ , high score, peer problems	OR 1.44 (0.47, 4.80)	No sig. assoc.	
	Fatty fish, late pregnancy, 32 GW	Meals/wk, 3 cat	≥3 vs never	217	9 yrs	SDQ ⁸ , high score, total difficulties	OR 1.20 (0.32, 4.49)	No sig. assoc.	No sig. assoc.
		Meals/wk, 3 cat	≥3 vs never	217	9 yrs	SDQ ⁸ , high score, emotional symptoms	OR 1.04 (0.23, 4.66)	No sig. assoc.	
		Meals/wk, 3 cat	≥3 vs never	217	9 yrs	SDQ ⁸ , high score, conduct problems	OR 0.31 (0.08, 1.10)	No sig. assoc.	
		Meals/wk, 3 cat	≥3 vs never	217	9 yrs	SDQ ⁸ , high score, hyperactivity	OR 0.72 (0.26, 1.98)	No sig. assoc.	
		Meals/wk, 3 cat	≥3 vs never	217	9 yrs	SDQ ⁸ , high score, peer problems	OR 0.82 (0.27 to 2.57)	No sig. assoc.	

GW=gestational week. ¹Social and Communication Disorders Checklist (poor social cognition, 10% worst), ²Child Communication Checklist (poor coherent speech, 10% worst), ³Emotionality, Activity, Sociability temperament traits (poor sociability, 10% worst), ⁴Repetitive behavior measure (10% worst),

⁵Childhood Asperger Syndrome Test (CAST), ⁶The Attention Network Test (ANT), HRT-SE and omission errors subtests, ⁷Conners' Parent Rating Scale Short Form Revised (CPRS-R:S), ⁸Strength and Difficulties Questionnaire (SDQ), emotional problems, conduct problems, hyperactivity/inattention, peer problems, total problems, high scores (upper 10-20%).

4.10.3.6 Summary estimates based **on VKM's inclusion** of primary studies

Due to heterogenous study outcomes and many studies reporting unstandardized linear regression coefficients, no summary estimates were calculated. Conclusions were therefore based on an overall qualitative evaluation of the results from the publications.

4.10.3.7 VKM's search compared to previous systematic reviews on neurodevelopment in children

Table 4.10.3.7-1 gives an overview of publications included in the Hibbeln (2019) on maternal fish consumption and child neurodevelopment that are not included by VKM. Notably, Hibbeln et al. (2019) report findings from publications on seafood intake and not only fish intake.

VKM excluded five of the publications at abstract screening since they did not fulfil the criteria for inclusion, and six were excluded at full text screening – all due to having MeHg measurements and not fish as exposure. One was excluded after it was rated C at the quality assessment step, and one paper did not appear in VKM's search. All publications identified by VKM were included in Hibbeln et al. (2019).

Table 4.10.3.7-1 Overview of publications in the Hibbeln et al. (2019) review not included by VKM.

Pregnancy	Excluded in selection process	Graded C	Did not appear in VKM's search
Barbone, 2019			X
Budtz-Jørgensen et al, 2007	X		
Davidson et al, 2011	X		
Davidson et al, 2008	X		
Furlong et al, 2018		X	
Golding et al, 2017*	X		
Hibbeln et al, 2018*	X		
Hu et al, 2016*	X		
Lederman et al, 2008	X		
Llop et al, 2017*	X		
Lynch et al, 2011*	X		
Valent et al, 2013*	X		
Williams et al, 2001	X		

*Methyl mercury as exposure

4.10.4 Heterogeneity maternal fish intake and child neurodevelopment

Study characteristics of the publications identified by VKM on maternal fish intake and neurodevelopment in children vary substantially, both in age of assessment, the

neurodevelopmental outcome measured and the methods of assessment strategy. There is a consistency in the findings however, in terms of the direction of the associations, where most are on the protective/beneficial side and few are adverse associations, suggesting little unexplained heterogeneity.

4.10.5 Dose-response relationship maternal fish intake and child neurodevelopment

The VKM project group did not identify meta-evidence on a dose-response relationship between maternal fish consumption and child neurodevelopment.

4.10.6 Weight of evidence for maternal fish intake and neurodevelopment in children

In this section, the evidence of the association between maternal fish intake and child neurodevelopment is weighted according to the WCRF criteria presented in Chapter 3.1.6, (Box 2).

Published evidence of fish intake and child neurodevelopment

The single identified systematic review of maternal seafood intake and child neurodevelopment (Hibbeln et al., 2019) concluded that there was moderate and consistent evidence indicating that consumption of seafood during pregnancy has beneficial associations with child neurodevelopment. In that systematic review, the evidence does not meet the criteria for “strong” evidence due to the absence of RCTs.

VKM evaluated 22 primary studies of the association between maternal fish consumption (total, or fatty or lean) and child neurodevelopment (categorized as early child development, cognition, and mental health). The published evidence was more limited for fatty and lean fish, than for total fish. Of 22 studies, four publications reported results on fatty fish (Julvez et al., 2016, Julvez et al., 2019, Gale et al., 2008, Golding et al., 2018) and five on lean fish (Julvez et al., 2016, Julvez et al., 2019, Golding et al., 2018, Kvestad et al., 2021, Markhus et al., 2020) with no more than two studies on fatty fish and three on lean fish for each outcome category (early child development, cognition, or mental health). Two of these publications reported findings from a RCT on maternal lean fish consumption and early child development. Summary estimates could not be calculated (see Chapter 4.10.3.6) and conclusions must be based on an overall evaluation of the results from the publications.

Twelve identified studies reported findings on *maternal total fish consumption and early child development*, two from a RCT and the remaining from prospective birth cohorts. Although, few of the identified studies use identical assessment tools, all outcomes express early child development, such as social and communicative skills, language and vocabulary and fine motor skills. Out of the twelve publications, two studies reported protective associations for all included outcomes (Oken et al., 2005; Oken et al., 2008a), and five studies reported significant protective results in some of the included comparisons (Daniels et al., 2004;

Hibbeln et al., 2007; Hamazaki et al., 2020; Oken et al., 2008b); Xu et al., 2015). Three studies reported no significant results (Suzuki et al., 2010; Julvez et al., 2016; Vecchione et al., 2021), and notably, findings from the one RCT reported conflicting findings with both protective (Kvestad et al., 2021) and adverse effects (Markhus et al., 2020) of the maternal lean fish intake on early child development. Overall, the results are from studies using different tests, and some of the identified studies include many outcomes without correcting for multiple comparisons, and not all comparisons reach significance. Hence, based on the current findings, we cannot rule out false positive results.

For maternal fish intake and cognition in children four years and above, eight studies, all prospective birth cohorts were identified. Two studies reported protective associations in all included outcomes (Mendez et al., 2008; Vejrup et al., 2018), two studies reported protective associations in some of the included comparisons (Hibbeln et al., 2007; Julvez et al., 2016) and four studies reported no significant associations (Deroma et al., 2013; Oken et al., 2016; Gale et al., 2008; Steenweg-De Graaff et al., 2015). Of the eight studies, three report multiple comparisons across neurodevelopmental sub-categories and ages (i.e., Gale et al., 2008; Hibbeln et al., 2007; Julvez et al., 2016). The cognitive outcomes addressed in this section were not set as primary outcome in these publications and there was no report of adjustments for the multiple comparisons. The age for the cognitive assessments in the included studies varied, and although studies used widely known tools of general abilities, the assessment strategy was not uniform across the studies. These factors represent potential biases to the findings, and hence, we cannot rule out false positive results.

For the mental health outcomes, seven studies report findings on maternal fish exposure (total, fatty and lean) and mental health. Although seven studies in total, these studies report findings on mental health conditions that differ and were categorized as autism, ADHD, and other mental health conditions. It could be questioned whether these sub-categories should be evaluated as one, or many categories. This will not impact the conclusion, however. Of the seven studies, two report of protective associations in all included outcomes (mental health problems (Mesirow et al., 2016) and autism (Julvez et al., 2016), three report of protective associations in some of the included outcomes (mental health problems (Hibbeln et al., 2007) and ADHD (Sagiv et al., 2012; Julvez et al., 2019)) and two report of no significant association (mental health problem (Gale et al., 2008) and autism (Steenweg-De Graaff et al., 2015)). Hence, evidence for associations between maternal fish intake and the mental health outcomes is considered to be scarce.

To sum up, the evidence for a beneficial association between maternal intake and early child development and cognition was the most convincing in the evaluations, while evidence for beneficial associations with mental health conditions (autism, ADHD, and other mental health conditions) was limited by the small number of studies and that in general few of the included comparisons yielded protective and significant findings. Evidence on the association between maternal fatty fish consumption and child neurodevelopment was limited, and the one identified RCT (two publications) on maternal lean fish consumption and early child development outcomes, suggested both protective and adverse effects of the maternal lean fish consumption and did not substantiate the suggestive evidence.

Heterogeneity

Unexplained heterogeneity in terms of the direction of the results is limited.

Mechanism

Fish contain several nutrients that are important for brain structural and functional development and at the same time neurotoxins that may have adverse effects on the developing brain. The impact may be greater in the fetal period due to the high metabolic demands of the brain and its increased susceptibility to adverse exposures.

Upgrading factors

No substantial upgrading factors were identified.

4.10.6.1 *Conclusion weight of evidence maternal fish intake and child neurodevelopment*

There is evidence from more than two independent prospective cohort studies (referring to the WCRF criteria); in total VKM identified 22 primary studies (20 publications reporting results from prospective cohort studies and two publications reporting results from an RCT). The direction of the associations is generally consistent (towards protective), suggesting limited unexplained heterogeneity, and there is evidence for biological mechanisms between the fish intake and neurodevelopment (biological plausibility).

The neurodevelopmental domains, age at assessment and assessment tools varied substantially from study to study. Moreover, ten of the identified studies included more than three comparisons, (Daniels et al., 2004; Hibbeln et al., 2007; Oken et al., 2008a; Oken et al., 2008b; Julvez et al., 2016; Oken et al., 2016; Sagiv et al., 2012; Gale et al., 2008; Golding et al., 2018), with significant beneficial associations in only part of these comparisons. Only two studies had a pre-defined primary outcome (Oken et al., 2008b; Oken et al., 2005) and none reported to adjustment for multiple comparisons. Hence, false positive findings cannot be ruled out. In conclusion, the evidence for a protective association between maternal total fish consumption and child neurodevelopment is graded "limited, suggestive".

There were fewer studies of fatty fish and lean fish than total fish and the evidence is graded "limited, no conclusion" for the effects of fatty fish and lean fish on child neurodevelopment.

4.11 Child fish intake and neurodevelopment in children

4.11.1 VKM's search for published meta-analyses or systematic reviews on fish intake and neurodevelopment in children

See Chapter 4.9.1.

4.11.2 VKM's systematic review of primary studies on child fish intake and child neurodevelopment

4.11.2.1 *Included studies from the search*

A total of 10 publications graded A or B on child fish intake and child neurodevelopment were included in the evaluation (Daniels et al., 2004; Aberg et al., 2009; Kim et al., 2010; Mesirov et al., 2016; Handeland et al., 2017; Skotheim et al., 2017; Øyen et al., 2018; Hysing et al., 2018; Demmelmair et al., 2019; Teisen et al., 2020).

A description of the publications with child fish consumption as exposure (study name, design, time-period, size, and dietary assessment methods) can be found in Table 4.11.2.1-1.

Table 4.11.2.1-1 Overview of primary studies included in the weight of evidence analysis of fish intake and neurodevelopmental outcomes in children.

Author, year, country	Study name	Design	Inclusion year(s), end, follow-up time (child age)	Study size	Dietary assessment method
Early child development (age ≤ 3 years)					
Daniels, 2004, UK	ALSPAC	Prospective cohort	1991-1992, 15 and 18 months old.	7421	FFQ, 6 and 12 months
Cognition (age 4-18 years)					
Aberg, 2009, Sweden	ALLERGY2000	Prospective cohort, males, military register	2000-2004, 3 years follow up, 18 years at follow up.	3971	Questionnaire including questions on fish consumption at 15 yrs
Demmelmair, 2019, Germany	Sister study of FINS:KIDS, using Atlantic salmon	RCT, fatty fish vs meat for lunch 3 times weekly	2014, 16 weeks intervention, pre-post testing, 4-6 years old.	205	NA
Handeland, 2017, Norway	FINS:TEENS	RCT, fatty fish vs meat for lunch 3 times weekly	2015, 12 weeks, pre-post testing, 14-15 years	426	Short FFQ

Author, year, country	Study name	Design	Inclusion year(s), end, follow-up time (child age)	Study size	Dietary assessment method
Kim, 2010, Sweden	ALLERGY2000	Prospective cohort, registry-based outcome	2000-2004, 1 years follow up, 16 years at follow up.	9448	Questionnaire including questions on fish consumption at 15 yrs
Teisen, 2020, Denmark	FISK Junior	RCT, fatty fish vs Poultry for dinner/lunch for 2/3 times weekly	2016, 12 weeks +/- 2 intervention, pre-post testing, 8-9 years old.	199	NA
Øyen, 2018, Norway	FINS:KIDS	RCT, fatty fish vs meat for lunch 3 times weekly	2015, 16 weeks intervention, pre-post testing, 4-6 years old.	232	Short FFQ
Mental health (from birth – 18 years)					
Hysing, 2018, Norway	FINS: KIDS	RCT, fatty fish vs meat for lunch 3 times weekly	2015, 16 weeks intervention, pre-post testing, 4-6 years old.	232	NA
Mesirow, 2016, UK	ALSPAC	Prospective cohort	1991–1992, 4-13 years old.	7218	FFQ, 3 yrs
Skotheim, 2017, Norway	FINS: TEENS	RCT, fatty fish vs meat for lunch 3 times weekly	2015, 12 weeks, pre-post testing, 14-15 years	478	NA

4.11.2.2 *Overlapping publications*

There were no overlapping publications. Although there were multiple publications from the same studies, each publication contributed unique results.

4.11.2.3 *Studies by design and geographic region*

Of the ten publications, six reported findings from four RCTs involving fatty fish intake and cognition (Handeland et al., 2017; Øyen et al., 2018; Demmelmair et al., 2019; Teisen et al., 2021) and three on mental health (Skotheim et al., 2017; Hysing et al., 2018, Teisen et al., 2021) in children and adolescents, and the remaining had a prospective cohort design (Daniels et al, 2004; Aberg et al., 2009; Kim et al, 2010; Mesirow et al., 2016).

Two of the RCTs are from Norway (Handeland et al., 2017; Skotheim et al., 2017; Øyen et al., 2018; Hysing et al., 2018), one from Denmark (Teisen et al., 2021) and one from Germany (Demmelmair et al., 2019). The prospective studies are from Sweden (Aberg et al., 2009; Kim et al., 2010) and UK (Daniels et al., 2004; Mesirow et al., 2016).

4.11.2.4 *Studies by sex, potential effect modification and other sub-groups*

All studies included both boys and girls, and two stratified the analyses by sex (Demmelmair et al., 2019; Kim et al., 2010). One study stratified the analyses by parental educational level (high vs. low) (Aberg et al., 2009).

In four out of six publications reporting findings from RCTs, estimates were presented unadjusted and adjusted for dietary compliance (i.e., the amount of the fish/meat intervention children/adolescents consumed during the intervention period) (Hysing et al., 2018; Øyen et al., 2018, Skotheim et al., 2017; Handeland et al., 2017).

4.11.2.5 *Studies by fish exposure*

The six publications based on RCTs presented the effect of fatty fish intake in children and adolescents. The prospective cohort studies studied the associations between the outcome and total fish intake.

4.11.3 Results from the included primary studies on child fish intake and child neurodevelopment

The results from the publications are presented categorized by child total fish intake and child fatty fish intake.

4.11.3.1 *Studies of child total fish intake and early child development, cognition, and mental health*

Four cohort studies on child total fish intake and the neurodevelopmental outcomes were evaluated. One of these was with early child development as an outcome (Daniels et al., 2004), two with cognition (Aberg et al., 2009; Kim et al., 2010) and one with mental health (Mesirow et al., 2016), see Table 4.11.3.1-1. Age at neurodevelopmental assessment ranged from 15 months to 18 years in these studies.

Daniels et al. (2004) reported associations between child total fish intake at six and 12 months (never/rarely vs. one meal or more per week) and early child development outcomes, with protective associations, although not all reached significance.

The two publications involving child cognition reported associations between total fish intake in adolescents at the age of 15 years and school grades at 16 years (Kim et al., 2010) and cognitive abilities at 18 years (Aberg et al., 2009). Both studies reported significant better scores with higher fish intake for all outcomes (Aberg et al., 2008; Kim et al., 2010).

For mental health problems, the single identified cohort study reported no significant associations between child total fish intake at three years and conduct problem trajectories (Mesirow et al., 2016).

Table 4.11.3.1-1 Results from prospective cohort studies included for weight of evidence analysis of child total fish intake and early child development, cognition, and mental health.

Author, year, country	Fish intake, timing	Intake unit	High-low intake	N	Child age	Outcome measures	Estimates high-low or continuous, 95 % CI	Overall results	Overall conclusion, domain
Cognition									
Aberg, 2009, Sweden	15 yrs	Meal/wk, 3 cat	>1/ wk vs <1/wk	3972	18 yrs	STANDARD NINE ³ , combined IQ	β 0.58 (0.39, 0.77)	Sig. assoc.	Sig. assoc.
	15 yrs	Meal/wk, 3 cat	>1/ wk vs <1/wk	3972	18 yrs	STANDARD NINE ³ , verbal	β 0.46 (0.29, 0.64)	Sig. assoc.	
	15 yrs	Meal/wk, 3 cat	>1/ wk vs <1/wk	3972	18 yrs	STANDARD NINE ³ , visuo-spatial	β 0.51 (0.32, 0.69)	Sig. assoc.	
Daniels, 2004, UK	6 mo	Meal/wk, 2 cat	≥ 1 meal/wk vs rarely/never	7421	15 mo	MCDI ¹ , low test score, vocabulary comprehension	OR 0.9 (0.8, 1.1)	No sig. assoc.	Suggestive beneficial assoc. overall. At 6 months: sig. assoc. in 5 of 10 comparisons for dichotomized outcomes, sig. assoc. in all comparisons for outcomes on continuous scale. At 12 months: sig. assoc. in 4 of 10 comparisons for dichotomized
	6 mo	Meal/wk, 2 cat	≥ 1 meal/wk vs rarely/never	7421	15 mo	MCDI ¹ , low test score, social activity	OR 0.8 (0.7, 1.0)	Borderline sig. assoc.	
	6 mo	Meal/wk, 2 cat	≥ 1 meal/wk vs rarely/never	7421	18 mo	DDST ² , low test score, total,	OR 0.9 (0.7, 1.1)	No sig. assoc.	
	6 mo	Meal/wk, 2 cat	≥ 1 meal/wk vs rarely/never	7421	18 mo	DDST ² , low test score, language	OR 0.8 (0.7, 1.0)	Borderline sig. assoc.	
	6 mo	Meal/wk, 2 cat	≥ 1 meal/wk vs rarely/never	7421	18 mo	DDST ² , low test score, social	OR 0.9 (0.7, 1.2)	No sig. assoc.	
	6 mo	Meal/wk, 2 cat	≥ 1 meal/wk vs rarely/never	7421	15 mo	MCDI ¹ , high test score, vocabulary comprehension	OR 1.3 (1.1, 1.3)	Sig. assoc.	
	6 mo	Meal/wk, 2 cat	≥ 1 meal/wk vs rarely/never	7421	15 mo	MCDI ¹ , high test score, social activity	OR 1.3 (1.1, 1.5)	Sig. assoc.	
	6 mo	Meal/wk, 2 cat	≥ 1 meal/wk vs rarely/never	7421	18 mo	DDST ² , high test score, total,	OR 1.1 (0.9, 1.3)	No sig. assoc.	
	6 mo	Meal/wk, 2 cat	≥ 1 meal/wk vs rarely/never	7421	18 mo	DDST ² , high test score, language	OR 1.1 (1.0, 1.3)	No sig. assoc.	
	6 mo	Meal/wk, 2 cat	≥ 1 meal/wk vs rarely/never	7421	18 mo	DDST ² , high test score, social	OR 1.2 (1.0, 1.3)	Borderline sig. assoc.	

Author, year, country	Fish intake, timing	Intake unit	High-low intake	N	Child age	Outcome measures	Estimates high-low or continuous, 95 % CI	Overall results	Overall conclusion, domain
	6 mo	Meal/wk, 2 cat	≥1 meal/wk vs rarely/never	7421	15 mo	MCDI ¹ , continuous, vocabulary comprehension	β 3.3 (SE=0.73), <i>P</i> =0.0001	Sig. assoc.	outcomes, and in 4 out of 5 for outcomes on a continuous scale)
	6 mo	Meal/wk, 2 cat	≥1 meal/wk vs rarely/never	7421	15 mo	MCDI ¹ , continuous score, social activity	β 0.49 (SE=0.13), <i>P</i> =0.0002	Sig. assoc.	
	6 mo	Meal/wk, 2 cat	≥1 meal/wk vs rarely/never	7421	18 mo	DDST ² , continuous score, total,	β 0.5 (SE=0.13), <i>P</i> =0.001	Sig. assoc.	
	6 mo	Meal/wk, 2 cat	≥1 meal/wk vs rarely/never	7421	18 mo	DDST ² , continuous score, language	β 0.16 (SE=0.06), <i>P</i> =0.006	Sig. assoc.	
	6 mo	Meal/wk, 2 cat	≥1 meal/wk vs rarely/never	7421	18 mo	DDST ² , continuous score, social	β 0.14 (SE=0.05), <i>P</i> =0.008	Sig. assoc.	
	12 mo	Meal/wk, 2 cat	≥1 meal/wk vs rarely/never	7421	15 mo	MCDI ¹ , low test score, vocabulary comprehension	OR 0.7 (0.5, 0.8)	Sig. assoc.	
	12 mo	Meal/wk, 2 cat	≥1 meal/wk vs rarely/never	7421	15 mo	MCDI ¹ , low test score, social activity	OR 0.7 (0.6, 0.9)	Sig. assoc.	
	12 mo	Meal/wk, 2 cat	≥1 meal/wk vs rarely/never	7421	18 mo	DDST ² , low test score, total,	OR 0.8 (0.8, 0.9)	Sig. assoc.	
	12 mo	Meal/wk, 2 cat	≥1 meal/wk vs rarely/never	7421	18 mo	DDST ² , low test score, language	OR 0.9 (0.7, 1.1)	No sig. assoc.	
	12 mo	Meal/wk, 2 cat	≥1 meal/wk vs rarely/never	7421	18 mo	DDST ² , low test score, social	OR 0.6 (0.4, 0.7)	Sig. assoc.	
	12 mo	Meal/wk, 2 cat	≥1 meal/wk vs rarely/never	7421	15 mo	MCDI ¹ , high test score, vocabulary comprehension	OR 1.2 (0.9, 1.5)	No sig. assoc.	
	12 mo	Meal/wk, 2 cat	≥1 meal/wk vs rarely/never	7421	15 mo	MCDI ¹ , high test score, social activity	OR 0.9 (0.7, 1.1)	No sig. assoc.	
	12 mo	Meal/wk, 2 cat	≥1 meal/wk vs rarely/never	7421	18 mo	DDST ² , high test score, total,	OR 0.9 (0.7, 1.2)	No sig. assoc.	
	12 mo	Meal/wk, 2 cat	≥1 meal/wk vs rarely/never	7421	18 mo	DDST ² , high test score, language	OR 1.0 (0.8, 1.2)	No sig. assoc.	

Author, year, country	Fish intake, timing	Intake unit	High-low intake	N	Child age	Outcome measures	Estimates high-low or continuous, 95 % CI	Overall results	Overall conclusion, domain
	12 mo	Meal/wk, 2 cat	≥1 meal/wk vs rarely/never	7421	18 mo	DDST ² , high test score, social	OR 1.0 (0.8, 1.2)	No sig. assoc.	
	12 mo	Meal/wk, 2 cat	≥1 meal/wk vs rarely/never	7421	15 mo	MCDI ¹ , continuous score, vocabulary comprehension	β 4.4 (SE=0.92), P=0.0001	Sig. assoc.	
	12 mo	Meal/wk, 2 cat	≥1 meal/wk vs rarely/never	7421	15 mo	MCDI ¹ , continuous score, social activity	β 0.44 (SE=0.16), P=0.006	Sig. assoc.	
	12 mo	Meal/wk, 2 cat	≥1 meal/wk vs rarely/never	7421	18 mo	DDST ² , continuous score, total,	β 0.50 (SE=0.17), P=0.004	Sig. assoc.	
	12 mo	Meal/wk, 2 cat	≥1 meal/wk vs rarely/never	7421	18 mo	DDST ² , continuous score, language	β 0.09 (SE=0.07), P=0.2	No sig. assoc.	
	12 mo	Meal/wk, 2 cat	≥1 meal/wk vs rarely/never	7421	18 mo	DDST ² , continuous score, social	β 0.2 (SE=0.07), P=0.005	Sig. assoc.	
Kim, 2010, Sweden	15 yrs	Meal/wk, 3 cat	>1/ wk vs <1/wk	9448	16 yrs	School grades ⁴ , total	β 19.9 (16.5, 23.3)	Sig. assoc.	Sig. assoc.
Mental health									
Mesirow, 2016, UK	3 yrs	Serving/wk, continuous		5348	4-13 yrs	Conduct problems (CP) trajectories ⁵ , EOP vs low CP	F-value fish intake in EOP vs for CP trajectories 2.46, P=0.12	No sig. assoc.	No sig. assoc.

¹MacArthur Communicative Development Inventory (MCDI), vocabulary comprehension, social activity; ²Denver Developmental Screening Test (DDST), language, social and total score; ³STANDARD NINE, 4 logic tests, 3 verbal tests, 2 visuospatial tests, nine-point standard scale, mean (SD) =5(2), collected from the Swedish Military Conscript Register; ⁴School grades, sum of 16 subjects, collected from national register; ⁵Strength and Difficulties Questionnaire, conduct problem subscale, trajectory 3-14 yrs; ⁶Strength and Difficulties Questionnaire, conduct problem subscale at 4, 7, 8, 10, 12 and 13 yrs to identify: early onset persistent (EOP) and low conduct problems (Low CP); ⁶Strength and Difficulties Questionnaire, emotional difficulties and hyperactivity subscales from 4 to 13 yrs.

4.11.3.2 *Child fatty fish intake, cognition, and mental health*

A total of six publications reporting results from four different RCTs were evaluated. Two of these publications reported the effect of fatty fish on cognition in preschool children (Øyen et al., 2018; Demmelmair et al., 2017), one in children at school age (Teisen et al., 2020) and one in adolescents (Handeland et al., 2017). One reported the effect on mental health in preschool children (Hysing et al., 2018), one in school aged children (Teisen et al., 2020) and one in adolescents (Skotheim et al., 2017). All publications included multiple outcomes and multiple comparisons, none stated a primary outcome or adjusted for multiple comparisons.

In four out of six studies, analyses were adjusted for dietary compliance in addition to the crude analysis (Øyen et al., 2018; Hysing et al., 2018; Handeland et al., 2017; Skotheim et al., 2017). The participants (i.e., preschool children and adolescents) consumed different amounts of their lunch interventions (both the fatty fish and meat (control)), and for each study meal, there were exact measures of how much of the intervention the child/adolescent consumed (constituting dietary compliance).

Table 4.11.3.2-1 Results from studies included in the weight of evidence analysis of child fatty fish intake, cognition, and mental health, results from RCTs.

Author, year, country	Intervention, fish intake, duration	Intake unit	N	Child age	Outcome measure	Estimates (95%CI), dietary intervention	Overall results	Overall conclusions
Demmelmair, 2019, Germany	Fatty fish vs meat, 16 wks	3 lunch meals/wk	205	4-6 yrs	WPPSI-III ¹ , IQ scores, FSIQ	MD 1.2 (0.6, 3.1) vs 1.0 (-0.2, 2-2), <i>P</i> =0.334	No sig. difference	Suggestive beneficial effect (sig. difference between groups in 3 out of 17 comparisons)
	Fatty fish vs meat, 16 wks	3 lunch meals/wk	205	4-6 yrs	WPPSI-III ¹ , IQ scores, VIQ	MD -0.4 (-1.8, 1.0) vs -0.3 (-1.6, 1.1), <i>P</i> =0.923	No sig. difference	
	Fatty fish vs meat, 16 wks	3 lunch meals/wk	205	4-6 yrs	WPPSI-III ¹ , IQ scores, PIQ	MD 3.4 (1.3, 5.6) vs 3.3 (1.1, 5.5), <i>P</i> =0.934	No sig. difference	
	Fatty fish vs meat, 16 wks	3 lunch meals/wk	205	4-6 yrs	WPPSI-III ¹ subtests, raw score, total	MD 17.4 (14.8, 20.1) vs 14.6 (11.9, 17.3), <i>P</i> =0.143	No sig. difference	
	Fatty fish vs meat, 16 wks	3 lunch meals/wk	205	4-6 yrs	WPPSI-III ¹ subtests, raw score, verbal	MD 2.4 (1.5, 3.4) vs 1.9 (0.9, 2.9), <i>P</i> = 0.444	No sig. difference	
	Fatty fish vs meat, 16 wks	3 lunch meals/wk	205	4-6 yrs	WPPSI-III ¹ subtests, raw score, information	MD 1.1 (0.6, 1.5) vs 0.6 (0.2, 1.0), <i>P</i> =0.142	No sig. difference	
	Fatty fish vs meat, 16 wks	3 lunch meals/wk	205	4-6 yrs	WPPSI-III ¹ subtests, raw score, vocabulary	MD 0.9 (0.2, 1.6) vs 0.4 (0.3, 1.1), <i>P</i> =0.329	No sig. difference	
	Fatty fish vs meat, 16 wks	3 lunch meals/wk	205	4-6 yrs	WPPSI-III ¹ subtests, raw score, word reasoning	MD 0.6 (0.1, 1.0) vs 0.8 (0.4, 1.3), <i>P</i> =0.407	No sig. difference	
	Fatty fish vs meat, 16 wks	3 lunch meals/wk	205	4-6 yrs	WPPSI-III ¹ subtests, raw score, performance	MD 5.0 (3.8, 6.2) vs 3.2 (2.1, 4.4), <i>P</i> =0.039	Sig. difference	
	Fatty fish vs meat, 16 wks	3 lunch meals/wk	205	4-6 yrs	WPPSI-III ¹ subtests, raw score, block design	MD 2.3 (1.4, 3.2) vs 1.5 (0.6, 2.4), <i>P</i> =0.222	No sig. difference	
	Fatty fish vs meat, 16 wks	3 lunch meals/wk	205	4-6 yrs	WPPSI-III ¹ subtests, raw score, matrix reasoning	MD 1.1 (0.7, 1.6) vs 1.0 (0.6, 1.5), <i>P</i> =0.718	No sig. difference	
	Fatty fish vs meat, 16 wks	3 lunch meals/wk	205	4-6 yrs	WPPSI-III ¹ subtests, raw score, picture concepts	MD 1.5 (1.0, 2.1) vs 0.7 (0.1, 1.3), <i>P</i> =0.038	Sig. difference	
	Fatty fish vs meat, 16 wks	3 lunch meals/wk	205	4-6 yrs	WPPSI-III ¹ subtests, raw score, processing speed	MD 10.1 (7.9, 12.3) vs 9.4 (7.1, 11.6), <i>P</i> =0.640	No sig. difference	
	Fatty fish vs meat, 16 wks	3 lunch meals/wk	205	4-6 yrs	WPPSI-III ¹ subtests, raw score, coding	MD 5.2 (3.3, 7.0) vs 5.4 (3.6, 7.3), <i>P</i> =0.833	No sig. difference	

Author, year, country	Intervention, fish intake, duration	Intake unit	N	Child age	Outcome measure	Estimates (95 %CI), dietary intervention	Overall results	Overall conclusions
	Fatty fish vs meat, 16 wks	3 lunch meals/wk	205	4-6 yrs	WPPSI-III ¹ subtests, raw score, symbol search	MD 5.0 (4.1, 6.0) vs 3.6 (2.6, 4.6), $P=0.04$	Sig. difference	
	Fatty fish vs meat, 16 wks	3 lunch meals/wk	205	4-6 yrs	Fine manual dexterity; 9-HPT ² , dominant hand	MD 2.0 (2.9, 1.1) vs 3.0 (3.8, 2.1), $P=0.149$	No sig. difference	
	Fatty fish vs meat, 16 wks	3 lunch meals/wk	205	4-6 yrs	Fine manual dexterity; 9-HPT ² , non-dominant hand	MD 3.6 (4.8, 2.4) vs 3.6 (4.8, 2.4), $P=0.976$	No sig. difference	
Handeland, 2017, Norway	Fatty fish vs meat, 12 wks	3 lunch meals/wk	426	14-15 yrs	Attention performance, d2 test of attention ³ , concentration performance	MD for meat vs fish (ref) -2.3 (-6.8, 2.2), $P=0.317$	No sig. difference	Suggestive beneficial effect (sig. difference between groups in 3 out of 6 outcomes)
	Fatty fish vs meat, 12 wks	3 lunch meals/wk	426	14-15 yrs	Attention performance, d2 test of attention ³ , total performance	MD for meat vs fish (ref) -7.9 (-17.4, 1.6), $P=0.103$	No sig. difference	
	Fatty fish vs meat, 12 wks	3 lunch meals/wk	426	14-15 yrs	Attention performance, d2 test of attention ³ , processing speed	MD for meat vs fish (ref) -11.8 (-23.3, -0.4), $P=0.042$	Sig. difference	
	Fatty fish vs meat, 12 wks	3 lunch meals/wk	426	14-15 yrs	Attention performance, d2 test of attention ³ , omission errors	IRR for meat vs fish (ref) 0.85 (0.74, 0.98), $P=0.026$	Sig. difference	
	Fatty fish vs meat, 12 wks	3 lunch meals/wk	426	14-15 yrs	Attention performance, d2 test of attention ³ , commission errors	IRR for mB/Geat vs fish (ref) 0.91 (0.59, 1.39), $P=0.648$	No sig. difference	
	Fatty fish vs meat, 12 wks	3 lunch meals/wk	426	14-15 yrs	Attention performance, d2 test of attention ³ , total errors	IRR for meat vs fish (ref) 0.88 (0.75, 1.02), $P=0.094$	Borderline sig. difference	
Hysing, 2018, Norway	Fatty fish vs meat, 16 wks	3 lunch meals/wk	232	4-6 yrs	Mental health problems SDQ ⁴ , total	MD 0.22 (-0.47, 0.91) vs -0.37 (-1.03, 0.30), $P=0.191$	No sig. difference	No sig. effects
	Fatty fish vs meat, 16 wks	3 lunch meals/wk	232	4-6 yrs	Mental health problems SDQ ⁴ , emotional	MD -0.02 (-0.29, 0.24) vs -0.08 (-0.33, 0.17), $P=0.765$	No sig. difference	
	Fatty fish vs meat, 16 wks	3 lunch meals/wk	232	4-6 yrs	Mental health problems SDQ ⁴ , conduct	MD 0.04 (-0.22, 0.30) vs -0.07 (-0.32, 0.18), $P=0.501$	No sig. difference	

Author, year, country	Intervention, fish intake, duration	Intake unit	N	Child age	Outcome measure	Estimates (95 %CI), dietary intervention	Overall results	Overall conclusions
	Fatty fish vs meat, 16 wks	3 lunch meals/wk	232	4-6 yrs	Mental health problems SDQ ⁴ , hyperactivity/inattention	MD 0.10 (-0.23, 0.42) vs -0.03 (-0.35, 0.28), <i>P</i> =0.536	No sig. difference	
	Fatty fish vs meat, 16 wks	3 lunch meals/wk	232	4-6 yrs	Mental health problems SDQ ⁴ , peer problems	MD 0.07 (-0.15, 0.29) vs -0.16 (-0.37, 0.05), <i>P</i> =135	No sig. difference	
Skotheim, 2017, Norway	Fatty fish vs meat, 12 wks	3 lunch meals/wk	425	14-15 yrs	Mental health problems SDQ ⁴ , high scores, total	MD -1.54 (-3.01, 0.08) vs -4.11 (-5.55, -2.67), <i>P</i> =0.02	Sig. difference	Suggestive beneficial effect (sig. difference between groups in 2 out of 6 outcomes)
	Fatty fish vs meat, 12 wks	3 lunch meals/wk	425	14-15 yrs	Mental health problems SDQ ⁴ , high scores, emotional	MD -0.31 (-0.92, 0.30) vs -1.20 (-1.75, -0.64), <i>P</i> =0.04	Sig. difference	
	Fatty fish vs meat, 12 wks	3 lunch meals/wk	425	14-15 yrs	Mental health problems SDQ ⁴ , high scores, conduct	MD -1.64 (-2.39, -0.89) vs -1.53 (-2.16, -0.91), <i>P</i> =0.83	No sig. difference	
	Fatty fish vs meat, 12 wks	3 lunch meals/wk	425	14-15 yrs	Mental health problems SDQ ⁴ , high scores, hyperactivity/inattention	MD -0.90 (-1.44, -0.37) vs -0.44 (-0.99, 0.11), <i>P</i> =0.23	No sig. difference	
	Fatty fish vs meat, 12 wks	3 lunch meals/wk	425	14-15 yrs	Mental health problems SDQ ⁴ , high scores, peer problems	MD -1.47 (-2.28, -0.65) vs -1.95 (-2.70, -1.20), <i>P</i> =0.78	No sig. difference	
	Fatty fish vs meat, 12 wks	3 lunch meals/wk	425	14-15 yrs	Mental health problems SDQ ⁴ , high scores, prosocial behavior	MD 0.84 (0.15, 1.52) vs 1.05 (0.35, 1.75), <i>P</i> =0.63	No sig. difference	
Teisen*, 2020, Denmark	Fatty fish vs poultry	~300g/wk	199	8-9 yrs	Overall cognitive performance ⁵	MD -0.17 (-0.35, 0.01)	Borderline sig. difference	Suggestive beneficial effect (sig. difference between groups in 9 out of 38 comparisons)
	Fatty fish vs poultry	~300g/wk	199	8-9 yrs	Speed-accuracy trade-off ⁶	MD 0.02 (-0.22, 0.27)	No sig. difference	
	Fatty fish vs poultry	~300g/wk	199	8-9 yrs	Processing speed ⁷ , d2 processing speed, characters	MD 2.5 (-4.7, 9.7)	No sig. difference	
	Fatty fish vs poultry	~300g/wk	199	8-9 yrs	Processing speed ⁷ , stroop color time, s	MD -2 (-5, 1)	No sig. difference	
	Fatty fish vs poultry	~300g/wk	199	8-9 yrs	Processing speed ⁷ , witch reaction time, ms	MD -39 (-83, 6)	No sig. difference	
	Fatty fish vs poultry	~300g/wk	199	8-9 yrs	Processing speed ⁷ , TI 5-choice reaction time median, ms	MD -3 (-12, 6)	No sig. difference	

Author, year, country	Intervention, fish intake, duration	Intake unit	N	Child age	Outcome measure	Estimates (95 %CI), dietary intervention	Overall results	Overall conclusions
	Fatty fish vs poultry	~300g/wk	199	8-9 yrs	Attention ⁷ , switch total error, %	OR 0.97 (0.86, 1.09)	No sig. difference	
	Fatty fish vs poultry	~300g/wk	199	8-9 yrs	Attention ⁷ , flanker total error, %	OR 0.90 (0.65, 1.25)	No sig. difference	
	Fatty fish vs poultry	~300g/wk	199	8-9 yrs	Attention ⁷ , RVP total error, %	OR 0.88 (0.79, 0.98)	Sig. difference	
	Fatty fish vs poultry	~300g/wk	199	8-9 yrs	Attention ⁷ , RVP misses, %	OR 0.87 (0.75, 1.02)	Borderline sig. difference	
	Fatty fish vs poultry	~300g/wk	199	8-9 yrs	Attention ⁷ , d2 inattention error, %	OR 1.11 (0.93, 1.33)	No sig. difference	
	Fatty fish vs poultry	~300g/wk	199	8-9 yrs	Attention ⁷ , RTI 5-choice reaction time SD, ms	MD 2 (-6, 11)	No sig. difference	
	Fatty fish vs poultry	~300g/wk	199	8-9 yrs	Impulsivity ⁷ , d2 impulsivity error, %	OR 0.65 (0.41, 1.02)	Borderline sig. difference	
	Fatty fish vs poultry	~300g/wk	199	8-9 yrs	Impulsivity ⁷ , RVP false alarm, %	OR 0.86 (0.73, 1.02)	Borderline sig. difference	
	Fatty fish vs poultry	~300g/wk	199	8-9 yrs	Impulsivity ⁷ , flankert incongruent error, %	OR 0.99 (0.89, 1.09)	No sig. difference	
	Fatty fish vs poultry	~300g/wk	199	8-9 yrs	Inhibition ⁷ , stroop effect, s	MD -2 (-6, 3)	No sig. difference	
	Fatty fish vs poultry	~300g/wk	199	8-9 yrs	Inhibition ⁷ , flankert effect, ms	MD 2 (-11, 15)	No sig. difference	
	Fatty fish vs poultry	~300g/wk	199	8-9 yrs	Cognitive flexibility ⁷ , switch cost, ms	MD -5 (-43, 32)	No sig. difference	
	Fatty fish vs poultry	~300g/wk	199	8-9 yrs	Cognitive flexibility ⁷ , mixing cost, ms	MD -51 (-94, -7)	Sig. difference	
	Fatty fish vs poultry	~300g/wk	199	8-9 yrs	Working memory ⁷ , SWM strategy score	MD 0.35 (-0.21, 0.92)	No sig. difference	
	Fatty fish vs poultry	~300g/wk	199	8-9 yrs	Working memory ⁷ , PAL memory score	MD 1.15 (0.92, 1.44)	Sig. difference	

Author, year, country	Intervention, fish intake, duration	Intake unit	N	Child age	Outcome measure	Estimates (95 %CI), dietary intervention	Overall results	Overall conclusions
	Fatty fish vs poultry	~300g/wk	199	8-9 yrs	Overall socioemotional problems ⁸	MD -0.13 (-0.26, 0.01)	No sig. difference	
	Fatty fish vs poultry	~300g/wk	199	8-9 yrs	Overall socioemotional problems, externalizing vs internalizing problems ⁹	MD 0.02 (-0.20, 0.24)	No sig. difference	
	Fatty fish vs poultry	~300g/wk	199	8-9 yrs	Overall socioemotional problems, SDQ externalizing problems ¹⁰	MD -0.24 (-0.69, 0.21)	No sig. difference	
	Fatty fish vs poultry	~300g/wk	199	8-9 yrs	Overall socioemotional problems, BRIEF impulsivity ¹¹	MD 0.13 (-0.68, 0.42)	No sig. difference	
	Fatty fish vs poultry	~300g/wk	199	8-9 yrs	Overall socioemotional problems, SDQ internalizing problems ¹⁰	MD -0.63 (-1.11, -0.16)	Sig. difference	
	Fatty fish vs poultry	~300g/wk	199	8-9 yrs	Overall socioemotional problems, BRIEF emotional control ¹¹	MD -0.04 (-0.63, 0.55)	No sig. difference	
	Fatty fish vs poultry	~300g/wk	199	8-9 yrs	Overall socioemotional problems, KINDLP emotional well-being ¹²	MD 1.04 (-1.57, 3.65)	No sig. difference	
	Fatty fish vs poultry	~300g/wk	199	8-9 yrs	Overall socioemotional problems, KINDLC emotional well-being ¹²	MD 1.55 (-1.44, 4.54)	No sig. difference	
	Fatty fish vs poultry	~300g/wk	199	8-9 yrs	Prosocial behavior, prosocial score ¹⁰	MD 0.17 (-0.12, 0.46)	No sig. difference	
	Fatty fish vs poultry	~300g/wk	199	8-9 yrs	Prosocial behavior, KINDLP friends ¹²	MD 0.43 (-2.20, 3.07)	No sig. difference	
	Fatty fish vs poultry	~300g/wk	199	8-9 yrs	Prosocial behavior, KINDLC friends ¹²	MD -0.03 (-3.61, 3.65)	No sig. difference	
	Fatty fish vs poultry	~300g/wk	199	8-9 yrs	Prosocial behavior, SDQ total difficulties ¹⁰	MD -0.89 (-1.60, -0.18)	Sig. difference	

Author, year, country	Intervention, fish intake, duration	Intake unit	N	Child age	Outcome measure	Estimates (95 %CI), dietary intervention	Overall results	Overall conclusions
	Fatty fish vs poultry	~300g/wk	199	8-9 yrs	Prosocial behavior, KINDLP total well-being ¹²	MD 0.21 (-1.62, 2.04)	No sig. difference	
	Fatty fish vs poultry	~300g/wk	199	8-9 yrs	Prosocial behavior, KINDLC total well-being ¹²	MD -0.18 (-2.14, 1.78)	No sig. difference	
	Fatty fish vs poultry	~300g/wk	199	8-9 yrs	Prosocial behavior, BRIEF global executive function ¹¹	MD -1.51 (-4.45, 1.43)	No sig. difference	
	Fatty fish vs poultry	~300g/wk	199	8-9 yrs	Prosocial behavior, BRIEF flexibility ¹¹	MD 0.20 (-0.32, 0.72)	No sig. difference	
	Fatty fish vs poultry	~300g/wk	199	8-9 yrs	Prosocial behavior, BRIEF working memory ¹¹	MD -0.29 (-0.95, 0.37)	No sig. difference	
Øyen, 2018, Norway	Fatty fish vs meat, 16 wks	3 lunch meals/wk	232	4-6 yrs	WPPSI-III ¹³ subtests, raw scores, total	MD 17.7 (14.8, 20.7) vs 17.8 (15.0, 20.6), $P=0.97$	No sig. difference	No sig. effect (borderline sig. effect in 2 out of 14 comparisons)
	Fatty fish vs meat, 16 wks	3 lunch meals/wk	232	4-6 yrs	WPPSI-III ¹³ subtests, raw scores, verbal	MD 3.8 (2.6, 5.0) vs 4.3 (3.1, 5.4), $P=0.59$	No sig. difference	
	Fatty fish vs meat, 16 wks	3 lunch meals/wk	232	4-6 yrs	WPPSI-III ¹³ subtests, raw scores, information	MD 1.0 (0.6, 1.4) vs 1.1 (0.8, 1.5), $P=0.63$	No sig. difference	
	Fatty fish vs meat, 16 wks	3 lunch meals/wk	232	4-6 yrs	WPPSI-III ¹³ subtests, raw scores, vocabulary	MD 1.1 (0.3, 1.9) vs 1.1 (0.4, 1.9), $P=0.99$	No sig. difference	
	Fatty fish vs meat, 16 wks	3 lunch meals/wk	232	4-6 yrs	WPPSI-III ¹³ subtests, raw scores, word reasoning	MD 1.8 (1.1, 2.4) vs 2.1 (1.4, 2.7), $P=0.50$	No sig. difference	
	Fatty fish vs meat, 16 wks	3 lunch meals/wk	232	4-6 yrs	WPPSI-III ¹³ subtests, raw scores, performance	MD 6.0 (4.7, 7.3) vs 5.6 (4.4, 6.8), $P=0.65$	No sig. difference	
	Fatty fish vs meat, 16 wks	3 lunch meals/wk	232	4-6 yrs	WPPSI-III ¹³ subtests, raw scores, block design	MD 1.7 (1.3, 2.1) vs 1.1 (0.7, 1.6), $P=0.07$	Borderline sig. difference	
	Fatty fish vs meat, 16 wks	3 lunch meals/wk	232	4-6 yrs	WPPSI-III ¹³ subtests, raw scores, matrix reasoning	MD 2.5 (1.8, 3.1) vs 2.2 (1.6, 3.1), $P=0.52$	No sig. difference	
	Fatty fish vs meat, 16 wks	3 lunch meals/wk	232	4-6 yrs	WPPSI-III ¹³ subtests, raw scores, picture concepts	MD 2.1 (1.1, 3.0) vs 2.0 (1.1, 2.9), $P=0.91$	No sig. difference	
	Fatty fish vs meat, 16 wks	3 lunch meals/wk	232	4-6 yrs	WPPSI-III ¹³ subtests, raw scores, processing speed	MD 8.1 (5.9, 10.3) vs 7.8 (5.7, 9.9), $P=0.83$	No sig. difference	

Author, year, country	Intervention, fish intake, duration	Intake unit	N	Child age	Outcome measure	Estimates (95 %CI), dietary intervention	Overall results	Overall conclusions
	Fatty fish vs meat, 16 wks	3 lunch meals/wk	232	4-6 yrs	WPPSI-III ¹³ subtests, raw scores, coding	MD 4.5 (2.9, 6.2) vs 5.2 (3.6, 6.8), $P=0.58$	No sig. difference	
	Fatty fish vs meat, 16 wks	3 lunch meals/wk	232	4-6 yrs	WPPSI-III ¹³ subtests, raw scores, symbol search	MD 3.6 (2.7, 4.5) vs 2.6 (1.7, 3.5), $P=0.12$	No sig. difference	
	Fatty fish vs meat, 16 wks	3 lunch meals/wk	232	4-6 yrs	Fine manual dexterity; 9-HPT ¹⁴ , dominant hand	MD -2.7 (-3.6, -1.8) vs -1.8 (-2.7, -1.0), $P=0.19$	No sig. difference	
	Fatty fish vs meat, 16 wks	3 lunch meals/wk	232	4-6 yrs	Fine manual dexterity; 9-HPT ¹⁴ , non-dominant hand	MD -4.2 (-5.3, -3.2) vs -2.7 (-3.8, 1.7), $P=0.05$	Borderline sig. difference	

¹Wechsler preschool and primary scale of intelligence, 3rd edition, Full scale IQ, verbal IQ, and performance IQ, ²Nine-hole peg test (dominant and non-dominant hand), ³d2 test of attention, ⁴Strength and Difficulties Questionnaire, ⁵PCA-generated component based on included measures in the study, ⁶PCA-generated component based on included measures in the study, ⁷a battery of cognitive tests including the d2 test of attention, the Stroop color-word test, a computer-based child-adapted Flanker task, and 4 tests from the Xambridge Neuropsychological Automated Battery, ⁸PCA-generated component based on included measures in the study, ⁹PCA-generated component based on included measures in the study, ¹⁰Strength and Difficulties Questionnaire, ¹¹Behavior Rating Inventory of Executive Function, ¹²KINDL Questionnaire of quality of life, ¹³Wechsler preschool and primary scale of intelligence, 3rd edition, Full scale IQ, verbal IQ, and performance IQ, ¹⁴Nine-hole peg test (dominant and non-dominant hand).

*~300g/wk=either dinner 2 times/wk or lunch 3 times/wk.

For the cognitive outcomes, one of the studies in preschool children reported significantly larger improvements in the fish intervention group compared to control in two subtests (picture concept and symbol search subtests raw score), but not for the 15 remaining comparisons (Demmelmair et al., 2019). The other study in preschool children reported no significant effects of the fish intervention compared to meat on any outcome (Øyen et al., 2018). Notably, both studies report the findings using raw scores, which makes the strength of findings hard to interpret. Demmelmair et al. (2019) also reported the results by three composite scores (IQ scores with a mean (SD) of 100 (15)) and in these comparisons, there were no difference between groups. The study by Teisen et al. (2020) included 21 subtests of different cognitive functions in a large battery of tests. Two of the comparisons (one test of attention and one on cognitive flexibility) were significant different between groups. In the study of adolescents 14-15 years, there were significantly larger improvements in the fish group compared to the meat group in two of six included subtests assessing attention skills (Handeland et al., 2017) (Table 4.11.3.2-1).

In sub-analysis, taking dietary compliance into account, Øyen et al. (2018) reported significant differences between groups in three of the comparisons, suggesting a beneficial association with the total raw (unstandardized) scores ((20.4 (17.5, 23.3) vs. 15.2 (12.4, 18.0), $P=0.006$ in the fish vs. meat group, respectively). These differences were further apparent in three of the subtests, namely the vocabulary, block design and symbol search subtest (P -values 0.04, 0.03 and 0.02, respectively). In Handeland et al. (2017), when adjusting for dietary compliance statistical significance was lost for one of the comparisons (for errors or omission the IRR (95% CI) became 0.88 (0.76, 1.02), $P=0.084$).

In the crude analyses for the mental health outcomes, the RCT in preschool children reported no significant effect on scores of the SDQ (Hysing et al., 2018). The RCT in school aged children reported significant difference between groups in two (SDQ internalizing problems and SDQ total difficulties) out of 17 included subtests (Teisen et al., 2020), while the RCT in adolescents reported a protective effect of fish on emotional problems and total problems in the dichotomized scores, but no such effects for the four remaining outcomes and no protective effect when scores were used on a continuous scale (Skotheim et al., 2017).

Adjusting for dietary compliance made no difference to the results in the preschool children (Hysing et al., 2018), while for the adolescents the significant finding in the crude analysis were no longer present for total difficulties (change (95% CI) -4.10 (-5.54, -2.65), $P=0.06$).

4.11.3.3 *Summary estimates based on VKM's inclusion of primary studies*

As for maternal intake of fish, no summary estimates were calculated due to heterogenous study outcomes (different assessment tools) and many studies reporting unstandardized linear regression coefficients.

4.11.3.4 VKM`s search compared to previous systematic reviews on child fish intake and neurodevelopment in children

Table 4.11.3.4-1 gives an overview of publications included in the systematic review by Hibbeln et al. (2019) that are not included by VKM on child fish consumption and neurodevelopment. Three of the publications were excluded at abstract screening since they did not fulfil the criteria for inclusion and four did not appear in VKM's search. VKM identified Demmelmair et al. (2019) which was probably too recent to be included in Hibbeln et al. (2019).

Table 4.11.3.4-1 Overview of publications included in the review by Hibbeln et al. (2019) but not by VKM.

Publications	Excluded at abstract screening	Did not appear in VKM search
Hertz-Picciotto et al, 2010		X
Liu et al., 2017		X
Ríos-Hernández, 2017	X	
San Mauro Martín et al., 2018		X
Sørensen et al., 2015	X	
Zhou et al., 2016	X	
Woo et al., 2014		X

4.11.4 Heterogeneity child fish intake and neurodevelopment

Study characteristics in publications identified by VKM on child fish intake and neurodevelopment in children vary, in particularly in terms of the included outcomes. For the findings, direction of the associations is generally consistent with few adverse associations.

4.11.5 Dose-response relationship child fish intake and neurodevelopment

The VKM project group did not identify meta-evidence on a dose-response relationship between maternal fish consumption and child neurodevelopment.

4.11.6 Weight of evidence child fish intake and neurodevelopment

In this section, we will weigh the evidence of the association between fish intake and child neurodevelopment according to the WCRF criteria presented in Chapter 3.1.6, (Box 2).

Published evidence of child fish intake and neurodevelopment

The single identified systematic review of child seafood intake and child neurodevelopment (Hibbeln et al., 2019) concludes that there are moderate and consistent evidence indicating

that consumption of seafood during childhood through adolescence has beneficial associations with child neurodevelopment. In this systematic review, the evidence does not meet the criteria for “convincing” evidence due to the insufficient number of RCTs for child and adolescent’s intake.

Among the four prospective cohort studies on the association between total child fish intake and neurodevelopment, two suggest a beneficial association in all included comparisons and one in parts of the comparisons, while the remaining reports of no significant associations. In these prospective studies, the age at outcome assessment ranged substantially (from 15 months to 18 years), and although the two studies in adolescents reported protective associations, overall conclusions are limited by the difference in outcome measures.

In addition to the four prospective cohort studies, VKM included four independent RCTs (six publications in total) on the effect of increased fatty fish intake and child cognitive abilities and mental health. Although there are some differences between groups in these RCTs, these are restricted to few of the included outcomes and with limited apparent pattern in terms of cognitive domains.

Heterogeneity

Unexplained heterogeneity is limited.

Mechanism

There are several plausible mechanisms for associations between child fish intake and neurodevelopment. Fish contains several nutrients that are important for brain structural and functional development and at the same time neurotoxins that may have adverse effects on the developing brain.

Upgrading factors

No substantial upgrading factors were identified.

4.11.6.1 *Conclusion weight of evidence child fish intake and neurodevelopment*

There is evidence from four independent RCTs involving fatty fish interventions and four independent prospective cohort studies on total fish intake (ten publications in total). The direction of the effects and associations are generally consistent towards protection from fish intake with little unexplained heterogeneity and there is evidence for biological mechanisms between child fish intake and neurodevelopment (biological plausibility). Findings are limited by multiple included outcomes measures, multiple methods of measurements of outcomes, multiple comparisons, and substantial age differences at outcome assessment in the identified studies. In conclusion, the evidence is graded “limited, suggestive” that child fish consumption (total and fatty fish) benefits neurodevelopment.

4.12 Introduction fish intake and neurocognitive and psychiatric endpoints in adults (age >18 years)

This chapter is an introduction to the weight of evidence analysis chapters for the included neurocognitive and psychiatric outcomes in adults (Chapters 4.13-4.14).

Overview of studies summarized according to neurocognitive and psychiatric endpoints in adults

VKM included primary studies of fish intake in relation to neurocognitive and psychiatric endpoints in adults graded A or B in the quality assessment. These publications included outcomes related to cognition and cognitive decline (including dementia and Alzheimer's disease), and to depression and other psychiatric symptoms. Cognition and cognitive decline are mainly presented as binary outcomes for dementia, Alzheimer's disease, and cognitive decline (no/yes), but also on a continuous scale for symptoms of cognitive decline. The psychiatric endpoints mainly include studies on depression and post-partum depression presented both as binary outcome (incidence of depression) and on a continuous scale for symptoms of depression.

In the following, the two main categories: "cognition and cognitive decline" and "depression and other psychiatric symptoms" will be presented and evaluated separately. Figure 4.12-1 shows an overview of the outcome categories.

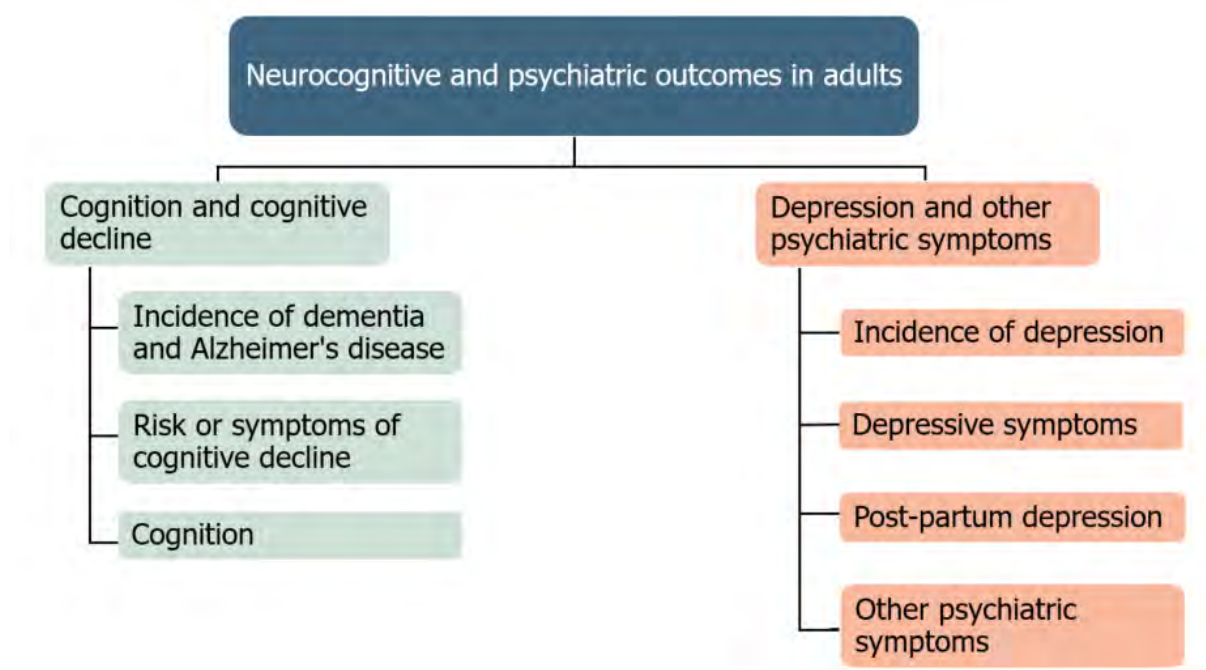


Figure 4.12-1 Overview of evaluated subcategories of the neurocognitive and psychiatric outcomes.

4.13 Fish intake and neurocognitive and psychiatric endpoints in adults

Mechanisms

Fish and other seafood consumption may prevent age-related cognitive decline. Potential mechanisms involve neuro- and cardioprotective pathways, including specific benefits of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), LC n-3 FA that are crucial for brain structure and function. Plausible mechanisms for a protective effect of LC n-3 FA are described in more detail in Chapter 5.2.

4.13.1 VKM's search for previous systematic reviews and meta-analyses of fish, cognition, and cognitive decline

4.13.1.1 *Description of the identified publications*

VKM's search for systematic reviews and meta-analyses (see Chapter 3.1.2.2) identified six publications on the association between fish intake and cognitive decline. One umbrella review, and four systematic reviews with meta-analyses were included. All included systematic reviews were given AMSTAR grade B for moderate quality by VKM, the excluded systematic review was graded C (see Table 4.13.1-1).

Table 4.13.1.1-1 Reasons for exclusion of identified papers from the search of systematic reviews and meta-analyses of fish intake and cognitive decline 2016-2021.

Included papers	Excluded paper and reasons for exclusions
Umbrella reviews Barbaresko et al., 2020	Cao et al., 2016: excluded, AMSTAR quality C
Systematic reviews Kosti et al., 2021 Bakre et al., 2018 Zeng et al., 2017 Zhang et al., 2016	

The meta-analyses are described in more detail below; first a main description of the methods used and then main/selected results from each meta-analysis are provided (see Table 4.13.1.2-1).

Umbrella review on incidence of neurodegenerative disorders

Barbaresko et al. (2020) provides an umbrella review of meta-analyses of prospective studies assessing fish intake (and other dietary exposures) related to the incidence of neurodegenerative disorders including cognitive decline, cognitive impairment, Alzheimer's disease, all-cause dementia, and Parkinson disease. When duplicate publications on the same exposure and outcome were found, this umbrella review selected the most recent

meta-analysis, the meta-analysis investigating dose-response relationships, or the meta-analysis including the highest number of prospective studies. The methodological quality of the included meta-analyses was assessed with the ROBIS tool, and the quality of the meta-evidence was assessed using the NutriGrade scoring system (type of bias estimation) (Schwingshackl et al., 2016). The study of total fish intake and risk of Alzheimer`s disease and all-cause dementia by Barbaresco et al. (2020) included the meta-analysis by Zhang et al. (2016) and Bakre et al. (2018) (see below for description of these studies). Barbaresco et al. (2020) rated the quality of the meta-evidence of fish intake and Alzheimer`s disease (based on n=6 studies) to be moderate, and to be low for all cause dementia (based on n=5 studies) based on the NutriGrade score.

Meta-analyses on cognitive decline, dementia, and Alzheimer`s disease

Kosti et al. (2021) conducted a systematic review and dose-response meta-analysis of observational and experimental studies to evaluate the association between fish intake, all-cause dementia, and Alzheimer`s disease. The authors performed a systematic literature search in PubMed, Scopus and Web of Science databases until March 2021. The quality of the eligible papers included was assessed by the Risk of Bias in Non-Randomized Studies of Exposures (ROBINS-E) for the observational studies and by the revised Cochrane Risk of Bias tool version 2.0 (RoB 2.0) for the randomized controlled trials (RCTs). Eleven studies were included to the quantitative synthesis, all prospective cohort studies on the risk of cognitive decline (both dementia and Alzheimer`s disease, n=6), the risk of dementia (n=3), and the risk of Alzheimer`s disease (n=2). No RCTs were included. Six of the studies were judged to have low risk of bias, while four were judged as having high risk of bias due to limited control of confounding and concerns regarding the exposure classification.

Bakre et al. (2018) conducted a meta-analysis of observational studies (cross-sectional, case-control and cohort studies) on the association between fish consumption and risk of dementia. In addition, they included a large cross-sectional study from China. The authors performed a systematic literature search in the MEDLINE, PubMed, CINAHL, PsychINFO, and Psychology and Behavioural Sciences Collection databases until November 2016. The quality of the eligible papers included in the meta-analysis was assessed by the Newcastle-Ottawa Scale criteria. Nine publications including 15 studies looking into fish intake and dementia were included in the meta-analysis on all-cause dementia (in total eleven publications (17 studies) were included in the paper. There were seven papers on association between fish intake and Alzheimer`s disease, and the quality of all the papers included in the meta-analysis were overall 7 high-quality articles and 3 medium-quality articles.

Zeng et al. (2017) conducted a meta-analysis of prospective cohort studies on the association between fish consumption and cognitive problems (Alzheimer`s disease, all-cause dementia, and mild cognitive impairment). The authors performed a systematic literature search in PubMed, Embase, and Web of Science until December 2014. The quality of the eligible papers included in the meta-analysis was assessed by the Newcastle-Ottawa Scale criteria. Seven eligible studies investigated the association between fish intake and all-

cause dementia (n=6), Alzheimer`s disease (n=7), or mild cognitive impairment (n=2). The results of quality assessment yielded scores of 6-7 points.

Zhang et al. (2016) conducted a meta-analysis of prospective cohort studies on the association between fish consumption and cognitive problems spanning from mild impairment to severe diseases. The authors performed a systematic literature search in MEDLINE, EMBASE, the Cochrane Library, and BIOSIS previews for studies published in the period from January 1997 to September 2014. The quality of the eligible papers included in the meta-analysis was assessed by the Newcastle-Ottawa Scale criteria. Six eligible studies investigated the association between fish intake and mild cognitive impairment (n=1), dementia (n=4), or Alzheimer`s disease (n=5). The results of the quality assessment yielded a score of 7 points or above for five of the six studies, indicating high quality.

4.13.1.2 *Results from the meta-analyses*

Below is a summary table for fish intake and cognitive decline in adults based on the identified meta-analyses (Table 4.13.1.2-1).

Table 4.13.1.2-1 Summary of results from meta-analyses on total fish intake and risk of cognitive decline (dementia and Alzheimer`s disease).

Author, year	Type of studies included	No. of studies	Outcome: no. of cases	Comparison	Summary RR (95% CI)	Heterogeneity	Overall results
Kosti, 2021	Prospective cohort studies	9	Dementia	Highest vs lowest	0.80 (0.69, 0.93)	$I^2 = 34\%$	Findings show a protective association of fish intake on dementia and Alzheimer`s. The dose response analyses show that a fish intake up to two portions a week was associated with a marginally non-statistically significant 10% reduction in all-cause dementia and a 30% reduction in the risk of Alzheimer`s disease
		7	Alzheimer`s	Highest vs lowest	0.74 (0.63, 0.87)	$I^2 = 52\%$	
Bakre, 2018	Prospective cohort and cross-sectional studies	15 (9 pub)	Dementia: 3139	Consumed fish (or consumed fish at a higher level) compared with those who did not eat fish (or who consumed fish at a lower level)	0.80 (0.74, 0.87)	$I^2 = 0.0\%$	A higher consumption of fish was associated with lower risk of dementia and Alzheimer`s disease
		7	Alzheimer`s: 1105		0.73 (0.65, 0.82)	$I^2 = 36.4\%$	
Zeng, 2017	Prospective cohort studies	6	Dementia	Highest vs lowest	0.86 (0.73, 1.02)	$I^2 = 0.0\%$	A higher consumption of fish was associated with lower risk of Alzheimer`s disease. There was also a protective association of dementia, although non-significant
		7	Alzheimer`s	Highest vs lowest	0.80 (0.65, 0.97)	$I^2 = 48.2\%$	
Zhang, 2016	Prospective cohort studies	4	Dementia: 1182	Increment of 1 serving/wk	0.95 (0.90, 0.99)	$I^2 = 63.4\%$	Higher consumption of fish was associated with lower risks of dementia and Alzheimer`s disease
		5	Alzheimer`s: 915	Increment of 1 serving/wk	0.93 (0.90, 0.95)	$I^2 = 74.8\%$	

4.13.2 VKM's systematic review of primary studies on fish intake and cognition and cognitive decline in adults

4.13.2.1 Included studies from search

A total of 24 publications, all quality-graded B, were originally included in the evaluation (Barberger-Gateau et al., 2007; Barberger-Gateau et al., 2002; Devore et al., 2009; Fischer et al., 2018; Kalmijn et al., 1997a; Kalmijn et al., 1997b; Larrieu et al., 2004; Morris et al., 2003; Ngabirano et al., 2019; Tsurumaki et al., 2019; Kesse-Guyot et al., 2011; An et al., 2016; Vercambre et al., 2010; Nooyens et al., 2016; Morris et al., 2005; Samieri et al., 2018; Qin et al., 2014; Van de Rest et al., 2009; Van Gelder et al., 2007; Kim et al., 2013; Hansen et al., 2015; Shao-Yuan Chuang et al., 2019; Keenan et al., 2020; Yeh et al., 2021). Of these, ten investigated incidence of dementia, Alzheimer's disease, or vascular dementia as outcomes, 14 had risk or symptoms of cognitive decline, and finally one had cognition as outcome.

Selected study characteristics (study name, design, time, size and age of the study population and dietary assessment method) are presented in Table 4.13.2.1-1.

Table 4.13.2.1-1 Overview of primary studies included in the weight of evidence analysis of fish intake and cognition and cognitive decline in adults.

Author, year, country	Study name	Design	Inclusion year(s), end, follow-up time	Study size	Dietary assessment method
Cognitive decline					
Incidence of dementia/Alzheimer's Disease/vascular dementia					
Barberger-Gateau, 2007, France	3-City study	Prospective cohort study	1999-2000, 4 years	8085	FFQ
Barberger-Gateau, 2002, France	PAQUID	Prospective cohort study	1991-1992, 7 years	1674	Semi-quantitative FFQ
Devore, 2009, Netherlands	The Rotterdam study	Prospective cohort study	1990-1991, 9.6 years	7983	Semi-quantitative FFQ
Fischer, 2018, Germany	German Study on Ageing, Cognition and Dementia in Primary Care Patients	Prospective cohort study	2003-2004, 10 years	3327	8-item questionnaire
Kalmijn, 1997a, Netherlands	The Rotterdam study	Prospective cohort study	1990-1991, 2.1 years	7983	Semi-quantitative FFQ
Larrieu, 2004, France	Synthesis of results in PAQUID study	Prospective cohort study	1991-1992, 7 years	1674	Semi-quantitative FFQ
Morris, 2003, US	The Chicago Health and Aging Project	Prospective cohort study	1993-1997, 3.9 years	815	FFQ
Ngabirano, 2019, France	3-City Study	Prospective cohort study	1999-2001, 9.8 years	9249	FFQ

Author, year, country	Study name	Design	Inclusion year(s), end, follow-up time	Study size	Dietary assessment method
Shao-Yuan Chuang, 2019, Taiwan	Nutrition and Health Survey in Taiwan (NAHSIT)	Prospective cohort study	1999-2000, 12 years	1436	FFQ
Tsurumaki, 2019, Japan	Ohsaki cohort 2006 study	Prospective cohort study	2006-2007, 5.7 years	13102	FFQ
Risk or symptoms of cognitive decline					
An, 2016, China	Chinese Longitudinal Healthy Longevity Survey (CLHLS)	Prospective cohort study	1998, 14 years	4749	Interview
Fischer, 2018, Germany	German Study on Ageing, Cognition and Dementia in Primary Care Patients	Prospective cohort study	2003-2004, 10 years	3327	8-item questionnaire
Kalmijn, 1997b, Netherlands	The Zutphen Elderly Study	Prospective cohort study	1985, 8 years	342	Cross-check dietary history method
Keenan, 2020, US	Age-Related Eye Disease Study 1 and 2	Cross-sectional study and prospective cohort study	1992-1998 and 2006-2008, 10 years	3074 and 3326	FFQ
Kesse-Guyot, 2011, France	SUVIMAX 2 study	Prospective cohort study	2006, 7.5 years	3294	24-hour recall
Kim, 2013, US	the Women's Health Study	Prospective cohort study	1992-1995, 6 years	5988	FFQ
Morris, 2005, US	Chicago Health and Aging Project (CHAP)	Prospective cohort study	1993-1997, 6 years	6158	FFQ
Nooyens, 2018 Netherlands	The Doetinchem Cohort Study (DCS)	Prospective cohort study	1995-2002, 5 years	2612	Semi-quantitative FFQ
Qin, 2014, China	China Health and Nutrition Survey (CHNS)	Prospective cohort study	1997, 5.3 years	2408	24-hour recall
Samieri, 2018, US	Synthesis of results from 5 previous studies: Three-City study (3C), Nurses' Health Study (NHS), Women's Health Study (WHS), Chicago Health and Aging Project (CHAP), and Rush Memory and Aging project (MAP)	Prospective cohort studies	1992-1999, 3.9-9.1 years	23688	FFQ
Vercambre, 2010, France	The E3N study	Prospective cohort study	1990, 16 years	4809	Semi-quantitative FFQ
Van De Rest, 2009, US	Veterans Affairs Normative Aging Study	Prospective cohort study	1963-1970, 6 years	2280	Semi-quantitative FFQ
Van Gelder, 2007, Netherlands	The Zutphen Elderly Study	Prospective cohort study	1985, 5 years	939	Cross-check dietary history method

Author, year, country	Study name	Design	Inclusion year(s), end, follow-up time	Study size	Dietary assessment method
Yeh, 2021, US	Nurses` Health Study and Health Professionals Follow-up Study	Prospective cohort studies	1976, 28 years and 1986, 16 years	49493 and 27842	Semi-quantitative FFQ
Cognition					
Hansen, 2015, US		RCT	NA	80	NA

4.13.2.2 *Overlapping and excluded publications*

Two studies (Larrieu et al., 2004; Samieri et al., 2018) reported synthesis of results from previous studies (the PAQUID study and Nurses' Health Study, Womens's Health Study, Chicago Health and Aging Project, and Rush Memory and Aging project) and were excluded from the evaluation. Two studies reported results from the same cohort (3-City study) (Barberger-Gateau et al., 2007, Ngabirano et al., 2019) and according to the protocol, the earliest study (Barberger-Gateau et al., 2007) was excluded from the study.

Moreover, the RCT by Hansen et al. (2015) was excluded. This was a trial on the effect of Atlantic salmon on cognition in a very selected population (80 US forensic inpatients with anti-social features and a history of alcohol and drug abuse) not representative for the general population. The RCT was also the only study on general cognition in young adults in this evaluation, supporting the exclusion of the study.

In the publications by Keenan et al. (2020), only results from the Age-Related Eye Disease Study 2 (AREDS 2) were included, the results from the AREDS 1 were excluded according to the study protocol being a cross-sectional study.

4.13.2.3 *Studies by design and geographic region*

All included studies in the evaluation were prospective cohort studies. Most of the studies originated from European countries such as France (Barberger-Gateau et al., 2007; Barberger-Gateau et al., 2002; Fischer et al., 2018; Ngabirano et al., 2019; Kesse-Guyot et al., 2011; Vercambre et al., 2010) and the Netherlands (Devore et al., 2009; Kalmijn et al., 1997a; Kalmijn et al., 1997b; Nooyens et al., 2016; Van Gelder et al., 2007). There were also studies from US (Morris et al., 2003; Morris et al., 2005; Van de Rest et al., 2009; Kim et al., 2013; Hansen et al., 2015; Keenan et al., 2020; Yeh et al., 2021) and from Asian countries such as Japan (Tsurumaki et al., 2019), China (An et al., 2016; Qin et al., 2014) and Taiwan (Shao-Yuan Chuang et al., 2019)

4.13.2.4 *Studies by sex, potential effect modification and other sub-groups*

Two studies reported findings from large female US cohorts (Kim et al., 2013; Vercambre et al., 2009), and three studies included men only (Kalmijn et al., 1997b; Van Gelder et al., 2007; Van de Rest et al., 2009). Some studies also reported the potential modifying effect of age (Barberger-Gateau et al., 2002; Vercrambe et al., 2009; Qin et al., 2014), sex and APOE-ε4 status (Ngabirano et al., 2019; Barberger-Gateau et al., 2007), APOE haplotypes (defined by rs429358 and rs7412) (Keenan et al., 2020), educational level (Vercambre et al., 2009), cognitive function (Kim et al., 2013), sleep (Tsurumaki et al., 2019) and vitamin E and aspirin supplements (Kim et al., 2013).

4.13.2.5 *Studies by fish exposure*

All studies included total fish as exposure (sum of all fish, unspecified fish, fish including seafood or shellfish), and one study included also fatty fish (Nooyens et al., 2016). One study included fatty fish only (Van de Rest et al., 2009), and one divided the total fish into “tuna and other dark-meat fish” and “light-meat fish and shellfish” (Kim et al., 2013).

4.13.3 Results from the included primary studies on fish intake and cognitive decline in adults

4.13.3.1 *Studies of fish intake and the incidence of dementia/Alzheimer’s disease/vascular dementia and symptoms of cognitive decline in adults*

We included 21 studies for the weight of evidence analysis (Barberger-Gateau et al., 2007; Barberger-Gateau et al., 2002; Devore et al., 2009; Fischer et al., 2018; Kalmijn et al., 1997a; Kalmijn et al., 1997b; Morris et al., 2003; Ngabirano et al., 2019; Tsurumaki et al., 2019; Kesse-Guyot et al., 2011; An et al., 2016; Vercambre et al., 2010; Nooyens et al., 2016; Morris et al., 2005; Qin et al., 2014; Van de Rest et al., 2009; Van Gelder et al., 2007; Kim et al., 2013; Shao-Yuan Chuang et al., 2019; Keenan et al., 2020; Yeh et al., 2021). These studies included clinical diagnoses as outcomes, i.e., incidence of dementia, Alzheimer’s disease and vascular dementia and a range of symptom measures of cognitive decline (e.g., memory tests and assessments as Mini-mental state examination). Results from studies using symptom measures as outcomes were both reported as binary outcomes (yes/no) based on cut off scores, and as continuous scores (more or less symptoms).

Eight studies reported incidence of dementia, Alzheimer’s disease, and vascular dementia. These include five hazard ratio (HR) estimates (Barberger-Gateau et al., 2002; Devore et al., 2009; Ngabirano et al., 2019; Tsurumaki et al., 2019; Shao-Yuan Chuang et al., 2019) and one odds ratio (OR) estimate (Kalmijn et al., 1997a) for incidence of dementia; four HR estimates (Barberger-Gateau et al., 2002; Devore et al., 2009; Fischer et al., 2018, Ngabirano et al., 2019) and two OR estimates (Morris et al., 2003; Kalmijn et al., 1997a) for incidence of Alzheimer’s disease, and one OR estimate (Kalmijn et al., 1997a) for vascular dementia (Table 4.13.3.1-1).

Seven studies reported ORs and HRs for poor scores on symptom measures of cognitive decline (Kim et al., 2013; Kesse-Guyot et al., 2011; Kalmijn et al., 1997b; Vercambre et al., 2010; An et al., 2016; Keenan et al., 2020; Yeh et al., 2021).

Six studies reported unstandardized linear regression coefficients (e.g., mean change, mean difference) using the measures on a continuous scale (Fischer et al., 2018; Nooyens et al., 2018; Morris et al., 2005; Qin et al., 2014; Van Gelder et al., 2007; Keenan et al., 2020) (Table 4.13.3.1-2).

One study (Van de Rest et al., 2009) reported associations between fatty fish only and symptoms of cognitive decline.

Of the six studies reporting findings using the symptom measures on a continuous scale (Table 4.13.3.1-2), three reported no significant associations between fish intake and memory decline (Fischer et al., 2018; Nooyens et al., 2016; Van Gelder et al., 2007), while Keenan et al. (2020) and Morris et al. (2005) reported significantly fewer symptoms of cognitive decline with increased fish intake. Qin et al. (2014) found significant reduced annual cognitive decline in adults 65 years and above, but not in adults less than 65 years. These studies used different assessment strategies (direct assessments with standardized tests, telephone interview and self-report).

The exposure levels and results with estimates for studies using incidence of dementia, Alzheimer`s disease and vascular dementia as outcome, and studies using symptoms measures of cognitive decline (both binary and continuous) are included in Tables 4.13.3.1-1 and 4.13.3.1-2, respectively.

Table 4.13.3.1-1 Results from prospective cohort studies included in the weight of evidence analysis of total fish intake and cognitive decline in men and women using incidence of dementia overall, Alzheimer's disease and vascular dementia as outcome.

Author, year, country	Intake unit	High-low intake	Total cases, population	Outcome measure	HR/RR high-low (95% CI)	Overall results	Overall conclusion
Barberger-Gateau, 2002, France	Times, 4 cat	Once a day vs never	170 M/W	Dementia ¹	HR 0.73 (0.52, 1.03)	Borderline sig. assoc.	Borderline protective assoc. for dementia overall and Alzheimer's disease
	Times, 4 cat	Once a day vs never	135 M/W	Alzheimer's disease	HR 0.69 (0.47, 1.01)	Borderline sig. assoc.	
Devore, 2009, Netherlands	g/d, 3 cat	29.6 vs 0 g/d	157 M/W	Dementia ²	HR 0.95 (0.76, 1.19)	No sig. assoc., <i>P</i> -trend=0.7	No sig. assoc.
	g/d, 3 cat	29.6 vs 0 g/d	117 M/W	Alzheimer's disease	HR 0.99 (0.76, 1.29)	No sig. assoc., <i>P</i> -trend=0.9	
Fischer, 2018, Germany	Times/wk, 5 cat	Every day vs never	418 M/W	Alzheimer's disease ³	HR 0.98 (0.87, 1.11)	No sig. assoc	No sig. assoc.
Kalmijn, 1997a, Netherlands	g/d, 3 cat	>18.5 vs <3 g/d	58 M/W	Dementia ⁴	OR 0.4 (0.2, 0.9)	Sig. assoc., <i>P</i> -trend=0.03	Sig. protective assoc. for dementia overall and Alzheimer's disease. No assoc. with vascular dementia
	g/d, 3 cat	>18.5 vs <3 g/d	37 M/W	Alzheimer's disease	OR 0.3 (0.1, 0.9)	Sig. assoc., <i>P</i> -trend=0.005	
	g/d, 3 cat	>18.5 vs <3 g/d	12 M/W	Dementia w/vascular component	OR 0.7 (0.2, 2.8)	No sig. assoc., <i>P</i> -trend=0.39	
Morris, 2003, US	Times/wk, 4 cat	2 times/wk vs never	131 M/W	Alzheimer's disease ⁵	OR 0.4 (0.2, 0.9)	Sig. protective assoc., <i>P</i> -trend=0.07	Sig. protective assoc. for Alzheimer's disease
Ngabirano, 2019, France	Times/wk, 4 cat	≥4 vs ≤1 time/wk	662 M/W	Dementia ⁶	HR 1.1 (0.7, 1.7)	No sig. assoc	No sig. assoc.
	Times/wk, 4 cat	≥4 vs ≤1 time/wk	466 M/W	Alzheimer's disease ⁶	HR 1.1 (0.7, 1.8)	No sig. assoc	
Shao-Yuan Chuang, 2019, Taiwan	Times/wk, 3 cat	≥4 vs <1 time/wk	114 M/W	Dementia ⁷	HR 0.70 (0.50, 0.99)	Sig. assoc., <i>P</i> -trend=0.03	Sig. lower risk for dementia with higher fish consumption

Author, year, country	Intake unit	High-low intake	Total cases, population	Outcome measure	HR/RR high-low (95 % CI)	Overall results	Overall conclusion
Tsurumaki, 2019, Japan	g/d, 4 cat	96.9 vs 20.4 g/d	1118 M/W	Dementia ⁸	HR 0.84 (0.71, 0.997)	Sig. assoc., <i>P</i> -trend=0.03	Sig. protective assoc. for dementia

¹Participants who had lost 3 points on the Mini-Mental State Examination (MMSE), or with suspected dementia and diagnosis confirmed by a neurologist; ²A 3-step protocol for diagnosing dementia including screening (MMSE), Camdex and evaluation by neurologist/neuropsychologist; ³Assessed with the SIDAM and diagnosed by consensus of the interviewer and a geriatrician/geriatric psychiatrist; ⁴A 3-stage procedure; initial screening (MMSE and GMS-A), an informant interview (CAMDEX) and clinical examination; ⁵Diagnosed by structured neurological clinical evaluations including medical history, neurologic examination, medication use, neuropsychological testing, informant interviews and laboratory testing; ⁶Three-step protocol: neuropsychological assessment, evaluation by a neurologist and committee of neurological experts for consensus of diagnosis; ⁷Identified based on physician diagnosis in the National Health Insurance Database; ⁸Incident dementia was defined as disabling dementia according to the criteria of the LTCI system used in Japan.

Table 4.13.3.1-2 Results from prospective cohort studies included in the weight of evidence analysis of total fish intake and symptom measures of cognitive decline in men and women (binary and continuous) as outcome.

Author, year, country	Intake unit	High-low intake	Total cases/N	Outcome measure, binary or continuous	Estimates high-low (95 % CI)	Overall results	Overall conclusion
An, 2016, China	Times, 3 cat	Almost every day vs rarely/never	4749 M/W	Cognitive impairment; MMSE ⁵	HR 0.91 (0.78, 1.06)	No sig. assoc.	No sig. assoc.
Fischer, 2018, Germany	Times/wk, 5 cat	Every day vs never	2622 M/W	Memory; CERAD ¹¹	β -0.03 (-0.14, 0.08)	No sig. assoc.	No sig. assoc.
Kalmijn, 1997b, Netherlands	g/d, 3 cat	>20 vs 0 g/d	153 M	Cognitive impairment; MMSE ⁶	OR 0.63 (0.33, 1.21)	No sig. assoc., <i>P</i> -trend=0.13	No sig. assoc.
	g/d, 3 cat	>20 vs 0 g/d	51 M	Cognitive decline; MMSE change score	OR 0.45 (0.17, 1.16)	No sig. assoc., <i>P</i> -trend=0.09	
Keenan, 2020, US	Servings/wk, 4 cat	Cat 4 vs 1	3326 M/W	Cognitive impairment ⁹ ; TICS-M<30	OR 0.5 (0.39, 0.65)	Sig. assoc., <i>P</i> -trend<0.001	Sig. reduced cognitive impairment

Author, year, country	Intake unit	High-low intake	Total cases/N	Outcome measure, binary or continuous	Estimates high-low (95 %CI)	Overall results	Overall conclusion
	Servings/wk, 4 cat	Cat 4 vs 1	3326 M/W	Cognitive impairment ⁹ ; TICS-M, continuous	β 1.03 (0.70, 1.36)	Sig. assoc., P -trend<0.001	
	Servings/wk, 4 cat	Cat 4 vs 1	3326 M/W	Cognitive impairment ⁹ ; composite score, lowest decile	OR 0.5 (0.38, 0.78)	Sig. assoc., P -trend=0.004	
	Servings/wk, 4 cat	Cat 4 vs 1	3326 M/W	Cognitive impairment ⁹ ; composite score, overall	β 1.50 (0.90, 2.10)	Sig. assoc., P -trend<0.001	
Kesse-Guyot, 2011, France	Daily intake, 5 cat	Q5 vs Q1	3294 M/W	Cognitive function; MMSE ²	OR 0.86 (0.60, 1.21) for poor scores	No sig. assoc., P -trend=0.29	No sig. assoc.
	Daily intake, 5 cat	Q5 vs Q1	3294 M/W	Cognitive function; verbal memory ³	OR 1.09 (0.78, 1.51) for poor scores	No sig. assoc., P -trend=0.41	
	Daily intake, 5 cat	Q5 vs Q1	3294 M/W	Cognitive function; CDS ⁴	OR 0.80 (0.63, 1.01) for poor scores	Borderline sig. assoc., P -trend=0.10	
Kim, 2013, US	Servings/wk, 4 cat	>2/wk vs <1/wk	5988 W	Cognitive decline; global cognition ¹	OR 0.80 (0.60, 1.06) for worse scores	No sig. assoc., P -trend=0.14	No sig. assoc.
Morris, 2005, US	Times/ wk, 3 cat	>2 vs 0 times/wk	3718 M/W	Cognitive decline ¹³	Change in cognitive score β 0.013	Sig. assoc., P =0.04	Sig. reduced cognitive decline with increased fish intake
Nooyens, 2018, Netherlands	Times, 4 cat	≥ 2 vs <1 time/mo	2604 M/W	Global cognitive function ¹²	Change score (β) - 0.02	No sig. assoc., P -linear trend=0.72	No sig. assoc.
				Memory	Change score (β) - 0.04	No sig. assoc., P -linear trend=0.61	
				Information processing speed	Change score (β) 0.01	No sig. assoc., P -linear trend=0.83	
				Cognitive flexibility	Change scores (β) 0.00	No sig. assoc., P -linear trend=0.79	
Qin, 2014, China	Serving/ wk, 2 cat	>1 vs ≤ 1 serving/wk	1566 M/W, all	Cognitive decline ¹⁴ , global cognitive score	β 0.11 (-0.03, 0.24), P =0.07	Borderline sig. assoc	Sig. reduced annual cognitive decline in adults

Author, year, country	Intake unit	High-low intake	Total cases/N	Outcome measure, binary or continuous	Estimates high-low (95 %CI)	Overall results	Overall conclusion
	Serving/ wk, 2 cat	>1 vs ≤1 serving/wk	M/W, <65 yrs	Cognitive decline ¹⁴ , global cognitive score	β -0.06 (-0.22, 0.11), $P=0.52$	No sig. assoc.	≥65 yrs but not in adults <65 at enrollment
	Serving/ wk, 2 cat	>1 vs ≤1 serving/wk	M/W, ≥65 yrs	Cognitive decline ¹⁴ , global cognitive score	β 0.34 (0.11, 0.56), $P=0.004$	Sig. assoc.	
	Serving/ wk, 2 cat	>1 vs ≤1 serving/wk	1566 M/W, all	Cognitive decline ¹⁴ , composite score	β 0.018 (-0.001, 0.038), $P=0.06$	Borderline sig. assoc.	
	Serving/ wk, 2 cat	>1 vs ≤1 serving/wk	M/W, <65 yrs	Cognitive decline ¹⁴ , composite score	β -0.005 (-0.0029, 0.019), $P=0.67$	No sig. assoc.	
	Serving/ wk, 2 cat	>1 vs ≤1 serving/wk	M/W, ≥65 yrs	Cognitive decline ¹⁴ , composite score	β 0.053 (0.020, 0.087), $P=0.002$	Sig. assoc.	
	Serving/ wk, 2 cat	>1 vs ≤1 serving/wk	1566 M/W, all	Cognitive decline ¹⁴ , verbal memory score	β 0.017 (-0.006, 0.040), $P=0.15$	No sig. assoc.	
	Serving/ wk, 2 cat	>1 vs ≤1 serving/wk	M/W, <65 yrs	Cognitive decline ¹⁴ , verbal memory score	β -0.006 (-0.035, 0.024), $P=0.71$	No sig. assoc.	
	Serving/ wk, 2 cat	>1 vs ≤1 serving/wk	M/W, ≥65 yrs	Cognitive decline ¹⁴ , verbal memory score	β 0.015 (0.013, 0.088), $P=0.008$	Sig. assoc.	
	Serving/ wk, 2 cat	>1 vs ≤1 serving/wk	1566 M/W, all	Cognitive decline ¹⁴ , immediate 10-word recall	β 0.020 (-0.003, 0.043), $P=0.10$	No sig. assoc.	
	Serving/ wk, 2 cat	>1 vs ≤1 serving/wk	M/W, <65 yrs	Cognitive decline ¹⁴ , immediate 10-word recall	β 0.001 (-0.029, 0.030), $P=0.91$	No sig. assoc.	
	Serving/ wk, 2 cat	>1 vs ≤1 serving/wk	M/W, ≥65 yrs	Cognitive decline ¹⁴ , immediate 10-word recall	β 0.046 (0.007, 0.085), $P=0.020$	Sig. assoc.	
	Serving/ wk, 2 cat	>1 vs ≤1 serving/wk	1566 M/W, all	Cognitive decline ¹⁴ , delayed 10-word recall	β 0.013 (-0.010, 0.036), $P=0.28$	No sig. assoc.	
	Serving/ wk, 2 cat	>1 vs ≤1 serving/wk	M/W, <65 yrs	Cognitive decline ¹⁴ , delayed 10-word recall	β -0.011 (-0.041, 0.020), $P=0.49$	No sig. assoc.	
	Serving/ wk, 2 cat	>1 vs ≤1 serving/wk	M/W, ≥65 yrs	Cognitive decline ¹⁴ , delayed 10-word recall	β 0.050 (0.013, 0.087), $P=0.008$	Sig. assoc.	
Van Gelder, 2007, Netherlands	g/d, 3 cat	>20 g/d vs none	210 M/W	Cognitive function, MMSE ¹⁵	Mean score 26.5 (26.0, 27.0) vs 26.4 (25.8, 26.9)	No sig. difference, P -trend=0.81	No sig. assoc.

Author, year, country	Intake unit	High-low intake	Total cases/N	Outcome measure, binary or continuous	Estimates high-low (95 %CI)	Overall results	Overall conclusion
	g/d, 3 cat	>20 g/d vs none	210 M/W	Cognitive decline, 5 years, MMSE, change score	Mean score -1.2 (-1.9, -0.6) vs -0.3 (0.9, 0.2)	No sig. difference, <i>P</i> -trend=0.07	
Vercambre, 2010, France	g/d, 3 cat	Cat 3 vs 1	598 W	Cognitive decline; DECO score ⁷	OR 0.80 (0.64, 0.99) for poor scores	Sig. assoc., <i>P</i> -trend=0.043	Sig. reduced cognitive decline, but not functional impairment
	g/d, 3 cat	Cat 3 vs 1	716 W	Functional impairment; 4 IADL score ⁸	OR 0.99 (0.81, 1.21) for poor scores	No sig. assoc., <i>P</i> -trend=0.939	
Yeh, 2021, US	Servings/d, 5 cat	Cat 5 vs 1 (median 0.81 vs 0.20 g/d)	W, Nurses' Health Study	Subjective cognitive decline ¹⁰	OR 0.83 (0.75, 0.90)	Sig. assoc., <i>P</i> -trend<0.0001	Sig. reduced cognitive decline
	Servings/d, 5 cat	Cat 5 vs 1 (median 0.81 vs 0.20 g/d)	M, Health Professionals Follow-up Study	Subjective cognitive decline ¹⁰	OR 0.89 (0.81, 0.94)	Sig. assoc., <i>P</i> -trend=0.0001	

¹Telephone Interview for Cognitive Status (TICS): A composite score of an adaptation of the Mini-Mental State Examination; immediate and delayed recalls of the East Boston Memory Test (EBMT) paragraph; a delayed recall of the TICS 10-word list; and category fluency, ²Mini mental state examination (French version) scores <27, ³Five-word test, immediate and delayed verbal memory, scores <18, ⁴McNair's Cognitive Difficulties Scale, scores >39, ⁵Mini mental state examination, scores <17, ⁶Mini mental state examination, scores >25 and change scores > 2 over 3 years, ⁷DEtéroration Cognitive Observée (range 0-38) scores <33, ⁸Instrumental Activities of Daily Living, ⁹Telephone Interview for Cognitive Status-modified (TICS-M) and a composite score of the sum of z-scores for each test within the battery, ¹⁰Subjective cognitive decline (SCD), mailed or online questionnaire with 6 (HPFS) or 7 (NHS) yes (1)/no (0) questions, decline defined as 3-unit increment in the score, ¹¹Three subtests from the CERAD neuropsychological assessment battery; ten-item Word List Immediate Recall subtest, Word List Delayed Recall subtest and Word List Recognition subtest, summed to a total memory score (range 0-100), ¹²Global function is a composite score of four tests: 15 Words Verbal Learning Test (memory), the Stroop Colour- Word Test, the Word Fluency test (information processing speed), and the Letter Digit Substitution Test (cognitive flexibility), scored were standardized, higher scores indicating better cognition, change score was baseline scores subtracted from follow up score, ¹³Sum of four standardized tests: the East Boston Tests of Immediate and Delayed Recall, the Mini-Mental State Examination, and the Symbol Digit Modalities Test, ¹⁴Telephone interview for Cognitive Status-modified, Global cognitive score is the sum of all tests and the primary outcome, Composite score was calculated by averaging z-scores for all tests, Verbal memory scores was calculated by averaging z-scores of the memory scores, ¹⁵Mini mental state examination, continuous. Studies on fatty and lean fish intake and cognitive decline

Three studies reported findings from fatty fish intake and cognitive decline/symptoms of dementia (Nooyens et al., 2016; Van de Rest et al., 2009; Kim et al., 2013). While Van de Rest et al. (2009) and Nooyens et al. (2016) reported no significant associations between the fatty fish intake and various tests of memory function, Kim et al. (2013) reported a significant reduced odds for cognitive decline with higher intake. The former study also reported findings on lean fish with no significant associations (Kim et al., 2013), see Table 4.13.3.1-3.

Table 4.13.3.1-3 Results from prospective observational (cohort or other prospective observational design) studies included in the weight of evidence analysis of fatty fish intake, dark and light-meat fish intake and symptoms measures of cognitive decline (binary and continuous) as outcome.

Author, year, country	Fish type	Intake unit	High-low intake	N	Outcome measure	Estimates high-low (95 % CI)	Overall results	Overall conclusion, domain
Kim, 2013, US	Dark meat fish	Servings/wk, 4 cat	≥1/wk vs <1/wk	5988 W	Cognitive decline, global cognition ⁴	OR 0.79 (0.61, 1.01) for worse score	Borderline sig. assoc. (high-low), sig. trend (<i>P</i> -trend=0.02)	Suggestive protective assoc.
		Servings/wk, 4 cat	≥1/wk vs <1/wk	5988 W	Cognitive decline, verbal memory	OR 0.84 (0.65, 1.08) for worse score	No sig. assoc., <i>P</i> -trend=0.08	
	Light meat fish	Servings/wk, 4 cat	≥1/wk vs <1/wk	5988 W	Cognitive decline, global cognition ⁴	OR 0.81 (0.52, 1.25) for worse score	No sig. assoc., <i>P</i> -trend=0.15	No sig. assoc.
		Servings/wk, 4 cat	≥1/wk vs <1/wk	5988 W	Cognitive decline, verbal memory	OR 1.03 (0.68, 1.56) for worse score	No sig. assoc., <i>P</i> -trend=0.89	
Nooyens, 2018, Netherlands	Fatty fish	Times, 4 cat	≥1 time/wk vs <1 time/mo	2604 M/W	Global cognitive function ¹	β -0.01	No sig. assoc., <i>P</i> -linear trend=0.63	No sig. assoc.
	Fatty fish	Times, 4 cat	≥1 time /wk vs <1 time/mo	2604 M/W	Memory	β -0.06	No sig assoc., <i>P</i> -linear trend=0.33	
	Fatty fish	Times, 4 cat	≥1 time /wk vs <1 time/mo	2604 M/W	Information processing speed	β -0.01	No sig. assoc., <i>P</i> -linear trend=0.69	
	Fatty fish	Times, 4 cat	≥1 time /wk vs <1 time/mo	2604 M/W	Cognitive flexibility	β -0.04	No sig. assoc., <i>P</i> -linear trend=0.75	

Author, year, country	Fish type	Intake unit	High-low intake	N	Outcome measure	Estimates high-low (95 % CI)	Overall results	Overall conclusion, domain
Van de Rest, 2009, US	Fatty fish	Servings/wk, quartiles	Q4 vs Q1 (median 2.79 vs 0.21 servings/wk, energy adjusted)	M 1025	Memory ² , word list memory test	Mean scores 8.7 (18.4, 19.1) vs 19.0 (18.7, 19.4)	No sig. assoc., <i>P</i> -trend=0.08	No sig. assoc.
		Servings/wk, quartiles	Q4 vs Q1 (median 2.79 vs 0.21 servings/wk, energy adjusted)	M 1025	Visuospatial ³ , spatial copying, sum of drawings	Mean score 5.8 (5.7, 6.0) vs 5.8 (5.7, 6.0)	No sig. assoc., <i>P</i> -trend=0.70	
		Servings/wk, quartiles	Q4 vs Q1 (median 2.79 vs 0.21 servings/wk, energy adjusted)	M 1025	Pattern comparison test, response time (sec)	Mean time 5.6 (5.5, 5.8) vs 5.7 (5.5, 5.8)	No sig. assoc., <i>P</i> -trend=0.18	

¹Global function is a composite score of four tests: 15 Words Verbal Learning Test (memory), the Stroop Colour- Word Test, the Word Fluency test (information processing speed), and the Letter Digit Substitution Test (cognitive flexibility), scored were standardized, higher scores indicating better cognition, change score was baseline scores subtracted from follow up score; ²Word list memory test (adapted from CERAD), range 0-30, ³Spatial copying task constructional praxis (CERAD), range 0-9 and Pattern comparison (NES2), ⁴Telephone Interview for Cognitive Status (TICS): A composite score of an adaptation of the Mini-Mental State Examination; immediate and delayed recalls of the East Boston Memory Test (EBMT) paragraph; a delayed recall of the TICS 10-word list; and category fluency.

4.13.3.2 *Summary relative risks (RR) based on VKM's inclusion of primary studies*

Below, VKM presents summary RRs by three endpoints: incidence of dementia, incidence of Alzheimer's disease, and risk of cognitive decline (yes/no) based on cut-off scores on symptom measures. Studies reporting HRs or ORs were summarized separately since the properties of the OR can overestimate and give a more extreme estimate than the HR, although the direction will be the same.

VKM calculated a summary relative risk (RR) for developing dementia in relation to the highest versus lowest intake of total fish, based on five prospective studies reporting HRs (Barberger-Gateau et al., 2002; Devore et al., 2009; Ngabirano et al., 2019; Tsurumaki et al., 2019; Shao-Yuan Chuang et al., 2019) (Table 4.13.3.1-1). The summary RR suggested a protective association for the highest intake that was statistically significant (RR=0.85, 95% CI: 0.75, 0.96) without significant heterogeneity ($P_{\text{heterogeneity}}=0.37$). One additional primary study on dementia reported an odds ratio (Kalmijn et al., 1997a). This study had a prospective design and found a statistically significant protective association (OR: 0.4, 95% CI 0.2, 0.9), supporting the summary RR.

VKM's summary of the RR for developing Alzheimer's disease, the most common cause of dementia, in relation to the highest versus lowest intake of total fish included four prospective studies reporting HRs (Barberger-Gateau et al., 2002; Devore et al., 2009; Fischer et al., 2018; Ngabirano et al., 2019) (Table 4.13.3.1-1). The summary RR was closer to unity than the summary RR for dementia overall, and not statistically significant (RR=0.95, 95% CI: 0.84, 1.08). Heterogeneity was not statistically significant ($P_{\text{heterogeneity}}=0.34$). Two primary studies reporting ORs based on prospective designs (Kalmijn et al., 1997a; Morris et al., 2003) reported protective associations that were statistically significant: OR=0.3 (95% CI 0.1, 0.9) (Kalmijn et al., 1997a) and OR=0.4 (95% CI 0.2, 0.9) (Morris et al., 2003).

VKM also summarized seven studies with eight estimates reporting ORs for the risk of cognitive decline as a binary outcome (yes/no) based on symptom measures in relation to the highest versus lowest intake of total fish (Kim et al., 2013; Kesse-Guyot et al., 2011; Kalmijn et al., 1997b; Vercambre et al., 2010; An et al., 2016; Keenan et al., 2020; Yeh et al., 2021). Outcomes were based on similar cut-off values for a low score on the Mini-Mental State Examination (MMSE), or a composite score including the MMSE, the Détérioration Cognitive Observée (DECO) score or a questionnaire of subjective cognitive decline (SCD). The summary RR suggested a protective association for the highest intake that was statistically significant with significant heterogeneity (RR=0.81, 95% CI: 0.73, 0.89, ($P_{\text{heterogeneity}}=0.004$). Heterogeneity was due to differences in the magnitude of the protective associations, not direction. In influence analysis, excluding Keenan (2020) with the lowest OR, heterogeneity was non-significant ($P_{\text{heterogeneity}}=0.65$). In a sensitivity analysis, the three studies that used either the DECO score (Vercambre et al., 2010), a composite score (Kim et al., 2013) or the SCD (Yeh et al., 2021) were excluded. The summary RR for the four

remaining studies that used the MMSE was slightly weaker than for all seven studies (RR=0.72, 95% CI: 0.51, 1.01). Heterogeneity was significant ($P_{\text{heterogeneity}}=0.001$) and explained by the study by Keenan et al. (2020).

4.13.3.3 VKM's search compared to previous meta-analyses on cognition and cognitive decline in adults

There are some differences between studies included by VKM and previous meta-analyses. While the meta-analyses focus on studies with incidence of dementia and Alzheimer's disease as outcomes, VKM also included 13 studies reporting findings based on symptom measures of cognitive decline (i.e., the risk of cognitive decline as binary outcomes based on cut off scores and the association between fish intake and symptoms of cognitive decline as continuous outcomes).

Discrepancies between primary studies on incidence of dementia and/or Alzheimer's disease included by VKM, and the identified meta-analyses are described in Table 4.13.3.3-1.

Table 4.13.3.3-1 Primary studies on incidence of dementia and/or Alzheimer's disease included by VKM compared with identified meta-analyses.

Author, year	VKM	Zhang, 2016	Zeng, 2017	Bakre, 2018	Kosti, 2021	Reason for exclusion by VKM
Albanese, 2009				X		Cross-sectional
Barberger-Gateau, 2007			X	X	X	Excluded due to duplicate study
Barberger-Gateau, 2002	X	X		X		
Chan, 2013			X			Cross-sectional
Conquer, 2000				X		Cross-sectional
Dever, 2009	X	X	X		X	
Fischer, 2018	X			X		
Huang, 2005		X	X	X	X	Cross-sectional
Kalmijn, 1997	X	X	X		X	
Kim, 2010				X		Cross-sectional
Larrieu, 2004				X	X	Reported synthesis of previous results
Lopez, 2011				X		Cross-sectional
Morris, 2003	X	X	X	X	X	
Ngabirano, 2019	X				X	
Nozaki, 2021					X	Graded C
Olsson, 2015			X			Cross-sectional
Roberts, 2010			X			Excluded at initial screening due to not relevant exposure
Schaefer, 2006			X	X		Cross-sectional
Shao-Yuan Chuang, 2019	X				X	
Tsurumaki, 2019	X				X	
Tully, 2003						Cross-sectional

Author, year	VKM	Zhang, 2016	Zeng, 2017	Bakre, 2018	Kosti, 2021	Reason for exclusion by VKM
Zhang, 2018					X	Not relevant outcome (included for mortality)

Three studies identified by VKM (Fischer et al., 2018; Ngabirano et al., 2019; Tsurumaki et al., 2019) were published after the meta-analyses by Zhang et al. (2016), Zeng et al. (2017) and Bakre et al. (2018). Two of these studies (Ngabirano et al., 2019; Tsurumaki et al., 2019) were included in the most previous meta-analysis by Kosti et al. (2021) however.

The differences in included studies between VKM and the meta-analyses seems to be, with some exceptions, mostly due to study design. The meta-analyses have included cross-sectional studies which were excluded from the current VKM evaluation in accordance with the protocol. One study on dietary patterns (Olsson et al., 2015) was included in the meta-analysis by Zeng et al. (2017), but not by the other meta-analyses or by VKM. One study (Roberts et al., 2010) included in Zeng et al. (2017) was excluded as it was not involving fish exposure and two studies (Nozaki et al., 2021; Zhang et al., 2018) included in Kosti et al. (2021) were excluded by VKM due to poor quality or because the health outcome was not relevant.

4.13.4 Heterogeneity fish intake and cognitive decline in adults

While the VKM`s summary RRs for incidence of dementia and Alzheimer`s disease estimated based on the included primary studies did not show significant heterogeneity, the summary RR for cognitive decline did. The heterogeneity for cognitive decline was no longer significant however, after removing a study from the analysis that had a very low OR.

The included meta-analyses reported non-significant heterogeneity.

4.13.5 Dose-response relationships in fish intake and cognitive decline in adults

The meta-analysis by Zhang et al. (2016) demonstrated a dose-response relationship of $RR=0.95$ (95%CI 0.90, 0.99), $I^2=63.4\%$ with every increment of one serving/week for dementia and a similar relationship for Alzheimer`s disease ($RR: 0.93$ (95%CI 0.90, 0.95), $I^2=74.8$). Dose-response analyses for curvilinear associations between fish servings/week and the RRs for both endpoints were shown.

In the meta-analysis by Zeng et al. (2017), the pooled effect estimates demonstrated that increased fish intake of 100 g/week was associated with a 12% reduced risk of Alzheimer`s disease.

In the meta-analysis by Bakre et al. (2018), the pooled data showed a dose-response relationship with a reduced RR of 0.84 (95 % CI 0.72, 0.98) for dementia in participants with a low level of fish consumption, RR of 0.78 (95 % CI 0.68, 0.90) with a middle level of fish

consumption and RR of 0.77 (95 % CI 0.61, 0.98) with a high level of fish consumption compared to those who did not eat fish (or who consume fish at lower levels).

The most recent meta-analyses by Kosti et al. (2021) examined the dose-response relationship between fish intake (grams/weekly) and the risk of dementia and the risk of Alzheimer`s disease. According to their findings, although higher fish intake is associated with lower dementia risk, the slope of risk reduction gradually reduces with intakes higher than approximately 250 g/week. Compared to no fish intake, an intake of 125 grams fish/week was associated with a marginally non-significant 6% lower risk of dementia (RR of 0.94 (95% CI 0.84, 1.05), a further increase to 250 grams/week was associated with a non-significant 12% reduction in the RR (RR=0.88 (95% CI 0.73, 1.07)) and an increase to 375 grams/week with a non-significant 16 % lower risk of dementia (RR=0.84 (95%CI 0.68, 1.03)). The gradual levelling off of the risk reduction as fish intake increases is more evident in the case of Alzheimer`s disease. Compared with no fish consumption, the risk of Alzheimer`s disease decreases by 24% at intakes of 125 grams/week (RR = 0.76, (95% CI 0.63, 0.93)) and by 31% at intakes of 250 g/week (RR=0.69 (95% CI: 0.54, 0.88)). Any increase beyond this intake does not seem to be related to additional benefit. In sensitivity analyses, the associations between fish intake and dementia and Alzheimer`s disease were attenuated after excluding studies with short follow-up.

4.13.6 Weight of evidence fish intake cognitive decline in adults

In this section, the evidence of the association between fish intake and incidence of dementia, Alzheimer`s disease and cognitive decline is weighed according to the WCRF criteria presented in Chapter 3.1.6, (Box 2).

Published evidence of maternal fish intake and cognitive decline in adults

The results in the four previous meta-analyses (Zhang et al., 2016; Zeng et al., 2017; Bakre et al., 2018; Kosti et al., 2021) suggested that a higher intake of fish is associated with lower risk for dementia and Alzheimer`s disease.

VKM`s summary RRs for developing dementia suggests a protective association of the fish intake. The summary RR for developing Alzheimer`s disease also suggests a protective association, although the estimate did not reach statistical significance. The summary RR for the risk of cognitive decline as a binary outcome supports the above results with a significant protective association. In the six studies reporting findings using continuous outcomes on the symptom measures of cognitive decline, three reported no significant associations between fish intake and cognitive decline and three reported significantly fewer symptoms of cognitive decline with increased fish intake.

Evidence was too scarce to conclude on the potential associations between fatty and lean fish intake and cognitive decline.

Based on VKM`s summary and previous meta-analyses there is evidence that total fish intake reduces the risk of dementia, Alzheimer`s disease and cognitive decline.

Heterogeneity

No substantial unexplained heterogeneity was observed in the VKM summary or in the previous meta-analyses.

Mechanism

There is evidence for several plausible mechanisms operating in humans.

Upgrading factors

The meta-analyses reported dose-response relationships between fish intake and dementia and Alzheimer's disease. In the most previous meta-analyses, however the dose-response relationship was only significant for Alzheimer's disease and not for dementia.

4.13.6.1 *Conclusion weight of evidence fish intake and cognitive decline in adults*

There is evidence from more than two independent and good quality prospective cohort studies (referring to the WCRF criteria for evaluation of evidence). Of the total identified 20 studies on total fish intake, three studies out of seven investigating the risk for incidence of dementia or Alzheimer's disease reported a reduced risk for dementia (n=1) or Alzheimer's disease (n=2) with increased fish intake. Five studies of the total of 12 investigating the risk for or association with cognitive decline in general, reported protective associations between fish intake and cognitive decline. The VKM summary RR for the risk of dementia, Alzheimer's disease and cognitive decline (binary outcomes) also suggest a protective association with fish intake, which is supported by the identified meta-analyses. The directions of the associations are generally consistent (towards protective) and there is no substantial unexplained heterogeneity across studies. There is evidence for biological plausibility, and dose-response relationships have been demonstrated, although only for Alzheimer's disease in the most previous meta-analyses. In conclusion, the evidence that consumption of total fish reduces the risk of dementia, Alzheimer's disease and cognitive decline is graded "probable".

There were fewer studies of fatty fish and lean fish than total fish and the evidence is graded "limited, no conclusion" for the effects of fatty fish and lean fish on risk of dementia, Alzheimer's disease and cognitive decline.

4.14 Fish intake and depression and other psychiatric symptoms in adults

Mechanisms

The exact biological mechanisms whereby high-fish intake reduce risk of depression are not well established (Li, 2016). But it has been proposed that LC n-3 FAs may have a beneficial effect by modifying serotonergic and dopaminergic neurotransmission (see Chapter 5.2 for a more detailed description of n-3 FA mechanisms). In addition, high-quality protein, vitamins, and minerals may have a protective effect on depression. Finally, high-fish consumption may also be related to a healthier diet and better nutritional status, which could contribute to the lower risk of depression.

4.14.1 VKM's search for published systematic reviews and meta-analyses on fish intake and depression and other psychiatric symptoms in adults

4.14.1.1 *Description of the identified publications*

VKM's search for systematic reviews and meta-analyses (see Chapter 3.1.2.2) identified 12 publications on the association between fish intake and depression and other psychiatric outcomes. Two umbrella reviews, and four systematic reviews with meta-analyses were included. All included systematic reviews were given AMSTAR grade B by VKM for moderate study quality. See Table 4.14.1.1-1 for reasons for exclusion of the remaining six.

Table 4.14.1.1-1 Reasons for exclusion of identified papers from the search of systematic reviews and meta-analyses of fish intake and depression 2016-2021.

Included papers	Excluded paper and reasons for exclusions
Umbrella reviews: Xu et al., 2021 Jayedi and Shab-Bidar, 2020	Kromhout et al., 2016: excluded, not correct outcome Molendijk et al., 2018: excluded, AMSTAR quality C Khan et al., 2020: excluded, not looking specifically on fish intake Ljungberg et al., 2020: excluded, not looking specifically on fish intake
Systematic reviews: Matison et al., 2021 Yang et al., 2018 Grosso et al., 2016 Li et al., 2016	Opie et al., 2020: excluded, not looking specifically on fish intake Zhao and Zhang, 2020: umbrella review looking at postpartum depression, no meta-analysis or systematic review looking on fish intake were identified.

The meta-analyses are described in more detail below; first a main description of the methods used and then main/selected results from each meta-analysis are provided (see Table 4.14.2-1).

Umbrella review of fish intake and depression

Xu et al. (2021) is an umbrella review of meta-analyses of prospective studies on dietary factors and the prevention and treatment of depression. The authors performed a systematic literature search in PubMed, Embase and Cochrane Library up to June 2021 to identify relevant meta-analyses. Twenty-eight meta-analyses with 40 summary estimates on various dietary factors were identified. For total fish intake and risk of depression, Xu et al. (2021) included the meta-analysis by Yang et al. (2018) (see below for description of the study). The NutriGrade score was used to evaluate the quality of the meta-evidence.

Jayedi and Shab-Bidar (2020) is an umbrella review of meta-analyses of prospective studies investigating fish intake and different outcomes (CVD, Type 2 diabetes (T2D), site-specific cancers, neurological disorders, all cause and cause-specific mortality, and any other diseases of aging). This review selected only the meta-analyses with the largest number of primary prospective cohort studies, one for each outcome. The quality of the meta-evidence was assessed using the NutriGrade scoring system (type of bias estimation) (Schwingshackl et al., 2016). For total fish intake and risk of depression, Jayedi and Shab-Bidar (2020) included the meta-analysis by Yang et al. (2018) (see below for description of the study). The quality of the meta-evidence of fish intake and depression (based on n=10 studies) was rated moderate based on the NutriGrade score.

Meta-analyses of fish intake and depression

Matson et al. (2021) conducted a systematic review and meta-analysis to examine the longitudinal evidence between diet and incidence of depression in adults 45 years and older. The authors performed a systematic literature search in the Medline Complete, Embase and PSYCHINFO electronic databases from January 2008 to December 2020. The quality of the included studies was assessed using a modified Newcastle-Ottawa scale for assessing the quality of non-randomized studies in meta-analysis. A total of 33 studies were included in the systematic review and meta-analysis, of these three studies looked at fish intake and depression.

Yang et al. (2018) conducted a meta-analysis of prospective cohort studies on the association between fish consumption and depression risk. The authors performed a systematic literature search in the Web of Science and PubMed databases until April 2018. The quality of the eligible papers included in the meta-analysis was assessed by the Newcastle-Ottawa Scale criteria (Wells et al., 2018). Ten studies looking into fish intake and depression were included. The results of quality assessment yielded a score of 8 points or above for all studies, indicating high quality.

Grosso et al. (2016) conducted a meta-analysis of observational studies (cross-sectional and cohort studies) on the association between fish consumption and depression risk. The authors performed a systematic literature search in MEDLINE, EMBASE, PsycInfo and the Cochrane Database of Systematic Reviews until August 2014. The quality of the eligible papers included in the meta-analysis was assessed by the Newcastle-Ottawa Scale criteria (Stang et al., 2010). Twenty-one studies looking into fish intake and depression were

included. The quality of all the papers included in the meta-analysis were 8 high-quality articles and 13 medium-quality articles.

Li et al. (2016) conducted a meta-analysis of observational studies (cross-sectional, case-control and cohort studies) on the association between fish consumption and depression risk. The authors performed a systematic literature search in the Web of Science, Embase and PubMed databases until March 2015. The quality of the eligible papers included in the meta-analysis was assessed by the Newcastle-Ottawa Scale criteria (Stang et al., 2010). Sixteen studies looking into fish intake and depression were included. The quality of all the papers included in the meta-analysis were 14 high-quality articles and two medium-quality articles.

4.14.2 Results from the meta-analyses

Below is a summary table for fish intake and depression based on the five identified meta-analyses.

Table 4.14.2-1 Summary of results from meta-analyses on total fish intake and risk of depression.

Author, year	Type of studies included	Total no studies	No of cases	Comparison	Summary RR (95 %CI)	Heterogeneity	Overall results
Matison, 2021	Prospective cohort studies evaluating the association between diet and depression	3			1.00 (0.80, 1.26)	$I^2 = 0.0\%$	Overall, there was no association between fish intake and incident depression
Yang, 2018	Prospective cohort studies evaluating the association between fish intake and depression	10 (8 pub)	6672	Highest vs lowest	0.89 (0.80, 0.99)	$I^2 = 0.0\%$	Higher fish intake reduces the risk of depression, especially in women
Grosso, 2016	Prospective cohorts and cross-sectional studies evaluating the association between fish intake and depression	21		Highest vs lowest	0.78 (0.69, 0.89)	$I^2 = 61\%$	Higher fish intake decreases the risk of depression
		10 cohorts		Highest vs lowest	0.83 (0.70, 0.97)	$I^2 = 42\%$	
Li, 2016	Prospective cohorts and cross-sectional studies evaluating the association between fish intake and depression	26 (16 pub)		Highest vs lowest	0.83 (0.74, 0.93)	$I^2 = 64.5\%$	Higher fish consumption reduces the risk of depression
		10 cohorts		Highest vs lowest	0.84 (0.75, 0.94)	$I^2 = 23.6\%$	

4.14.3 VKM's systematic review of primary studies on fish and depression and other psychiatric symptoms in adults

4.14.3.1 *Included studies from search on depression and other psychiatric symptoms in adults*

A total of 17 publications, all graded B, were evaluated (Astorg et al., 2008; Choda et al., 2020; Colangelo et al., 2009; Elstgeest et al., 2019; Kyrozis et al., 2009; Li et al., 2011; Lucas et al., 2011; Matsuko et al., 2017; Smith et al., 2014; Tsai et al., 2012; Hakkarainen et al., 2004; Strøm et al., 2009; Hamazaki et al., 2019; Hamazaki et al., 2018; Hansen et al., 2014; Sanches-Villegas et al., 2007; Hedelin et al., 2011). Of these, eleven publications concerned depression, three post-partum depression and three other diagnoses. In studies of depression, the methods of measurements varied between physician-diagnosed depression, medication use or hospital/out-patient admission as a marker of depression, and self-reported depressive symptoms.

Four studies were excluded because the evidence base was evaluated to be too scarce to make a conclusion. These studies were on other psychiatric diagnoses such as anxiety (Hansen et al., 2015), a composite score of different mental disorders (Sanchez-Villegas et al., 2007), general mental health (Choda et al., 2020) and psychotic-like symptoms (Hedelin et al., 210). Thus, 13 studies were left for further analysis on depression, of which three were on postpartum depression.

Selected study characteristics (study name, design, time-period, size and age of the study population and dietary assessment method) of the included studies are presented in Table 4.14.3.1-1.

Table 4.14.3.1-1 Overview of primary studies evaluated in weight of evidence analysis of fish intake and depression and other psychiatric symptoms in adults.

Author, year, country	Study name	Design	Inclusion year(s), end, follow-up time	Study size	Dietary assessment method
Depression					
Astorg, 2008, France	The SU.VI.MAX study	Prospective observational	1994-1995, 8 years	1864	24-hour recall
Colangelo, 2009, US	CARDIA study	Prospective observational	1985-1986, 20 years	5115	FFQ
Elstgeest, 2019, Italy	InCHIANTI study	Prospective observational	1998-2000, 3,6 and 9 years	1453	FFQ
Hakkarainen, 2004, Finland	ATBC study	Prospective observational	1985, 5-8 years (median 6 years)	84181	FFQ
Kyrozis, 2009, Greece	ILIDA: EPIC-Greece cohort	Prospective observational	1994-1999, 6.4-12.6 years (median 8 years)	1225	Semi-quantitative FFQ

Author, year, country	Study name	Design	Inclusion year(s), end, follow-up time	Study size	Dietary assessment method
Li, 2011, US	NHEF	Prospective observational	1971-1975, 11 years	5068	FFQ
Lucas, 2011, US	Nurse Health Study	Prospective observational	1976, 10 years	121700	FFQ
Matsuko, 2017, Japan	Japan Public Health Center – Based Study	Prospective observational	1990, 34 years	1299	FFQ
Smith, 2014, Australia	CDAH study	Prospective observational	1985, 26 years	1386	FFQ
Tsai, 2012, Taiwan	Survey of Health and Living Status of the Elderly in Taiwan	Prospective observational	1999, 4 years	1609	Semi-quantitative FFQ
Postpartum depression					
Hamazaki, 2019, Japan	The Japan Environment and Children's Study (JECS).	Prospective observational	2011-2014, 6- and 12-months post-partum	84181	FFQ
Hamazaki, 2018, Japan	The Japan Environment and Children's Study (JECS).	Prospective observational	2011-2014 first, second/third trimester, 1-month post-partum	104102	FFQ
Strøm, 2009, Denmark	DNBC cohort	Prospective observational	1996-2002, 12- and 30-months gestation and 6- and 12-months post-partum	86435	FFQ
Other psychiatric symptoms					
Choda, 2020, Japan	Japan Multi-Institutional Collaborative Cohort Study	Prospective observational	2008-2010, 5 years	4701	FFQ
Hansen, 2014, US		RCT	NA	95	NA
Hedelin, 2010, Sweden	Scandinavian Women's Lifestyle and Health Cohort,	Prospective observational	1991-1992, 11 years	34310	FFQ
Sanchez-Villegas, 2007, Spain	SUN cohort	Prospective observational	1999-2007, 2 years	10096	FFQ

4.14.3.2 *Overlapping publications*

No publications were found to be overlapping.

4.14.3.3 *Studies by design and geographic region*

All the 13 included studies on depression or postpartum depression had prospective cohort designs. Four of the studies were from European countries, four from USA, one from Australia and the remaining from Asian countries (Japan (three) and Taiwan (one)) (Table 4.14.2.1-1).

4.14.3.4 *Studies by sex, potential effect modification and other sub-groups*

Of ten studies on depression (not counting three studies of postpartum depression), eight studies reported findings from both men and women (Smith et al., 2014; Matsuko et al., 2017; Astorg et al., 2008; Colangelo et al., 2009; Li et al., 2011; Tsai et al., 2012; Elstgeest et al., 2019; Kyrozi et al., 2009). Of these, four presented the results for the total group, and separately for men and women (Smith et al., 2014; Astorg et al., 2008; Colangelo et al., 2009; Li et al., 2011). In addition to the three studies in post-partum depression (Strøm et al., 2009; Hamazaki et al., 2018; Hamazaki et al., 2019), one study reported results from women only (Lucas et al., 2011), and one study reported findings in men only (Hakkarainen et al., 2004).

4.14.3.5 *Studies by fish exposure*

While all the prospective cohort studies reported findings from total fish consumption, one also reported findings for fatty fish (Astorg et al., 2008).

4.14.3.6 *Studies with converted risk estimates*

Two studies (Li et al., 2011; Strøm et al., 2009) presented estimates for low (< once a week or 0-3 g/day) compared to high (> once a week or >30 g/day) intake. Estimates were converted to high versus low for comparability with other studies. Both the original and converted estimates are included in the result presentation.

4.14.4 Results from the included primary studies on depression in adults

4.14.4.1 *Studies of fish intake and depression*

Of the 13 publications included in the weight of evidence analysis, three studies reported HRs for depressive disorders; by Cox regression (Lucas et al., 2011; Hakkarainen et al., 2004) and by Poisson regression (Smith et al., 2014). Five studies reported the OR for depressive disorder (Matsuko et al., 2017; Astorg et al., 2008; Colangelo et al., 2009; Li et al., 2011; Tsai et al., 2012), while the remaining two studies reported depressive symptoms on a continuous scale using unstandardized linear regression coefficients (Elstgeest et al., 2019; Kyrozi et al., 2009).

In addition, three studies reported ORs for post-partum depression (Strøm et al., 2009; Hamazaki et al., 2018; Hamazaki et al., 2019).

Results from the studies can be found in Tables 4.14.4.1-1 and 4.14.4.1-2 for total fish and fatty fish and depression and Table 4.14.4.1-3 for post-partum depression.

Table 4.14.4.1-1 Results from prospective observational studies included in the weight of evidence analysis of total fish intake and depression.

Author, year, country	Intake unit	High-low intake	Cases	Outcome measure	Estimates high low or continuous (95%CI)	Overall results	Overall conclusion, domain
Astorg, 2008, France	g/d, tertiles	NA	307 M/W	≥1 depressive episode ⁶	OR 0.71 (0.52, 0.97)	Sig. assoc., <i>P</i> -trend=0.029	Sig. protective assoc. with recurrent depressive episodes, but not single depressive episodes
	g/d, tertiles	Mean 87.9 vs 14.9	78 M	≥1 depressive episode	OR 0.68 (0.38, 1.21)	No sig. assoc., <i>P</i> -trend=0.18	
	g/d, tertiles	Mean 71.6 vs 10.7	232 W	≥1 depressive episode	OR 0.70 (0.48, 1.02)	No sig. assoc., <i>P</i> -trend=0.056	
	g/d, tertiles	NA	177 M/W	Single depressive episode	OR 0.77 (0.52, 1.15)	No sig. assoc., <i>P</i> -trend=0.20	
	g/d, tertiles	88.6 vs 15.5	47 M	Single depressive episode	OR 1.11 (0.53, 2.36)	No sig. assoc., <i>P</i> -trend=0.78	
	g/d, tertiles	71.3 vs 10.7	130 W	Single depressive episode	OR 0.62 (0.38, 1.01)	Borderline sig. assoc., <i>P</i> -trend=0.052	
	g/d, tertiles		127 M/W	Recurrent depressive episodes	OR 0.64 (0.41, 1.01)	Borderline sig. assoc., <i>P</i> -trend=0.05	
	g/d, tertiles	87.9 vs 14.9	31 M	Recurrent depressive episodes	OR 0.39 (0.16, 0.97)	Sig. assoc., <i>P</i> -trend=0.025	
	g/d, tertiles	72.0 vs 11.2	96 W	Recurrent depressive episodes	OR 0.77 (0.45, 1.32)	No sig. assoc., <i>P</i> -trend=0.34	
Colangelo, 2009, US	g/mo, quintiles	Q5 vs Q1	744 M/W	Depressive symptom ⁷ , above cut off	OR 0.97 (0.73, 1.28) for having depressive symptoms in year 10	No sig. assoc., <i>P</i> -trend=0.59	Significant protective assoc. in women, but not in men
	g/mo, quintiles	Q5 vs Q1	3317 M/W	Chronic depressive symptoms ⁸	OR 0.80 (0.64, 1.01) for number of visits with depressive symptoms	Borderline sig. assoc., <i>P</i> -trend=0.07	
	g/mo, quintiles	Q5 vs Q1	1481 M	Chronic depressive symptoms	OR 0.89 (0.62, 1.28) for number of visits with depressive symptoms	No sig. assoc., <i>P</i> -trend=0.96	
	g/mo, quintiles	Q5 vs Q1	1836 W	Chronic depressive symptoms	OR 0.75 (0.55, 1.01) for number of visits with depressive symptoms	Borderline sig. assoc., <i>P</i> -trend=0.02	
	g/mo, quintiles	Q5 vs Q1	3317 M/W	Depressive symptom, continuous, year 20	β -0.70, <i>P</i> =0.10	No sig. assoc.	

Author, year, country	Intake unit	High-low intake	Cases	Outcome measure	Estimates high low or continuous (95 % CI)	Overall results	Overall conclusion, domain
	g/mo, quintiles	Q5 vs Q1	1481 M	Depressive symptom, continuous, year 20	β 0.21, $P=0.70$	No sig. assoc.	
	g/mo, quintiles	Q5 vs Q1	1836 W	Depressive symptom, continuous, year 20	β -1.59, $P=0.01$	Sig. assoc.	
Elstgeest, 2019, Italy	g/d, 4 cat	Cat 4 vs 1	1058 M/W	Depressive symptoms, CES-D ¹²	β -0.97 (-1.74, -0.21)	Sig. assoc., P -trend=0.013	Sig. protective assoc. with fish intake and depressive symptoms
Hakkarainen, 2004, Finland	Intake; 3 cat	3 vs 1	8612 M	Depressed mood ³	HR 1.06 (1.00, 1.12)	No sig. assoc.	No sig. assoc.
	Intake; 3 cat	3 vs 1	246 M	Major depression ⁴	HR 0.97 (0.70, 1.33)	No sig. assoc.	
Kyrozis, 2009, Greece	g/d, continuous		610 M/W	Geriatric depressive scale score ¹³	β -0.08 (-0.03, 0.15)	No sig. assoc.	No sig. assoc.
Li, 2011, US	Intake, 3 cat	<1/wk vs >1/wk	2039 M	Severely depressed mood ¹⁰	OR 0.48 (0.25, 0.94) reported as 2.08 (1.08, 4.09) for low vs high	Sig. assoc., P -trend=0.03	Sig. protective assoc. with depression in men, but not in women
	Intake, 3 cat	<1/wk vs >1/wk	3029 W	Severely depressed mood	OR 0.87 (0.63, 1.20) reported as 1.15 (0.83, 1.59) for low vs high	No sig. assoc., P -trend=0.40	
Lucas, 2011, US	Time/wk, 5 cat	≥ 5 /wk vs <1/wk	2823 W	Clinical depression ¹	HR 1.07 (0.74, 1.55)	No sig. assoc.	No sig. assoc.
Matsuko, 2017, Japan	g/d, 4 cat	152.6 vs 57.2	95 M/W	Major depressive disorder ⁵	OR 0.73 (0.41, 1.28)	No sig. assoc., P -trend=0.15	No sig. assoc.
Smith, 2014, Australia	Times/wk, continuous		70 M	Episodes of depression ²	RR (Poisson) 1.02 (0.95, 1.10), $P=0.54$	No sig. assoc.	Sig. reduced risk for depression in intake 2 times/wk in women, but not in men. No assoc. with intake on a continuous variable.
			160 W	Episodes of depression	RR (Poisson) 0.94 (0.87, 1.01), $P=0.07$	No sig. assoc.	
	Times/wk, 2 cat	2/wk vs <2/wk	70 M	Episodes of depression	RR (Poisson) 1.17 (0.74, 1.86), $P=0.49$	No sig. assoc.	
			160 W	Episodes of depression	RR (Poisson) 0.75 (0.57, 0.99), $P=0.04$	Sig. assoc.	

Author, year, country	Intake unit	High-low intake	Cases	Outcome measure	Estimates high low or continuous (95 % CI)	Overall results	Overall conclusion, domain
Tsai, 2012, Taiwan	Times/wk, 2 cat	≥3 times/wk vs <3 times/wk	1609 M/W	Risk of depression ¹¹	OR 0.91 (0.62, 1.14)	No sig. assoc., <i>P</i> -trend=0.62	No sig. assoc.

¹Physician-diagnosed depression and regular antidepressant medication use, ²Participants who had experienced an episode of major depression or dysthymic disorder as defined by the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV), ³Self-reported feelings of depressed mood the last 4 months, ⁴Data on hospital treatment due to depressive disorder derived from the National Hospital Discharge Register, ⁵Psychiatric assessment according to DSM-IV criteria for major depressive disorder (MDD): CES-D score ≥16 or PHQ-9 score ≥10 and diagnosed with current MDD by a trained psychiatrist, ⁶One antidepressant or lithium prescription as a marker of a depressive episode, ⁷The 20-item Center for Epidemiologic Studies Depression Scale (CES-D), cut off > 16, ⁸Total number of exams (1-3) having depressive symptom above cut off, ⁹The 20-item Center for Epidemiologic Studies Depression Scale (CES-D), year 10 and 20, ¹⁰Epidemiologic Studies Depression Scale (CES-D) questionnaire, scores of 22 and more considered severely depressed or taking anti-depression medication, ¹¹The ten-item CES-D, cut off scores 10 or higher, ¹²The 20-item CES-D, cut off scores of 20 and higher, ¹³Geriatric depressive scale score.

Table 4.14.4.1-2 Results from prospective observational study included in the weight of evidence analysis of fatty fish intake and depression.

Author, year, country	High-low intake	Cases	Outcome measure	Estimates high low (95 % CI)	Overall results	Overall conclusion, domain
Astorg, 2008, France	Intake vs no intake	307 M/W	>1 Depressive episode ¹	OR 0.70 (0.53, 0.92)	Sig. assoc., <i>P</i> -trend=0.012	Sig. protective assoc. for recurrent depressive episodes in women, but not in men
	Intake vs no intake	78 M	>1 Depressive episode	OR 0.50 (0.30, 0.83)	Sig. assoc., <i>P</i> -trend=0.007	
	Intake vs no intake	232 W	>1 Depressive episode	OR 0.80 (0.58, 1.12)	No sig. assoc., <i>P</i> -trend=0.20	
	Intake vs no intake	177 M/W	Single depressive episode	OR 0.73 (0.52, 1.04)	Borderline sig. assoc., <i>P</i> -trend=0.082	

Author, year, country	High-low intake	Cases	Outcome measure	Estimates high low (95 % CI)	Overall results	Overall conclusion, domain
	Intake vs no intake	47 M	Single depressive episode	OR 0.58 (0.31, 1.09)	Borderline sig. assoc., <i>P</i> -trend=0.090	
	Intake vs no intake	130 W	Single depressive episode	OR 0.81 (0.53, 1.23)	No sig. assoc., <i>P</i> -trend=0.32	
	Intake vs no intake	127 M/W	Recurrent depressive episode	OR 0.65 (0.44, 0.97)	Sig. assoc., <i>P</i> -trend=0.036	
	Intake vs no intake	31 M	Recurrent depressive episode	OR 0.41 (0.19, 0.88)	Sig. assoc., <i>P</i> -trend=0.022	
	Intake vs no intake	96 W	Recurrent depressive episode	OR 0.77 (0.48, 1.24)	No sig. assoc., <i>P</i> -trend=0.29	

¹One antidepressant or lithium prescription as a marker of a depressive episode.

Table 4.14.4.1-3 Results from prospective cohort studies included in the weight of evidence analysis of total fish intake and post-partum depression.

Author, year, country, timing	Intake unit	High-low intake	Cases	Outcome measure	Estimates high-low (95% CI)	Overall results	Overall conclusion, domain
Hamazaki, 2018, Japan, per-conception period	g/d, 5 cat	Q5 vs Q1 (F: 67.5 vs 4.7 g; M: 90.3 vs 4.7 g)	2527 M/W	Maternal psychological distress ³ , early pregnancy	OR 0.99 (0.94, 1.26)	No sig. assoc., <i>P</i> -trend=0.55	Sig. assoc. between fish intake and maternal psychological distress in late pregnancy, but not in early pregnancy, for the father and not for post-partum depression
	g/d, 5 cat	Q5 vs Q1 (F: 67.5 vs 4.7 g; M: 90.3 vs 4.7 g)	2475 M/W	Maternal psychological distress ³ , late pregnancy	OR 0.87 (0.76, 0.99)	Sig. assoc., <i>P</i> -trend=0.01	
	g/d, 5 cat	Q5 vs Q1 (F: 67.5 vs 4.7 g; M: 90.3 vs 4.7 g)	10732 W	Maternal post-partum depression ⁴	OR 0.94 (0.88, 1.01)	No sig. assoc., <i>P</i> -trend=0.23	

Author, year, country, timing	Intake unit	High-low intake	Cases	Outcome measure	Estimates high-low (95 % CI)	Overall results	Overall conclusion, domain
	g/d, 5 cat	Q5 vs Q1 (F: 67.5 vs 4.7 g; M: 90.3 vs 4.7 g)	776 M	Paternal psychological distress ¹	OR 1.00 (0.80, 1.25)	No sig. assoc., <i>P</i> -trend=0.82	
Hamazaki, 2019, Japan, mid/late pregnancy	g/d, 5 cat	Q5 vs Q1 (69.3 vs 5.2 g)	9761 W	Post-partum depression ⁴ , 6 months after delivery	OR 0.84 (0.78, 0.90)	Sig. assoc., <i>P</i> -trend <0.0001	Significant protective association with postpartum depression 6 and 12 months after delivery
	g/d, 5 cat	Q5 vs Q1 (69.3 vs 5.2 g)	2127 W	Post-partum psychological distress ³ , 12 months after delivery	OR 0.73 (0.64, 0.84)	Sig. assoc., <i>P</i> -trend <0.0001	
Strøm, 2009, Denmark, mid-pregnancy	g/d, 5 cat	>30 g/d (ref) vs 0-3 g/d	159 W	Post-partum depression, admission ¹	OR 1.22 (0.62, 2.41) reported as 0.82 (0.42, 1.64) for low vs high	No sig. assoc., <i>P</i> -trend=0.50	Sig. protective assoc. for prescription, no assoc. for admission
	g/d, 5 cat	>30 g/d (ref) vs 0-3 g/d	866 W	Post-partum depression, prescription ²	OR 0.68 (0.53, 0.89) reported as 1.46 (1.12, 1.90) for low vs high	Sig. assoc., <i>P</i> -trend= 0.04	

¹A case of post-partum depression admission was defined as a person admitted to the hospital or an outpatient contact with a diagnosis of a depressive episode (ICD-10) up to 1 year post-partum identified through the Danish Psychiatric Central register, ²A case of post-partum prescription was defined as a person who filled a prescription for an antidepressant identified through the Register of Medicinal Product Statistics, ³Kessler Psychological Distress Scale (K6) ≥ 13 ; ⁴Edinburgh Postnatal Depression Scale score ≥ 9 .

4.14.4.2 *Studies of fish intake and continuous outcomes*

Two studies reported findings of the association between fish intake and depressive symptoms on a continuous scale by unstandardized β s (Elstgeest et al., 2019; Kyrozis et al., 2009). While Elstgeest et al. (2019) demonstrated a significant decrease in depressive symptoms with higher fish intake, Kyrozis et al. (2009) reported no significant associations in a geriatric population.

4.14.4.3 *Summary relative risks (RR) based on VKM's inclusion of primary studies*

Below, VKM presents summary RRs for developing depression, and post-partum depression (summarized separately). HRs were equated with relative risk (RR) estimates from Poisson regression.

VKM calculated a summary RR for three studies reporting HRs or RR for clinical or major depression (Hakkarainen et al., 2004; Lucas et al., 2011; Smith et al., 2014) (Table 4.14.3.1-1). The summary RR was on the protective side, but not statistically significant for the highest versus lowest fish intake (RR=0.92, 95% CI: 0.78, 1.09). Heterogeneity was non-significant ($P_{\text{heterogeneity}}=0.53$). Smith et al. (2014) found a statistically significant protective association in women, but not in men.

The summary RR for developing depression in relation to the highest versus lowest intake of total fish, based on five prospective studies reporting ORs (Matsuko et al., 2017; Astorg et al., 2008; Colangelo et al., 2009; Li et al., 2011; Tsai et al., 2012) (Table 4.14.3.1-1) suggested a protective association for the highest intake that was statistically significant (RR=0.85, 95% CI: 0.74, 0.98) without significant heterogeneity ($P_{\text{heterogeneity}}=0.50$).

VKM additionally calculated a summary RR for all estimates in the eight above-mentioned studies (combining HRs and ORs). This summary RR supported the protective association of fish intake on risk for depression described in the meta-analyses (RR=0.88, 95% CI: 0.79, 0.98, ($P_{\text{heterogeneity}}=0.64$)).

The summary RR for developing postpartum depression (defined by prescription medication or the Edinburgh Postnatal Depression Scale) in relation to the highest versus lowest intake of total fish in mid-, or mid-late pregnancy included two studies reporting ORs (Strøm et al., 2009; Hamazaki et al., 2019). The summary RR was statistically significant on the protective side (RR= 0.79, 95% CI: 0.66, 0.95). Heterogeneity was non-significant ($P_{\text{heterogeneity}}=0.14$).

4.14.4.4 *VKM's search compared to previous meta-analyses on fish intake and depression in adults*

There are some differences in the primary studies included by VKM and previous systematic reviews (Grosso et al., 2016; Li et al., 2016; Matison et al., 2021; Yang et al., 2018).

Two of the meta-analyses (Li et al., 2016; Grosso et al., 2016;) included both cross-sectional studies and prospective cohort studies, but stratified results by study design. According to the current protocol, VKM does not consider evidence from cross-sectional studies. Hence, only estimates for the prospective cohort studies from these meta-analyses are used in the comparison.

Table 4.14.4.4-1 Overview of prospective cohort studies included by VKM compared with four identified meta-analyses.

Author, year	VKM	Li, 2016	Grosso, 2016	Yang, 2018	Matisson, 2021
Almeida, 2013					X
Astorg, 2008	X	X	X		
Colangelo, 2009	X	X	X	X	
Elstgeest, 2019	X				
Hakkarainen, 2004	X		X	X	
Hamazaki, 2019	X				
Hamazaki, 2018	X				
Kyrozis, 2009	X				
Li, 2011	X	X	X	X	
Lucas, 2011	X	X	X	X	X
Matsuko, 2017	X			X	
Mihrshahi, 2015		X		X	
Sanchez-Villegas, 2009		X	X		
Smith, 2014	X	X		X	
Strøm, 2009	X		X		
Tsai, 2012	X	X		X	X

Table 4.14.4.4-1 provides an overview of prospective cohort studies included by VKM and in the meta-analyses. The studies by Matsuko et al. (2017), Elstgeest et al. (2019) and Hamazaki et al. (2018/2019) were not included in the other meta-analyses probably due to the date of the publication. Strøm et al. (2009) was only included in Grosso et al. (2016). This study was on post-partum depression which could explain the reason for not being included in the other meta-analyses (which could also be the case for Hamazaki et al. (2018/2019)). None of the meta-analyses included Kyrozis et al. (2009), which was on a geriatric population included by VKM. Astorg et al. (2008), Lucas et al. (2011), Smith et al. (2014), Tsai et al. (2012) and Hakkarainen et al. (2004) were single studies included by VKM, but not in the identified meta-analyses.

Sanchez-Villegas et al. (2009) was excluded by VKM as the focus was on dietary patterns, and Mihrshahi et al. (2015), Huddy et al. (2016) and Almeida et al. (2013) were excluded due to the cross-sectional study design.

Overall, Yang et al. (2018) is the meta-analysis that seems to best mirror the primary studies identified by VKM.

None of the previous meta-analyses evaluated fish intake and post-partum depression.

4.14.5 Heterogeneity fish intake and depression in adults

There was no significant heterogeneity between studies included in VKM's summary RRs for either incidence of depression, or for post-partum depression.

The identified meta-analyses varied in terms of observed heterogeneity in their analysis. No heterogeneity was observed by Yang et al. (2018), while Li et al. (2016) and Grosso et al. (2016) reported moderate heterogeneity. In sensitivity analysis, Li et al. (2016) removed three studies, and the observed heterogeneity decreased.

4.14.6 Dose-response relationship for fish intake and depression in adults

Yang et al. (2018) found a non-significant dose-response relationship for 1 serving/week increment in fish intake and the risk of depression (pooled RR of 0.89 (95%CI: 0.75, 1.04), with no significant heterogeneity ($P=0.109$, $I^2=54.8\%$)). These analyses are based on 3 prospective studies only. Grosso et al. (2016) performed a dose-response analysis based on 16 studies (both cross-sectional and prospective observational studies) resulting in a non-significant overall linear association between fish and depression. Specific dose analyses revealed a significant dose-response relationship at 50 g/day (RR=0.84, 95%CI 0.72, 0.99), but not lower and higher (range 25-125g/day with 0 g/day as reference). These dose-response analyses were based on both cross-sectional and prospective studies, and the dose-response relationship based on the prospective studies only is uncertain.

The remaining three meta-analyses did not assess for dose-response relationships.

4.14.7 Weight of evidence fish intake and depression in adults

In this section, the evidence of the association between fish intake and risk for depression in adults is weighed according to the WCRF criteria presented in Chapter 3.1.6 (Box 2).

Published evidence of fish intake and depression in adults

Overall, results from three (Li et al., 2016; Grosso et al., 2016; Yang et al., 2018) of the four included meta-analysis, suggest a protective association of fish intake on depression, while one meta-analysis suggests no association (Matison et al., 2021). Matison et al. (2021) based their conclusion on only three studies. The fifth meta-study, a systematic review, presented no estimates, but concluded based on two publications that fish consumption reduced the risk of depression.

The two meta-studies that assessed for a dose-response relationship between fish intake and depression were not able to demonstrate a significant relationship overall (Yang et al., 2018; Grosso et al., 2016).

VKM's summary RRs for developing depression suggest a protective association for the highest intake of fish using data from studies reporting results in OR (RR=0.85, 95% CI: 0.74, 0.98), but no significant association combining the three studies reporting HRs or RR (RR=0.92, 95% CI: 0.78, 1.09). When combining all studies (reporting ORs and HRs (Cox and Poisson)), the summary RR suggested a protective association (RR=0.88, 95% CI: 0.79, 0.98, ($P_{\text{heterogeneity}}=0.64$), which is comparable to findings in the previous meta-analyses.

Using two identified studies on post-partum depression, VKM's summary RR suggest a protective association with reported fish intake during pregnancy and markers of post-partum depression. We have not identified previous meta-analyses evaluating the protective associations of fish on post-partum specifically.

Heterogeneity

Two of the meta-analyses reported moderate heterogeneity. In Yang et al. (2018), which perhaps is the meta-analysis most similar to that performed by VKM, heterogeneity is limited. Similarly, no substantial heterogeneity was observed between studies included in VKM's summary RR.

Mechanisms

Some plausible mechanisms have been proposed although not fully established.

Upgrading factors

The described dose-response relationships were not found to be an upgrading factor. No other upgrading factors were considered.

4.14.7.1 *Conclusion weight of evidence fish intake and depression in adults*

There is evidence from more than two independent and good quality prospective cohort studies on fish intake and the risk of developing depression (referring to the WCRF criteria). Of the eleven included studies on depression disorder or symptoms of depression, one study found an overall significant association between fish consumption and depressive symptoms, two studies found a protective association in women but not in men, one found a protective association in men but not in women, and one found protection for recurrent depression, but not for single episodes.

Although the single studies seem divergent in terms of the results, the summary RRs calculated by VKM and in three meta-analyses (high vs. low intake) suggest a protective association between fish intake and risk of depression. The direction of the associations is generally consistent (towards protective) and there is little unexplained heterogeneity. Plausible mechanisms are not fully established however, and hence the biological plausibility is not strong. Moreover, the demonstrated dose-response relationships in two meta-analyses are non-significant overall.

Overall, the evidence that consumption of total fish reduces the risk of depression is graded “limited, suggestive”.

There were fewer studies of fatty fish and lean fish than total fish and the evidence is graded “limited, no conclusion” for the effects of fatty fish and lean fish on adult depression.

For post-partum depression, the evidence is limited since there are findings from only two independent prospective cohort studies. VKM conclude that there is “limited, suggestive” evidence that consumption of total fish reduces the risk of post-partum depression.

4.15 Fish intake and type 2 diabetes in adults

This chapter summarizes the epidemiological evidence on fish intake and risk of developing type 2 diabetes (T2D).

The overall burden of T2D has increased in the world over the past few decades. In Norway, around 5% of the population (270 000 people) has diagnosed diabetes, and T2D accounts for around 90% of these. In addition, it is estimated that diabetes may be undiagnosed in around 60 000 people (source: Norwegian Institute of Public Health, Public Health Report, Diabetes in Norway, Updated 08.08.2017). Although more people are living with diabetes due to ageing, the number of new annual cases in Norway appears to have stabilized.

Based on epidemiological studies, lifestyle factors including diet, have been associated with risk of T2D (DeFronzo et al., 2015). Adiposity (higher BMI levels) is the most important risk factor.

In this chapter, we summarize primary studies of fish intake in relation to risk of developing T2D that were graded A or B in the quality assessment. These publications include latent autoimmune diabetes (LADA), and T2D. LADA is characterized as a hybrid between type 1 and type 2 diabetes. Like type 1 it is an autoimmune form of disease but with a slower progression than type 1. In addition, LADA patients have features of T2D like overweight and insulin resistance.

Mechanisms

T2D is a multifactorial disease involving both genetic and environmental factors. T2D is characterized by dysregulation of carbohydrate, lipid, and protein metabolism because of impaired insulin secretion, insulin resistance, or a combination of these (DeFronzo et al., 2015). The pathophysiological changes are characterized by β -cell dysfunction, insulin resistance and chronic inflammation. These conditions gradually reduce the control of blood glucose levels and lead to the development of micro- and macrovascular complications. Insulin resistance in muscle and the liver, and impaired insulin secretion by the pancreatic β cells are core defects in T2D.

Certain dietary components are associated with a reduced risk of T2D. Potential mechanisms underlying favourable effects of LC n-3 FA are discussed in Chapter 5.2. Concerns that LC n-3 FAs may have a negative effect on diabetes control by raising fasting glucose, have not been supported in LC n-3 supplantation trials.

Also, some studies have found reduced T2D risk with consumption of lean fish such as cod (e.g. Rylander et al., 2014; Øyen et al., 2021), and certain nutrients found in lean fish protein such as taurine have been implicated in the reduced risk (Liaset et al., 2019; Imae et al., 2014; Øyen et al., 2021). However, the exact mechanisms by which these nutrients may protect against T2D are not yet known.

Epidemiological and toxicological studies suggest that environmental contaminants could play a role in causing diabetes and obesity (Legler et al., 2015; Yang et al., 2017; Heindel et al., 2019). Seafood, including fish, are important sources for exposure to contaminants for humans. Several environmental contaminants have been suggested to contribute to T2D development, including heavy metals, pesticides, and persistent organic pollutants (Legler et al., 2015; Heindel et al., 2019). Multiple mechanisms by which chemicals may induce diabetes and obesity have been identified in toxicological studies, e.g., through endocrine and metabolic disruption of adipogenesis, but also through epigenomic, transgenerational effects (Heindel et al., 2019).

4.15.1 VKM's search for previous systematic reviews and meta-analyses of fish intake and T2D

4.15.1.1 *Description of the identified publications*

VKM's search for systematic reviews and meta-analyses (see Chapter 3.1.2.2) identified six publications on the association between fish intake and T2D that fulfilled the inclusion criteria and were read as full papers. Two papers were excluded, see Table 4.15.1.1-1 for reason for exclusions.

Table 4.15.1.1-1 Reasons for exclusion of identified papers from the search of systematic reviews and meta-analyses of fish intake and T2D 2016-2021.

Included papers	Excluded paper and reasons for exclusions
Umbrella review D'Alessandro et al., 2019	Yang et al., 2020, graded C Ibsen et al., 2020, not relevant for our purpose
Systematic reviews Namazi et al., 2019 Schwingschackl et al., 2017	
Federated meta-analysis ¹ Pastorino et al., 2021	

¹Consortium with remote access to individual level data, pooled by meta-analysis.

One of the four included studies was an umbrella review (D'Alessandro et al., 2019), building on one relevant meta-analysis for fish intake and T2D, which was also identified in VKM's search (Schwingschackl et al., 2017). VKM identified one additional meta-analysis not included in the umbrella review, by Namazi et al. (2019).

The meta-analyses are described in more detail below; first a main description of the methods used and then main/selected results from each analysis are provided (see Table 4.15.1.2-1).

Pastorino et al. (2021) performed meta-analysis of individual data from prospective cohort studies to examine the association between different types of fish intake and T2D, as part of the InterConnect project. InterConnect is a European Commission-funded project facilitating

cross-cohort analyses without pooling of data at a central site. Published articles in PubMed containing information on T2D incidence and dietary fish intake were searched, and 43 studies were invited to join the consortium. Twenty-eight prospective cohort studies were finally included, and incidence rate ratios (IRRs) and 95% confidence intervals were derived using piecewise Poisson regression for each study.

The umbrella review by D'Alessandro et al. (2019) included Medline and Google Scholar searches for dose-response meta-analyses investigating the association between food groups and CVD, CHD, stroke, T2D, colorectal and breast cancer risk, from inception up to December 2018. One of the inclusion criteria was that the meta-analyses should include linear and/or non-linear dose-response meta-analyses of prospective studies (cohort studies, nested case-control studies). Nine meta-analyses were identified for fish intake, and one of these had T2D as an outcome (Schwingshackl et al., 2017).

Schwingshackl et al. (2017) conducted a literature search in PubMed, Embase, and Google Scholar from inception until February 2017, and included 39 prospective observational studies from 37 publications. The study by Schwingshackl et al. (2017) had a good methodological quality (AMSTAR assessment done by Jayedi and Shab-Bidar (2020) assigned 10 out of 11 points). The quality of the meta-evidence of fish intake and T2D was rated moderate based on the NutriGrade score (Schwingshackl et al., 2017). We used the AMSTAR tool to assess the methodological quality of Schwingshackl et al. (2017), and the study was found to have a moderate quality (quality level B).

Namazi et al. (2019) conducted a systematic review and meta-analysis by performing a systematic search of PubMed/Medline, Scopus, and Web of Science (ISI) databases for cohort studies, published in English, until 1 September 2017 (no start date given), examining the relationship between different types of fish and seafood intake and risk of T2D in adult populations. We used the AMSTAR tool to assess the methodological quality of Namazi et al. (2019), and the study was found to have a moderate quality (quality level B).

4.15.1.2 *Results from the meta-analyses*

Table 4.15.1.2-1 summarizes data on fish intake and risk of T2D from the identified meta-analyses. Pastorino et al. 2021 reported statistically significant effect modification by sex, and therefore presented all estimates separately for men and women.

Table 4.15.1.2-1. Summary of results from meta-analyses on fish intake and risk of T2D.

Author, year	Type of studies included	Total no. of studies	No of cases	Comparison	Summary RR (95% CI)	Heterogeneity	Overall conclusion
Pastorino et al., 2021	Prospective cohort studies (men and women)	28	48 084				
	Prospective cohort studies (men)	19	NA	Total fish, per 100 g/wk	1.00 (1.00, 1.01)	$I^2=33.6\%$	No sig. assoc.
	Prospective cohort studies (women)	24	NA	Total fish, per 100 g/wk	1.02 (1.01, 1.03)	$I^2=61\%$	Sig. adverse assoc.
	Prospective cohort studies (men)	13	NA	Fatty fish, per 100 g/wk	0.99 (0.98, 1.01)	$I^2=0.0\%$	No sig. assoc.
	Prospective cohort studies (women)	17	NA	Fatty fish, per 100 g/wk	1.04 (1.01, 1.07)	$I^2=46\%$	Sig. adverse assoc.
	Prospective cohort studies (men)	13	NA	Lean fish, per 100 g/wk	1.01 (0.99, 1.04)	$I^2=55\%$	No sig. assoc.
	Prospective cohort studies (women)	17	NA	Lean fish, per 100 g/wk	1.02 (1.00, 1.04)	$I^2=33\%$	Sig. adverse assoc.
	Prospective cohort studies (men)	13	NA	Fried fish, per 100 g/wk	1.01 (0.97, 1.06)	$I^2=41\%$	No sig. assoc.
	Prospective cohort studies (women)	15	NA	Fried fish, per 100 g/wk	1.04 (0.98, 1.10)	$I^2=64\%$	No sig. assoc.
	Prospective cohort studies (men, America)	4	NA	Total fish, per 100 g/wk	1.02 (1.00, 1.05)	$I^2=53\%$	Borderline sig. adverse assoc.
	Prospective cohort studies (women, America)	5	NA	Total fish, per 100 g/wk	1.03 (1.02, 1.04)	$I^2=0.0\%$	Sig. adverse assoc.
	Prospective cohort studies (women, America)	5	NA	Highest vs lowest (total fish)	1.22 (1.02, 1.46)	NA	Sig. adverse assoc.
	Prospective cohort studies (women, America)	3	NA	Fatty fish, per 100 g/wk	1.03 (1.001, 1.064)	$I^2=0\%$	Sig. adverse assoc., not sig in other geographical areas (results not shown)

Author, year	Type of studies included	Total no. of studies	No of cases	Comparison	Summary RR (95 % CI)	Heterogeneity	Overall conclusion
	Prospective cohort studies (women, Asia and Australia)	4	NA	Total fish, per 100 g/wk	1.00 (0.99, 1.02)	$I^2=0.0\%$	No sig. assoc.
	Prospective cohort studies (women, Europe)	14	NA	Total fish, per 100 g/wk	1.01 (0.99, 1.03)	$I^2=66.3\%$	No sig. assoc.
Namazi et al., 2019	Prospective cohort studies	4	13 917	Highest vs lowest (fatty fish)	0.89 (0.82, 0.98)	$I^2=0\%$	Sig. protective assoc. (non-sig. after removing one study in sensitivity analysis)
	Prospective cohort studies	4	13 917	Highest vs lowest (lean fish)	1.03 (0.87, 1.22)	$I^2=51\%$	No sig. assoc.
	Prospective cohort studies	2	4 349	Highest vs lowest (fried fish)	1.02 (0.83, 1.26)	$I^2=71.2\%$	No sig. assoc.
Schwingschackl et al. 2017	Prospective observational studies	16	45 029	Highest vs lowest	1.04 (0.95, 1.13)	$I^2=76\%$	No sig. assoc.
	Prospective observational studies	15		Dose-response (100 g/d increase in fish)	1.09 (0.93, 1.28)	$I^2=84\%$	No sig. assoc.
	Prospective observational studies (America)	6		Dose-response (100 g/d increase in fish)	1.44 (1.19, 1.74)	$I^2=66\%$	Sig. strong adverse assoc.
	Prospective observational studies (Asia and Australia)	3		Dose-response (100 g/d increase in fish)	0.87 (0.80, 0.95)	$I^2=0\%$	Protective assoc.
	Prospective observational studies (Europe)	6		Dose-response (100 g/d increase in fish)	0.94 (0.78, 1.14)	$I^2=48\%$	No sig. assoc.

The two meta-analyses on the association between fish intake and risk of T2D found no overall significant association. However, Schwingschackl et al. (2017) noted significant associations when stratified by geographic region, where dose-response analyses of cohorts from Asia and Australia showed a protective association of fish intake and T2D risk. On the other hand, similar analyses of American cohorts showed a strong adverse association. Namazi et al. (2019) found a significant protective association of consumption of oily fish with T2D, but not after eliminating one study as a sensitivity analysis.

In the more comprehensive federated meta-analysis by Pastorino et al. (2021), a neutral association between total fish intake and T2D was found in men, whereas a modest adverse association was observed in women. In women, heterogeneity was observed that was partly explained by geographical location and types of fish intake. Similar to Schwingschackl et al. (2017), a significant adverse association was observed in women from the Americas, but not in women from the other regions (Europe, Asia/Australia). In contrast to both Schwingschackl et al. (2017) and Namazi et al. (2019), Pastorino et al. (2021) did not find evidence for protective associations among European nor among Asian cohorts. Furthermore, they observed adverse associations with both fatty and lean fish intake and T2D risk in women across all cohorts.

4.15.2 VKM's systematic review of primary studies on fish intake and T2D

4.15.2.1 *Included studies from search*

We evaluated 17 publications graded A or B with diabetes in adults as outcome; either total diabetes (type 1 and type 2 combined), T2D only, or latent LADA (Baghdasarian et al., 2018; Chen et al., 2020; Djousse et al., 2011; Du et al., 2020; Kaushik et al., 2009; Löfvenborg et al., 2014; Löfvenborg et al., 2021; Nanri et al., 2011; Patel et al., 2012; Rylander et al., 2014; Talaei et al., 2017; van Woudenberg et al., 2009; Villegas et al., 2011; Virtanen et al., 2014; Wallin et al., 2017; Zhang et al., 2019; Øyen et al., 2021).

Du et al. (2020) reported on total adult-onset diabetes which was summarized with studies of T2D. LADA was only presented in one study (Löfvenborg, 2014) and not summarized, but the study also contributed with results on T2D and was kept.

A description of the studies (study name, design, time period, size and age of the study population, and dietary assessment method) can be found in (Table 4.15.2.1-1).

Table 4.15.2.1-1 Overview of primary studies included in the weight of evidence analysis of fish intake and risk of T2D.

Author, year country	Study name	Design	Inclusion year(s), end, follow-up time	Study size, age	Dietary assessment method	Dietary assessment period
Baghdasarian 2018, USA	Framingham Offspring Study	Cohort	1971-1975, 20 yrs follow-up	2192 individuals, 35-64 (mean age 49)	Three-day dietary records, repeated	3-day dietary records, at year 12 and 20 of follow-up
Chen 2020, UK	UK Biobank	Cohort	2006-2010, 10.1 yrs follow-up (median)	392,287 participants, 37-73 yrs	FFQ at baseline, 24-hour recall using Oxford WebQ at follow-up	5 follow-up rounds 2009-2012
Djousse 2011, USA	Women's Health Study (WHS)	Cohort, based on RCT	1992-1995 to 2004, 12.4 yrs follow-up (mean)	36,328 female health professionals, ≥ 45, mean age 54.6 yrs	FFQ, semi-quant	Average intake previous year, at baseline
Du 2020, China	China Kadoorie Biobank	Cohort	2004-2008, 9 yrs follow-up	512,713, 35-74 yrs	Interviewer administered questionnaire for past year, quantitative recording of food-groups in resurveys	2013-2014
Kaushik 2009, USA	Nurses' Health Study (NHS), the Nurses' Health Study 2 (NHS2), and the Health Professionals Follow-Up Study (HPFS)	Cohorts, pooled	NHS: 1976-1986, NHS2: 1989-1991, HPFS: 1986-1986; end of follow-up: 2004, 2005, 2004	195,204 participants, Mean age NHS: 52, NHS2: 36, HPFS: 53	Semi-quant. FFQ, repeated at 4-year intervals	Average intake prev year, at baseline
Löfvenborg 2014, Sweden	ESTRID	Case-control	2010-2013	1558 individuals, 60 yrs	FFQ	Average intake prev year, at baseline
Löfvenborg 2021, Europe (8 countries)	EPIC-InterAct	Case-cohort	1991-2007, 9 yrs follow-up	340,234 in population (25,535 in sample), mean age 52-56 yrs	Quantitative dietary questionnaires or FFQ, country specific	At baseline
Nanri 2011, Japan	Japan Public Health Center-based Prospective Study	Cohort	1990 -1993, 2000-2003, 10 yrs follow-up	22,921 men, 29,759 women, 40-69 yrs	FFQ, including 19 items of fish/seafood	Average intake at baseline (second survey)

Author, year country	Study name	Design	Inclusion year(s), end, follow-up time	Study size, age	Dietary assessment method	Dietary assessment period
Rylander 2014, Norway	Norwegian Woman and Cancer Study (NOWAC)	Cohort	1996-1998, follow-up 2002-2005	33,740 women, 30-70 yrs	NOWAC FFQ	Usual intake, at baseline
Talaei 2017, Singapore	Singapore Chinese Health Study	Cohort	1993-1998, follow-up mean 10.9 yrs	45,411 participants (eller 63,257?), 45-74, mean age 55.2	FFQ, semi-quant	Usual diet in past year, at baseline
van Woudenberg 2009, Netherlands	Rotterdam Study	Cohort	1990-1993, follow-up mean 12.4 yrs	7,983 participants, average age 67.2±7.7 yrs	Self-adm. questionnaire combined with dietitian interview, and semi-quant FFQ	Intake in previous year, at baseline
Villegas 2011, China	Shanghai Women's Health Study (SWHS) and Shanghai Men's Health Study (SMHS)	Cohort	SWHS: 1996-2000, SMHS: 2002-2006, biennial follow-up until 2004-2006 and 2004-2008, 8.9 yrs and 4.1 yrs, respectively	SWHS: 74,942 women, SMHS: 61,500 men, 40-70 (74) yrs	FFQ by interview, repeated	Usual intake, at baseline and first follow-up
Virtanen 2014, Finland	Kuopio Ischemic Heart Disease Risk Factor (KIHD) study	Cohort	1984-1989, follow-up time 19.3 yrs	2,212 men, 42-60 yrs	4-day food recording, household measures	4 days at baseline
Wallin 2017, Sweden	The Cohort of Swedish Men	Cohort	1998-2012 (15 yrs)	35,583 men, 45-79 yrs	FFQ	Average intake prev year, at baseline
Zhang 2019, China	China Health and Nutrition Survey (CHNS)	Cohort	1997-2011 (14 yrs median follow-up)	15,100 participants, ≥20 yrs (mean age 42-43 yrs)	24-hour recall by interview, 3 consecutive days, repeated. Combined with weighted household data	3 days at baseline and follow-up (2000, 04, 06, 09, 11), cumulative average
Øyen 2021, Norway	Norwegian Mother, Father and Child Cohort Study (MoBa)	Cohort	1999-2008 (7.5 yrs follow-up)	60,831 mothers (median age 31 at delivery)	FFQ, semi-quantitative	At baseline
Excluded due to overlap						

Author, year country	Study name	Design	Inclusion year(s), end, follow-up time	Study size, age	Dietary assessment method	Dietary assessment period
Patel 2012, Europe (8 countries)	EPIC-InterAct Study	Case-cohort	1991-1993 to 2007	27,779 participants, 40-60 yrs, mean age 50-53	Quantitative dietary questionnaires or FFQ, country specific	Usual intake, at baseline

4.15.2.2 *Overlapping publications*

There were two studies from EPIC-InterAct, a pooled analysis of data from 8 European countries (Patel et al., 2012; Löfvenborg 2021). Löfvenborg 2021 was similar to Patel et al. (2012) but also investigated the role of antibody positivity (GAD65) in the association of fish intake with risk of T2D. Only Löfvenborg 2021 was kept for further analysis. The Norwegian sub-cohort of EPIC (Norwegian Woman and Cancer Study, NOWAC) was not part of EPIC-Interact, so there was no overlap with the publication from the NOWAC study (Rylander et al., 2014).

4.15.2.3 *Studies by design and geographic region*

The studies covered populations from USA (4 studies), Europe (9 studies) and Asia (5 studies, 1 Japanese, 3 Chinese, and 1 Singapore-Chinese). Except one case-control study, all studies had prospective, observational designs (cohort or case-cohort). One cohort was based on an RCT.

4.15.2.4 *Studies by sex, potential effect modification, and other sub-groups*

Twelve of the studies were conducted in both men and women, of which nine reported results for sexes combined (Baghdasarian et al. 2018; Kaushik et al. 2009; Löfvenborg et al. 2014; Löfvenborg et al. 2021, Talaei et al., 2017; van Woudenbergh et al., 2009), whereas three reported data on both sexes but separately (Nanri et al., 2011; Villegas et al., 2011; Zhang et al., 2019). Two studies reported data on men only (Virtanen et al., 2014; Wallin et al., 2017), and three studies reported women only (Djousse et al., 2011; Rylander et al., 2014; Øyen et al., 2021).

Löfvenborg et al. (2021) investigated the relationship between GAD65 antibody status and diabetes incidence. Du et al. (2020) reported on differences between rural and urban Chinese population subgroups. Øyen et al. (2021) stratified by pre-pregnancy BMI and Baghdasarian et al. (2018) by dietary cholesterol (DC).

4.15.2.5 *Studies by fish exposure*

The studies reported different aspects of fish intake. Total fish intake was reported in eleven studies (Du et al., 2020; Kaushik et al., 2009; Löfvenborg et al., 2021; Nanri et al., 2011; Patel et al., 2012; Rylander et al., 2014; van Woudenbergh et al., 2009; Villegas et al., 2011; Virtanen et al., 2014; Wallin et al., 2017; Zhang et al., 2019). Three studies reported total intake of fish and shellfish (Djousse et al., 2011; Talaei et al., 2017; Øyen et al., 2021). Eight studies reported intake of lean fish and fatty fish (Chen et al., 2020; Löfvenborg et al., 2014; Löfvenborg et al., 2021; Nanri et al., 2011; Patel et al., 2012; Rylander et al., 2014; van Woudenbergh et al., 2009; Øyen et al., 2021). One study reported on fish size (small, medium, large) (Nanri et al., 2011), one on freshwater vs. saltwater fish (Villegas et al., 2011). Two studies reported on fried fish vs. non-fried fish (Wallin et al., 2017; Zhang et al., 2019), one study on fish products alone (Rylander et al., 2014), and one study on fish

products including canned tuna, in addition to preserved fish (Nanri et al., 2011). As noted above, Baghdasarian et al. (2018) reported total fish intake categorized by dietary cholesterol.

4.15.2.6 *Studies adjusting for contaminants*

Two studies reported on contaminants. Wallin et al. (2017) assessed PCB and methyl mercury (MeHg) exposure based on recipe-based databases created for the FFQ used and reported contaminant-adjusted risk ratios for fish intake and diabetes. Øyen et al. (2021) derived the intake of MeHg and sum of dioxins and dl-PCBs from the FFQ and a contaminant database.

4.15.2.7 *Studies assessing potential non-linearity*

Two studies assessed potential non-linearity of the dose-response relationships for risk of T2D vs. fish intake, using restricted-cubic-spline regression (Rylander et al., 2014; Zhang et al., 2019 (figures not shown)).

4.15.2.8 *Studies with converted risk estimates*

Baghdasarian et al. (2018) reported HRs based on low vs. high intake. These have been converted to high vs. low intake values. Both the reported and converted estimates are found in Table 4.15.3.1-1.

4.15.3 Results from the included primary studies on type 2 diabetes (T2D)

4.15.3.1 *Studies of total fish intake and T2D in the general population*

We included 14 publications with 19 estimates of the association between total fish intake and T2D in the weight of evidence analysis. The exposure levels and results (high-low relative risk, and overall association) are included in Table 4.15.3.1-1.

Table 4.15.3.1-1 Results from studies included in the weight of evidence analysis of total fish intake and T2D in the general population.

Author, year, country	Study design	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low or continuous (95% CI)	Overall result
Baghdasarian 2018, USA	Cohort	Fish, dietary cholesterol <300 mg/d, M/W	Servings (ounce-eq)/wk, binary	≥1 vs. <1 servings/wk, ≥28 vs <28 g/wk	579	HR 0.80 (0.65, 0.97) reported as 1.25 (1.03, 1.53) for <1 (low) vs ≥1 (high) servings/day	Sig. protective assoc.
		Fish, dietary cholesterol ≥300 mg/d, M/W	Servings (ounce-eq)/wk, binary	≥1 vs <1 servings/wk	579	HR 1.02 (0.78, 1.33) reported as 0.98 (0.75, 1.28) for < 1 (low) vs. ≥ 1 (high) servings/day	No sig. assoc.
Djousse 2011, USA	Cohort, based on RCT	Fish, incl shellfish, W	Servings/wk, 5 cat	Quintile 5 vs 1, 3.93 vs 0.47 (median servings/wk)	2370	HR 1.49 (1.30, 1.70)	Sig. adverse assoc. for intake in Q3-Q5 vs. Q1, <i>P</i> -trend < 0.0001
Du 2020, China	Cohort	Fish, incl shellfish, M/W	days/wk, 4 cat	≥4 days/wk (regular) vs never/rarely		HR 1.06 (1.00, 1.13),	Borderline adverse assoc. However, <i>P</i> -trend 0.14 when adjusted for BMI
Kaushik 2009, USA	Cohorts, pooled	Fish, M/W	Intake/mo or wk, 5 cat	>5 times/wk (500 g/wk) vs <1 time/mo (<100 g/mo)	9380	HR/RR 1.22 (1.08, 1.39), pooled analysis	Sig. adverse assoc. for fish intake 2–4 times/wk and ≥ 5 times/wk
Löfvenborg 2021 Europe (8 countries)	Case-cohort	Fish, M/W	3 cat, high vs low	≥223 vs <69 g/wk	11247	HR 1.03 (0.93, 1.15)	No sig. assoc.
Nanri 2011, Japan	Cohort	Fish, M	g/d, quartiles	Quartile 4 vs 1	572	OR 0.89 (0.66, 1.18)	No sig. assoc., <i>P</i> -trend 0.33
		Fish, W	g/d, quartiles	Quartile 4 vs 1	399	OR 0.93 (0.66, 1.31)	No sig. assoc., <i>P</i> -trend 0.72
Rylander 2014, Norway	Cohort	Fish, W	g/d, 10 cat (per 25 g/d)	225 vs 0 g/d	479	RR (Poisson) 0.66 (0.36, 1.21)	No sig. assoc.
Talaei 2017, Singapore	Cohort	Fish, incl shellfish, M/W	g/d, quartiles	Quartile 4 vs 1, 89 g/d vs 25 g/d	5207	HR 1.07 (0.99, 1.16)	No sig. assoc.

Author, year, country	Study design	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low or continuous (95 % CI)	Overall result
van Woudenberg 2009, Netherlands	Cohort	Fish, M/W	g/d, 4 cat (0 intake, tertiles)	Upper tertile of intake vs no intake, ≥ 28 vs 0 g/d	463	HR 1.32 (1.02, 1.70)	Sig. adverse assoc. for highest intake of total fish (81% lean), P -trend 0.04
Villegas 2011, China	Cohort	Fish, W	g/d, quintiles	Quintile 5 vs 1, 80.2 vs 9.5 g/d (median values)	3034	HR 0.89 (0.78, 1.01)	Sig. or borderline protective assoc. for intake in quintiles 3 to 5 vs 1, P -trend 0.003
		Fish, M	g/d, quintiles	Quintile 5 vs 1, 79 vs 9.7 g/d (median values)	833	HR 0.94 (0.74, 1.17)	No sig. assoc., P -trend 0.50
Virtanen 2014, Finland	Cohort	Fish, M	g/d, quartiles	Quartile 4 vs 1, >75 vs <5 g/d	411	HR 0.89 (0.68, 1.18)	No sig. assoc., P -trend 0.40
Wallin 2017, Sweden	Cohort	Fish, M	Servings/wk, 5 cat	≥ 4 (median 5) vs <1 (median 0.9) servings/wk	3624	HR 1.00 (0.85, 1.18)	No sig. assoc., P -trend 0.48
		Fish - contaminant adjusted, M	Servings/wk, 5 cat	≥ 4 (median 5) vs <1 (median 0.9) servings/wk	3624	HR 0.79 (0.60, 1.04)	Borderline protective assoc. for ≥ 4 vs <1 servings/wk, P -trend 0.13
Zhang 2019, China	Cohort	Fish, M	g/day, 4 cat (no intake, low-med-high)	≥ 77.8 vs 0 g/d, cumulative average,	492	HR 0.97 (0.68, 1.34)	Adverse assoc. for low and moderate but not highest intake, P -trend 0.80
		Fish, W	g/day, 4 cat (no intake, low-med-high)	≥ 66.7 vs 0 g/d, cumulative average	525	HR 0.97 (0.73, 1.33)	Adverse assoc. for low and moderate but not highest intake, P -trend 0.81
Øyen 2021, Norway	Birth cohort	Fish, incl shellfish, W	g/d, continuous	Per 25 g total fish /1000 kcal	683	HR 0.91 (0.75, 1.10)	No sig. assoc., P -trend 0.325

Two studies reported protective associations between fish intake and risk of T2D (Baghdasarian et al., 2018, in low DC group; Villegas et al., 2011, in women), one reported borderline protective association after adjustment for contaminant (PCBs and MeHg) exposure (Wallin et al.,

2017), four studies showed adverse associations (Djousse et al., 2011; Kaushik et al., 2009; van Woudenberg et al., 2014; Zhang et al., 2019, for both sexes), whereas thirteen studies found no significant association.

4.15.3.2 *Studies of fatty fish intake and T2D in the general population*

We included seven publications with eight estimates of the association between fatty fish intake and T2D in the weight of evidence analysis. The exposure levels and results (high-low relative risk, and overall association) are included in Table 4.15.3.2-1.

Table 4.15.3.2-1 Results from studies included in the weight of evidence analysis of fatty fish intake and T2D in the general population.

Author, year, country	Study design	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low or continuous (95 % CI)	Overall result
Chen 2021, UK	Cohort	Fatty fish, M/W	Servings/wk, 4 cat	≥2 vs 0 servings/wk	7262	HR 0.79 (0.72, 0.87)	Protective assoc., <i>P</i> -trend <0.001
Löfvenborg 2021 Europe (8 countries)	Case-cohort	Fatty fish, M/W	3 cat, high vs low	≥78 vs <12 g/wk	9724	HR 0.96 (0.87, 1.05)	No sig. assoc.
Löfvenborg 2014, Sweden	Case-control	Fatty fish, M/W	Servings/wk, 3 cat	>2 vs <1 servings/wk	431	OR 0.97 (0.65, 1.45)	No sig. assoc.
Nanri 2011, Japan	Cohort	Fatty fish, M	g/d, quartiles	Quartile 4 vs 1	572	OR 0.79 (0.59, 1.05)	Borderline protective assoc. for intake in quartile 4 vs 1, <i>P</i> -trend 0.098
		Fatty fish, W	g/d, quartiles	Quartile 4 vs 1	399	OR 0.93 (0.67, 1.29)	No sig. assoc., <i>P</i> -trend 0.46
Rylander 2014, Norway	Cohort	Fatty fish, W	g/d, 3 cat (per 25 g/d)	50 vs 0 g/d	479	RR (Poisson) 1.1 (0.81, 1.50)	No sig. assoc.
van Woudenberg 2009, Netherlands	Cohort	Fatty fish, M/W	g/day: 4 cat (0 intake, tertiles)	Upper tertile of intake vs no intake, ≥7 vs 0 g/day	463	HR 0.99 (0.71, 1.38)	No sig. assoc., <i>P</i> -trend 0.93
Øyen 2021, Norway	Birth cohort	Fatty fish, W	g/d, continuous	Per 25 g total fish /1000 kcal	683	HR 0.94 (0.67, 1.32)	No sig. assoc., <i>P</i> -trend 0.704

In the seven studies reporting T2D risk based on fatty fish intake, there was one study reporting a protective association (Chen et al., 2021), and one study reporting borderline protective association in quartile 4 (Nanri et al., 2011, men). The three other studies found no significant association between fatty fish intake and risk of T2D (Löfvenborg et al., 2014; Nanri et al., 2011, women; Rylander et al., 2014).

4.15.3.3 *Studies of lean fish intake and T2D in the general population*

All studies on fatty fish intake also presented results on lean fish intake. Thus, we included seven studies with nine estimates, see Table 4.15.3.3-1.

Table 4.15.3.3-1 Results from studies included in the weight of evidence analysis of lean fish intake and type 2 diabetes in the general population.

Author, year, country	Study design	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low or continuous (95 % CI)	Overall result
Chen 2021, UK	Cohort	Lean fish, M/W	Servings/wk, 4 cat	≥2 vs 0 servings/wk	7262	HR 0.92 (0.80, 1.04)	No sig. assoc., <i>P</i> -trend 0.45
Löfvenborg 2021 Europe (8 countries)	Case-cohort	Lean fish, M/W	3 cat, high vs low	≥125 vs <13 g/wk	9724	HR 1.02 (0.92, 1.14)	No sig. assoc.
Löfvenborg 2014, Sweden	Case-control	Lean fish, M/W	Servings/wk, 2 cat	≥1 vs <1 servings/wk	431	OR 0.87 (0.63, 1.19)	No sig. assoc.
Nanri 2011, Japan	Cohort	Lean fish, M	g/d, quartiles	Quartile 4 vs 1	572	OR 1.05 (0.80, 1.38)	No sig. assoc., <i>P</i> -trend 0.83
		Lean fish, W	g/d, quartiles	Quartile 4 vs 1	399	OR 1.02 (0.75, 1.40)	No sig. assoc., <i>P</i> -trend 0.98
Rylander 2014, Norway	Cohort	Lean fish, W	g/d, 5 cat (per 25 g/d)	100 vs 0 g/d	479	RR (Poisson) 0.67 (0.46, 0.98)	No sig. assoc., <i>P</i> -trend 0.57
van Woudenberg 2009, Netherlands	Cohort	Lean fish, M/W	g/day, 4 cat (0 intake, tertiles)	Upper tertile of intake vs no intake, ≥23 vs 0 g/d	463	HR 1.30 (1.01, 1.68)	Protective assoc. for lean fish consumption of 75-100 vs 0 g/d
Øyen 2021, Norway	Birth cohort	Lean fish, incl shellfish, W	g/d, continuous	Per 25 g total fish /1000 kcal	683	HR 0.71 (0.53, 0.95)	Adverse assoc. for highest intake of lean fish, <i>P</i> -trend 0.06

4.15.3.4 *Studies of fried and non-fried fish intake and T2D in the general population*

We included two studies with four estimates of fried fish, see Table 4.15.3.4-1. The estimate for non-fried fish (Zhang, 2019 only) was included for comparison.

Table 4.15.3.4-1 Results from studies included in the weight of evidence analysis of fried fish intake and T2D in the general population.

Author, year, country	Study design	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low or continuous (95% CI)	Overall result
Wallin 2017, Sweden	Cohort	Fried fish, M	Servings/mo, 5 cat	≥6 (median 8) vs ≤1 (median 0) servings/mo	3624	HR 1.14 (1.03, 1.31)	Adverse association (sig or borderline) for 2 servings or more per month, <i>P</i> -trend 0.004
	Cohort	Fried fish - contaminant adjusted, M	Servings/mo, 5 cat	≥6 (median 8) vs ≤1 (median 0) servings/mo	3624	HR 1.13 (1.00, 1.28)	Adverse association (sig or borderline) for 2 servings or more per month, <i>P</i> -trend 0.004
Zhang 2019, China	Cohort	Fried fish, M	g/d, 4 cat (no intake, low-med-high)	>34.7 vs 0 g/d, cumulative average	492	HR 0.93 (0.64, 1.34)	Adverse association (sig or borderline) for 2 servings or more per month, <i>P</i> -trend 0.01
	Cohort	Fried fish, W	g/d, 4 cat (no intake, low-med-high)	>33.3 vs 0 g/d, cumulative average	525	HR 1.03 (0.73, 1.49)	Adverse association for low and moderate but not high intake, <i>P</i> -trend 0.30
	Cohort	Non-fried fish, M	g/d, 4 cat (no intake, low-med-high)	>66.7 vs 0 g/d, cumulative average	492	HR 0.94 (0.66, 1.32)	Adverse association for low and moderate but not high intake, <i>P</i> -trend 0.02
	Cohort	Non-fried fish, W	g/d, 4 cat (no intake, low-med-high)	>55.6 vs 0 g/d, cumulative average	525	HR 0.88 (0.61, 1.18)	No sig. assoc., <i>P</i> -trend 0.87

Both studies (Wallin et al., 2017, and Zhang et al., 2019) reported adverse associations between fried fish intake and T2D risk. Wallin et al. (2017) found significant or borderline associations for two servings or more per month, both before and after contaminant-adjustment. Zhang et al. (2019) found adverse associations for low and moderate, but not high fried fish intake, in both men and women. With non-fried fish intake, they found no significant association with T2D risk in men, but an adverse association in women for low and moderate intake, but not high intake.

4.15.3.5 *Studies of fish intake, environmental contaminants and T2D*

Wallin et al. (2017) reported results on total fish and fried fish intake before and after adjustment for environmental contaminants (PCBs and MeHg) calculated from FFQs (Table 4.15.3.1-1). Although finding correlations between fish intake and dietary contaminant intake (Spearman r : 0.77 for PCB and 0.70 for MeHg), only small changes were found in the overall association with T2D risk upon contaminant adjustment. They found that the association changed from no association to a borderline protective association (not statistically significant) after taking calculated dietary contaminant exposure into account for total fish intake, whereas the association for fried fish intake was not markedly influenced by this adjustment.

Øyen et al. (2021) investigated the influence of dietary exposure to MeHg and dioxins and DL-PCBs on T2D risk, using FFQ results and databases of concentrations of Hg (Jenssen et al., 2012), and dioxins and DL-PCBs in Norwegian food (Kvalem et al., 2009). Whereas the dietary exposure to MeHg (mostly from lean fish) was below TWI, the exposure to dioxins and DL-PCBs from fatty fish was higher than the recent TWI set by EFSA. However, no associations between fatty fish intake and T2D risk were observed.

4.15.3.6 *Summary relative risks (RR) based on VKM's inclusion of primary studies*

VKM calculated a summary RR for developing T2D in relation to the highest versus lowest intake of total fish, based on thirteen prospective studies (Table 4.15.3.1-1). One study reporting fish intake on a continuous scale could not be included. The summary RR showed no statistically significant association (RR=1.04, 95% CI: 0.96, 1.14). Among the primary studies there was substantial heterogeneity with reports of both protective or borderline protective associations, and adverse associations, contributing to a highly significant P -value for heterogeneity ($p_{\text{heterogeneity}} < 0.001$). No study dominated in terms of the relative weight (%). One recent publication from the Norwegian MoBa study (Øyen et al., 2021) reported results for fish intake on a continuous scale (per 25 g/1000 kcal) which could not be included in the high-low summary RR, but the results supported no significant association.

VKM's summary RRs for developing T2D in relation to the highest versus lowest intake of fatty fish or lean fish were based on five prospective studies (Table 4.15.3.3-1). The RR for fatty fish intake suggested a protective association with T2D (RR=0.88, 95% CI: 0.78, 0.99) with borderline significant heterogeneity ($p_{\text{heterogeneity}} = 0.06$). The heterogeneity was driven by

differences in the magnitude of association in the two largest studies, the UK biobank study reporting a protective association (Chen et al., 2021, 34% relative weight) and EPIC-Interact study reporting a non-significant association closer to null (Löfvenborg 2021 et al., 35% relative weight). One case-control study not included in the summary RR (Löfvenborg et al., 2014) reported an OR close to unity (no association), and the results from the Norwegian MoBa study with fatty fish on a continuous scale (Øyen et al., 2021) was also consistent with no association.

The RR for lean fish intake suggested no association with T2D (RR= 0.99, 95% CI: 0.87, 1.13) but there was significant heterogeneity ($p_{\text{heterogeneity}}=0.04$) with reports of both protective and adverse associations among the primary studies. The case-control study (Löfvenborg et al., 2014) not included in the summary RR, reported an OR on the protective side, but not statistically significant. The Norwegian MoBa study (Øyen et al., 2021) found a statistically significant protective association for lean fish on a continuous scale but limited to women with a pre-pregnancy BMI ≥ 25 kg/m² in sensitivity analyses. There was no association in women with pre-pregnancy BMI < 25 kg/m².

Two prospective primary studies reported on fried fish intake (Table 4.15.3.4-1, Wallin et al., 2017; Zhang et al., 2019). The summary RR for T2D diabetes was on the adverse side, but not statistically significant (RR=1.10, 95% CI: 0.97, 1.25, with $p_{\text{heterogeneity}}=0.31$). The result was dominated by Wallin et al. (2017), the one study reporting an adverse association, and which contributed 77% relative weight.

4.15.3.7 VKM's search compared to previous meta-analyses on T2D

Pastorino et al. (2021) included 28 studies in their federated meta-analysis, including eight sub-cohorts of the EPIC-Interact study. Eleven of these 28 studies had not published on this association earlier and were therefore not identified in VKM's literature search. Publications from the remaining seven studies were all identified and included by VKM: Du et al. (2020), Löfvenborg et al. (2021); Nanri et al. (2011), Patel et al. (2012) and Rylander et al. (2014), Villegas et al. (2011), Wallin et al. (2017).

Namazi et al. (2019) included seven cohort studies in their meta-analysis. All were found by VKM, but one was considered to be overlapping (Patel et al. 2012 overlapping with Patel et al. 2009 and the oldest study was excluded). VKM's search identified seven papers published after Namazi's search (Talaei et al., 2017; Baghdasarian et al., 2018, Zhang et al., 2019; Chen et al., 2020; Du et al., 2020; Löfvenborg et al., 2021; Øyen et al., 2021), but also found three additional publications not included in the Namazi et al. (2019) meta-analysis (Djousse et al., 2011; Kaushik et al., 2009; Virtanen et al., 2014).

Schwingschackl et al. (2017) included 13 papers (16 studies) in their meta-analysis. Of these, five were either not found in VKM's search or excluded in study selection as fish was not main exposure (Ericson et al., 2015; Krishnan et al., 2010; Lacoppidan et al., 2015; Montonen et al., 2005; Vang et al., 2008), whereas VKM identified seven papers published after Schwingschackl's search (Talaei et al., 2017; Baghdasarian et al., 2018, Zhang et al.,

2019; Chen et al., 2020; Du et al., 2020; Löfvenborg et al., 2021; Øyen et al., 2021), in addition to Virtanen et al. (2014) which was not included in Schwingshackl et al. (2017).

An overview of overlap between studies included in VKMs weight of evidence analysis of fish intake and T2D and the included systematic reviews/ meta-analyses is presented in Table 4.15.3.7-1. D'Alessandro et al. (2019) is an umbrella review and includes only Schwingshackl et al. (2017) for T2D.

Table 4.15.3.7-1 Overview of studies included by VKM compared with four identified meta-analyses on type 2 diabetes.

Publication	Included by VKM	Meta-analyses		
		Pastorini 2021 ¹	Namazi 2019	Schwingshackl 2017
Baghdasarian 2018, Framingham Offspring Study	X			
Chen 2020, UK Biobank	X			
Djousse 2011, WHS	X			X
Du 2020, CKB	X	X		
Ericson 2015, MDC				X
Kaushik 2009, NHS	X			X
Kaushik 2009, NHSII	X			X
Kaushik 2009, HPFS	X			X
Krishnan 2010				X
Lacoppidan 2015				X
Löfvenborg 2021, EPIC-InterAct	X	X		
Löfvenborg 2014, ESTRID	X			
Montonen 2005				X
Nanri 2011, JPHC	X	X	X	X
Patel 2012			X	X
Patel 2009			X	
Rylander 2014, NOWAC	X	X	X	X
Talaei 2017, Singapore Chinese Health Study	X			
Vang 2008				X
Villegas 2011, SWHS	X	X		X
Villegas 2011, SMHS	X	X	X	X
Virtanen 2014, KIID study	X			
Wallin 2017, The COSM	X	X	X	X
van Woudenberg 2009, Rotterdam Study	X		X	X
Zhang 2019, CHNS	X			
Øyen 2021, MoBa	X			

¹Consortium with remote access to individual level data, pooled by meta-analysis. Pastorini et al. 2021 additionally includes data from the following studies: ARIC—Atherosclerosis Risk in Communities; ELSA Brasil—Brazilian Longitudinal Study of Adult Health; CARDIA—Coronary Artery Risk Development in Young Adults Study; MESA—Multi-Ethnic Study of Atherosclerosis; PRHHP—Puerto Rico Heart Health Program; WHI—Women Health Initiative; Eastern Mediterranean Golestan (Iran); FMC—Finnish Mobile Clinic Health Examination Survey; Hoorn (the Netherlands); SMC—Swedish Mammography Cohort; SUN—Seguimiento Universidad de Navarra (University of Navarra Follow-up); Whitehall II (UK); Zutphen Study (the Netherlands); AusDiab—Australian Diabetes, Obesity and Lifestyle Study; NHAPC—Nutrition and Health of Aging Population of China Study.

4.15.4 Heterogeneity fish intake and T2D

Previous meta-analyses on fish intake and T2D have reported moderate to substantial heterogeneity between studies (Table 4.15.1.2-1) and also examined potential sources of heterogeneity in sub-group analyses.

Pastorino et al. (2021) found evidence of an overall neutral association between fish intake and T2D for men with low heterogeneity. In women, an adverse association with fish intake was found, but with evidence of heterogeneity between studies ($I^2=61$), mainly caused by geographical location of cohorts, and type of fish (fatty vs. lean fish). Women from American cohorts showed statistically significant adverse association between fish intake and T2D ($I^2=0\%$), whereas in European and Asian/Australian women there was no evidence of any association, but with higher heterogeneity in European studies ($I^2=66\%$ and $I^2=0\%$, respectively) (Table 4.15.1.2-1).

Namazi et al. (2019) performed a heterogeneity analysis by study quality (Ottawa checklist with range 6 to 8 stars, with a cut-off at ≥ 7 stars) in five studies of fatty fish and lean fish. Heterogeneity was low in studies of fatty fish and lower than in studies of lean fish. Study quality did not explain the relatively high heterogeneity among studies on lean fish. The I^2 was close to 50% in both strata of study quality.

Schwingschackl et al. (2017) found statistically significant heterogeneity between subgroups of geographic location and length of follow-up. Studies from Asia and Australia showed a stronger protective association and no heterogeneity ($I^2=0$), European studies showed no significant association and heterogeneity was moderate ($I^2=48$), whereas studies from USA had stronger adverse association, but with $I^2=66$ (Table 4.15.1.2-1). There was no evidence for small study effects.

4.15.5 Dose-response relationships fish intake and T2D

Schwingschackl et al. (2017) found no departure from a linear dose-response association in the overall meta-analysis (RR = 1.09, 95% CI .93, 1.28), but a strong adverse association in the dose-response analysis of studies conducted in America (RR = 1.44, 95% CI = 1.19, 1.74), and a protective association in Asian studies (RR = 0.87, 95% CI = 0.80, 0.95). Pastorino et al. (2021) reported associations to be linear and presented risk estimates for 100 g/week higher fish intake.

Of the primary studies, Rylander et al. (2014) performed a dose-response modelling and reported significant protective association of T2D risk and intake of lean fish in the 75-100 g/day range. Zhang et al. (2019) also performed a dose-response modeling and found a bell-shaped curve showing significant non-linearity and indicating adverse effects at low to moderate total fish intake for both sexes. Du et al. (2020) performed a regression analysis of T2D risk vs. fish intake stratified by sex and region, resulting in a significant increase in T2D risk with fish intake in urban men. This association was not observed in urban women nor in rural participants.

4.15.6 Weight of evidence fish intake and T2D

In this section, the evidence for an association between fish intake and T2D is weighed according to the WCRF criteria presented in Chapter 3.1.6 (Box 2).

Published evidence of fish intake and T2D

The two literature-based meta-analyses of the association between risk of T2D and fish intake (Namazi et al., 2019; Schwingschackl et al., 2017) did not present strong evidence in either direction (Table 4.15.1.2-1). Schwingschackl et al., 2017 (16 studies) found no overall association with total fish intake, but high heterogeneity. After geographical stratification, there was a statistically significant adverse association in US cohorts (n=6), a significant protective association in Asian cohorts (n=3) and a non-significant association in European cohorts (n=6). Namazi et al., 2019 found a protective association with fatty fish intake (n=4), and but not lean fish (n=4) intake. Total fish was not presented.

However, in the federated meta-analysis by Pastorino et al. (2021), stronger conclusions were drawn. Using individual-level data of 956,122 adults with 48,084 confirmed T2D cases, a statistically significant, although very modest, adverse association between total, fatty and lean fish intake and T2D incidence was found in women, but not in men. After geographical stratification, cohorts of American women continued to display adverse associations, both for total fish and fatty fish, which were stronger at high intakes.

The summary relative risk (RR) based on the primary studies included by VKM on total fish intake and T2D similarly suggested no association, although the estimate was on the adverse side (RR=1.04, 95% CI: 0.96, 1.14) with high heterogeneity. Among the primary studies, both adverse associations (4 studies) and protective or borderline protective associations (3 studies) were reported, as well as no significant associations (13).

The summary RR of seven primary studies on fatty fish intake indicated a protective association (RR=0.88, 95% CI: 0.78, 0.99), with borderline significant heterogeneity. No significant association was found with lean fish intake and T2D, but here significant heterogeneity was found. Pastorino et al. (2021), on the other hand, reported adverse associations between both fatty and lean fish intake and T2D in women in their federated meta-analysis.

Two primary studies on fried fish intake both reported adverse associations, indicating that intake of fried fish is a risk factor for T2D. However, in the Zhang et al. (2019) study, adverse associations were found in the low-medium intake range, and the summary RR based on high intake, although on the adverse side, did not reveal a significant association (RR=1.10, 95% CI: 0.97, 1.25).

Taken together, also considering the latest data from the federated meta-analysis of Pastorino et al. (2021), evidence for a neutral association of fish intake and T2D is relatively strong, but subgroup analyses indicate that various types and patterns of fish intake (e.g.,

based on geographical stratification, lean vs. fatty fish, or fried vs. non-fried fish) could be linked to adverse rather than protective outcomes, especially in women.

Heterogeneity

Significant heterogeneity was observed between studies in the included meta-analyses and VKM's summary RR of studies on fish intake and T2D.

Mechanisms/biological plausibility

Several plausible mechanisms linking dietary intake of fish and both decreased and increased risk of T2D in humans exist.

Upgrading factors

No upgrading factors were identified.

4.15.6.1 *Conclusions weight of evidence fish intake and T2D*

There is evidence from more than two independent and good quality cohort studies on total fish intake (VKM included 14 publications on the general population and three previous meta-analyses, including dose-response meta-analyses).

VKM's summary RR for primary studies of T2D is not statistically significant for total fish but is on the adverse side for the highest versus lowest intake. Previous meta-analyses have reported overall summary associations for total fish intake that indicate either a neutral or modestly adverse effect on T2D, but with substantial heterogeneity. Summary associations for fatty fish have indicated a protective effect (in line with VKM's summary estimate) or adverse effect. The largest study to date, a federated meta-analysis of 28 cohorts and over 900 000 participants (Pastorini et al. 2021) reported significant effect modification by gender and a slight increased risk of T2D limited to women. The risk was slightly higher for intake of fatty fish than lean fish, but with heterogeneity between studies. Subgroup analyses by Pastorini et al. indicate that various types and patterns of fish intake (e.g. based on geographical stratification, lean vs. fatty fish or fried vs. non-fried fish) may be linked to the observed increased risk of T2D, especially in women. However, the reasons for the observed adverse associations are not clear and could relate both to dietary misreporting, dietary patterns, cooking methods, fish-associated contaminants as well as to heterogeneity of the disease itself. In conclusion, the evidence that consumption of fish in general, or of fatty or lean fish, is associated with risk of T2D is graded "limited, no conclusion" due to inconsistent results and currently unexplained heterogeneity.

4.16 Fish intake and rheumatoid arthritis

The current chapter summarizes the epidemiological evidence for associations between fish intake and the risk of rheumatoid arthritis (RA). RA is a chronic inflammatory joint disease that in Norway affects 0.5-1% of the population and has an annual incidence rate of approximately 25 per 100 000 individuals. In the included studies in this report, the disease outcome is incident RA verified in medical journals. In the diagnostics of RA, both blood samples and clinical examination of joints are used. Diagnosis including positive blood antibodies of RA is classified as seropositive RA, while diagnoses including negative blood antibodies of RA is classified as seronegative RA.

Mechanisms

Intake of fatty fish and fish oil supplements have been reported to improve the course of established RA with a mechanism related to the long-chain n-3 fatty acids (LC n-3 FA). These fatty acids are precursors of anti-inflammatory eicosanoids and thus may reduce inflammatory activity. LC n-3 FA may have protective biologic effects in the phases prior to the onset of clinically apparent RA. Mechanisms for LC n-3 FA are described in more detail in Chapter 5.2.

4.16.1 VKM's search for previous systematic reviews and meta-analyses of fish intake and rheumatoid arthritis

4.16.1.1 *Description of the identified publications*

VKM's search for systematic reviews and meta-analyses (see Chapter 3.1.2.2) identified three publications on the association between fish intake and RA that were assumed to fulfill the inclusion criteria and were read as full papers. Two of these papers were excluded, see Table 4.16.1.1-1 for reason for exclusions.

Table 4.16.1.1-1 Reasons for exclusion of identified papers from the search of systematic reviews and meta-analyses of fish intake and RA 2016-2021.

Included papers	Excluded paper and reasons for exclusions
Asoudeh et al., 2021	Focus on treatment of RA patients: Nelson et al., 2020 Philippou et al., 2020

The included systematic review and meta-analysis is described below; first the methods used and then the main results.

Asoudeh et al. (2021) conducted a literature search in MEDLINE, Scopus and Embase up to October 2020 to review the evidence on the relationship of animal protein sources and risk of rheumatoid arthritis. Risk of bias in the eligible studies was assessed with use of ROBINS-E tool for non-randomized studies (Bero et al., 2018). The included papers on the association between intake of fish and risk of rheumatoid arthritis were cohort studies (n=5) and case-

control studies (n=5). None of the studies were found to have no risk of bias, while six had moderate and four had high risk of bias. Fish intake in the lowest intake groups ranged from 0 and 10 g/day and in the groups with the highest intake between 33 and 67.85 g/day. The outcome in all studies was a RA diagnosis. The risk of RA was compared between the high and low intake groups, as well as in a dose-response analysis per 100g/d increment in fish intake. The main meta-analysis was conducted for all papers together, and sub-analyses were conducted for cohort and case-control studies separately.

The meta-analysis included all the seven primary studies included in the present summary (Benito-Garcia et al., 2017; Di Giuseppe et al., 2013; Linos et al., 1999; Pedersen et al., 2005; Shapiro et al., 1996; Sparks et al., 2019, Nguyen et al., 2021), and two additionally case-control studies by Roswell et al. (2009) and Linos et al. (1991).

4.16.1.2 *Results from the meta-analysis*

Below is a summary table for fish intake in the included meta-analysis (Asoudeh et al. 2021).

Table 4.16.1.2-1 Results in meta-analysis on fish intake and risk of rheumatoid arthritis (RA) by Asoudeh et al. (2021).

Author, year	Type of studies included	Total no. of studies	No. of cases	Comparison	Summary RR (95 % CI)	Heterogeneity	Overall conclusion
Asoudeh, 2021	Cohort and case-control	10	5874	High vs low intake of fish	0.89 (0.80 to 0.99)	$I^2=0.0\%$, $P=0.71$	Sig. protective assoc. for intake of fish and risk of RA Sig. non-linear dose-response (P -non-linearity=0.04); lower risk of developing RA for fish intake up to 25 g/d
		10	5874	Per 100 g/d increment in fish intake	0.85 (0.73 to 0.98),	$I^2=0.0\%$, $P=0.50$	
	Cohort studies	5	2380	High vs low intake of fish	0.93 (0.82 to 1.05)	$I^2=0.0\%$, $P=0.75$	No sig. assoc. between intake of fish and risk of RA
		5	2380	Per 100 g/d increment in fish intake	0.92 (0.74 to 1.14)	$I^2=0.0\%$, $P=0.55$	No sig. assoc. or linear trend for intake of fish and risk of RA
	Case-control studies	5	3494	High vs low intake of fish	0.82 (0.68 to 0.99)	$I^2=0.0\%$, $P=0.51$	Sig. protective assoc. for intake of fish and risk of RA
		5	3494	Per 100 g/d increment in fish intake	0.79 (0.64 to 0.96)	$I^2=4.5\%$, $P=0.38$	Sig. protective assoc. and liner trend for intake of fish and risk of RA

The meta-analysis by Asoudeh et al. (2021) showed a significant protective association between fish intake and the risk of RA in all studies combined (cohort and case-control studies). The relative risk of RA in the high vs. low fish intake group was 0.89 (95% CI, 0.80 to 0.99). There was no significant heterogeneity between the studies. However, in analyses stratified by study-design, the protective association was stronger and only statistically significant in case-control studies (n=5 studies) compared with the cohort studies (n=5 studies). In linear dose-response meta-analysis (all studies), a 100 g/day increment in fish intake was significantly associated with a lower relative risk of RA (RR 0.85, 95% CI 0.73 to 0.98). A non-linear meta-analysis showed a U-shaped association between fish intake and risk of RA, with the lowest risk approximately at 25 g/day intake).

4.16.2 VKM's systematic review of primary studies on fish intake and rheumatoid arthritis

4.16.2.1 *Included studies from search*

VKM evaluated seven publications graded A or B with RA incidence as outcome (Benito-Garcia et al., 2017; Di Giuseppe et al., 2013; Linos et al., 1999; Ngyuen et al., 2021; Pedersen et al., 2005; Shapiro et al., 1996; Sparks et al., 2019).

There were overlapping publications were from the same study and one was excluded (Benito-Garcia et al. (2017) as described below, leaving six for further analysis.

A description of the studies (study name, design, time period, size and age of the study population, and dietary assessment method) can be found in Table 4.16.2.1-1.

Table 4.16.2.1-1 Overview of primary studies included in the weight of evidence analysis of fish intake and rheumatoid arthritis.

Author, year, country	Study name	Study design	Inclusion year(s), end, follow-up time	Study size, age	Dietary assessment method	Dietary assessment period
Di Giuseppe, 2013, Sweden	Swedish Mammography Cohort (SMC)	Prospective cohort	2003 (baseline for analysis) to 2010, 7.5 yrs follow-up (mean)	32 232 women, 54–89 yrs	Repeated FFQ (1987, 1997)	Average intake during previous yr
Linou, 1999, Greece		Case-control	Inclusion not stated, cases and control recruited at the same time	145 cases /188 controls, 18-80 yrs (mean 49.2 yrs)	An interviewer-administered, detailed, validated questionnaire	From childhood until their current disease was diagnosed (RA in cases or the disease that brought the controls to the hospital)
Nguyen, 2021, France	E3N-EPIC	Prospective cohort	Inclusion 1990, baseline 1993-5, end of follow-up 2014, mean follow-up time until diagnosis 11.7y	98,995 women, 52.5 yrs (mean)	FFQ	Average intake during previous yr
Pedersen, 2005, Denmark	Diet, Cancer and Health cohort	Prospective cohort	Inclusion 1993-7, end of follow-up 2001, mean 5.3 years (range < 1 mo to 7.7 yrs)	57 053 individuals, 50-64 yrs	FFQ	Previous yr
Shapiro, 1996, USA		Case-control	Inclusion 1986-91	324 cases/1245 controls, 18-64 yrs	FFQ	A 1-yr period 5 years before inclusion in the study
Sparks, 2019, USA	NHS and NHS II	Prospective cohorts	Inclusion 1976 (NHS) and 1989 (NHS II), baseline 1984 and 1991, end 2014 and 2015, respectively. A total of 3,863,909 person-years of follow-up	166,013 female nurses, 30–55 and 25-42 yrs at enrolment in NHS and NHS II, respectively	Repeated FFQ	Previous yr
Excluded due to overlap						
Benito-Garcia, 2007, USA	Nurses' Health Study (NHS)	Prospective cohort, occupational	1980 (baseline for analysis) to 2002, 22 yrs follow-up	82,063 female nurses, 30–55 yrs (at enrolment in 1976)	Repeated FFQ (1980, 1984, 1986, 1990, 1994, and 1998), semi-quant	NA. Described in other NHS publications as average intake during previous yr

4.16.2.2 *Overlapping publications*

Two publications used data from the Nurses' Health Study (NHS) I (Benito-Garcia et al. 2017; Sparks et al. 2019). Benito-Garcia et al. (2017) used total incident RA as the outcome, while Sparks et al. (2019) additionally stratified the outcome by seropositive and –negative RA and by age of RA onset ≤ 55 years and > 55 years. Sparks et al. 2019 also included a population from NHS II and therefore had more cases in addition to more detailed results and was kept for further analysis.

4.16.2.3 *Studies by design and geographic region*

Of the six publications (excluding one overlapping), two were from the USA (Shapiro et al., 1996; Sparks et al., 2019), one from Sweden (Di Giuseppe et al., 2013), one from Denmark (Pedersen et al. 2005), one from Greece (Linos et al., 1999) and one from France (Nguyen et al., 2021). Four of the studies were prospective cohort studies (Di Giuseppe et al., 2013; Pedersen et al., 2005; Sparks et al., 2019; Nguyen et al., 2021) of which one was in an occupational group (female nurses) (Sparks et al., 2019) and three were in population-based cohorts (Di Giuseppe et al., 2013; Pedersen et al., 2005; Nguyen et al., 2021). Two of the studies had a case-control design; one with hospital-based controls (Linos et al., 1999) and one with population-based controls (Shapiro et al., 1996).

4.16.2.4 *Studies by sex, potential effect modification and other sub-groups*

Five of the studies included only women (Benito-Garcia et al., 2017; Di Giuseppe et al., 2013; Shapiro et al., 1996; Sparks et al., 2019; Nguyen et al., 2021), while two (one prospective cohort and one case-control study) included both men and women (Linos et al., 1999; Pedersen et al., 2005). None of the studies examined sex differences. Except from Sparks et al. (2019), studies examined total RA incidence as the outcome. Sparks et al. (2019) reported results stratified by outcome groups seropositive and –negative RA, and further by age of RA onset ≤ 55 years and > 55 years.

4.16.2.5 *Studies by fish exposure*

All studies included total fish intake (either sum of all fish, unspecified fish or fish including shellfish and/or fish products). The Danish study (Pedersen et al., 2005) presented additionally sub-classification of fish intake by fat content; fatty, medium fat, and lean fish (Pedersen et al., 2005). The US case-control study (Shapiro et al., 1996) additionally classified results by intake of 1) fried fish or fish sandwich, 2) tuna, tuna salad or casserole and 3) broiled or baked fish (Shapiro et al., 1996).

4.16.2.6 *Studies assessing potential non-linearity*

None of the primary studies presented a non-linear dose-response analysis.

4.16.3 Results from the included primary studies on fish intake and rheumatoid arthritis

4.16.3.1 Studies of total fish intake and rheumatoid arthritis

We included six publications with seven estimates of the association between total fish intake and RA in the weight of evidence analysis. The exposure levels and results (high-low relative risk and overall) are included in Table 4.16.3.1-1.

Table 4.16.3.1-1 Results from studies included in the weight of evidence analysis of total fish intake and rheumatoid arthritis (RA) risk in the general population.

Author, year, country	Study design	Fish exposure, sex	Intake unit	High-low intake	Total cases	HR/OR high low or continuous (95 % CI)	Overall result
Di Giuseppe, 2013, Sweden	Prospective cohort	Fish, W	Servings/wk, binary	≥1 vs <1 serving/wk	205	HR=0.71 (0.48, 1.04)	Borderline protective assoc.
Linos, 1999, Greece	Case-control	Fish, M/W	Servings/mo, quartiles	10 vs 3 servings/mo	145	OR=0.95 (0.46, 1.96)	No sig. assoc., <i>P</i> -trend 0.65
Nguyen 2021	Prospective cohort	Fish, W	g/d, 3 cat	>25g/d vs <9 g/d	480	HR=0.99 (0.81, 1.22)	No sig. assoc.
Pedersen, 2005, Denmark	Prospective cohort	Fish, M/W	g/d, continuous	per 30 g/d increase	69	HR=0.91 (0.68, 1.23)	No sig. assoc., <i>P</i> -trend 0.55
Shapiro, 1996, USA	Case-control	Fish, incl. shellfish, W	Servings/wk, 3 cat	≥2 vs <1 serving/wk	324	OR=0.92 (0.67, 1.25)	No sig. assoc.
Sparks 2019, USA	Prospective cohort	Fish, incl. shellfish, W	Servings/mo or wk, 5 cat	≥4 servings/wk vs <1 serving/mo	1080	HR=0.93 (0.67, 1.28)	No sig. assoc., <i>P</i> -trend 0.42

There were no significant associations for total fish intake and RA risk, except in one study. Di Giuseppe et al. (2013) observed a borderline protective association for total fish intake (≥1 vs. <1 serving/week) in women (HR 0.71, 95% CI 0.48, 1.04).

4.16.3.2 Studies of intake of fatty and lean fish and rheumatoid arthritis

In two of the studies, fish intake was divided according to fat content (Pedersen et al., 2005) or fish type and preparation method (Shapiro et al., 1996). The exposure levels and results (high-low relative risk and overall) are included in Table 4.16.3.2-1.

Table 4.16.3.2-1 Results from studies included in the weight of evidence analysis of fatty and lean fish intake and rheumatoid arthritis (RA) risk in the general population.

Author, year, country	Study design	Fish exposure, sex	Intake unit	High-low intake	Total cases	HR/OR* high low or continuous (95 % CI)	Overall result
Fatty fish							
Pedersen, 2005, Denmark	Prospective cohort	Fatty fish, M/W	g/d, continuous	Per 30g/d increase	69	HR=0.51 (0.25, 1.03)	Borderline protective assoc., <i>P</i> -trend 0.06
Medium fat fish							
Pedersen, 2005, Denmark	Prospective cohort	Medium fat fish, M/W	g/d, continuous	Per 30g/d increase	69	HR=2.74 (1.39, 5.42)	Adverse assoc., <i>P</i> -trend 0.004
Lean fish							
Pedersen, 2005, Denmark	Prospective cohort	Lean fish, M/W	g/d, continuous	Per 30g/d increase	69	HR=0.83 (0.47, 1.46)	No sig. assoc., <i>P</i> -trend 0.52
Fried fish or fish sandwich							
Shapiro, 1996, USA	Case-control	Fried fish or fish sandwich, W	Servings/wk, 3 cat	≥2 vs <1 serving/wk	324	OR=1.00 (0.55, 1.82)	No sig. assoc.
Tuna, tuna salad or casserole							
Shapiro, 1996, USA	Case-control	Tuna, tuna salad or casserole, W	Servings/wk. 3 cat	≥2 vs <1 serving/wk	324	OR=1.19 (0.83, 1.72)	No sig. assoc.
Broiled or baked fish							
Shapiro, 1996, USA	Case-control	Broiled or baked fish, W	Servings/wk, 3 cat	≥2 vs <1 serving/wk	324	OR=0.57 (0.35, 0.93)	Protective assoc.

While intake of fatty fish was associated with a lower risk of RA (HR= 0.51, 95% CI 0.25, 1.03 per 30 g/day increase, P -trend 0.06) in the prospective cohort study of Pedersen et al. (2005), intake of medium fat fish in the same study was associated with increased risk of RA (HR= 2.74, 95% CI 1.39, 5.42 per 30 g/day increase, P -trend 0.004). In the case-control study of Shapiro et al. (1996), a protective association was observed between intake of broiled or baked fish and risk of RA (OR 0.57, 95% CI 0.35, 0.93 for ≥ 2 vs < 1 serving/week). No other significant associations for sub-types of fish with risk of RA were observed.

4.16.3.3 *Summary relative risk (RR) based on VKM's inclusion of primary studies*

The high-low summary RR calculated by VKM included three cohort studies (Benito-Garcia et al., 2017; Di Giuseppe et al., 2013; Sparks et al., 2019) and indicated no statistically significant association (RR= 0.91, 95% CI: 0.77, 1.09, $P_{\text{heterogeneity}}=0.32$). The estimate was similar to the result for cohort studies in the meta-analysis by Asoudeh et al. (2021) (RR= 0.93, 95% CI: 0.82, 1.05, $I^2=0.0\%$, $P_{\text{heterogeneity}}=0.75$).

4.16.3.4 *Heterogeneity fish intake and rheumatoid arthritis*

In the previous meta-analysis by Asoudeh et al. 2021 (10 included studies, 5 cohorts and 5 with a case-control design) there was no statistically significant heterogeneity ($I^2=0.0\%$, $P_{\text{heterogeneity}}=0.71$), or between the three studies in VKMs summary RR ($P_{\text{heterogeneity}}=0.32$). Associations were null or on the protective side but appeared to be of larger magnitude in case-control studies.

4.16.4 Dose-response relationship fish intake and rheumatoid arthritis

Asoudeh et al. 2021 performed a linear and non-linear meta dose-response analysis and reported a statistically significant linear trend, but also with significant departure from linearity ($P_{\text{non-linearity}}=0.04$). The lower risk of developing RA was seen for fish intake up to 25 g/d. When limited to cohort studies, there was no statistically significant association or linear trend.

4.16.5 Weight of evidence for fish intake and rheumatoid arthritis

Published evidence of fish intake and RA

One systematic review and meta-analysis of the association between fish intake and risk of RA was included. The meta-analysis (five cohort and five case control studies) showed a significant 11% lower risk of RA among those with high vs. low intake of fish, but not when limited to cohort studies. Case-control studies showed stronger associations. VKM included

seven studies (five cohort studies and two case-control studies). The summary RR (three cohort studies) showed no statistically significant association or heterogeneity.

Heterogeneity

No statistically significant heterogeneity was observed between studies included in one previous meta-analysis, or the summary RR calculated by VKM.

Mechanisms/biological plausibility

Plausible mechanisms have been presented previously in section on Mechanisms.

Upgrading factors

Evidence of dose-response was not found to be an upgrading factor in this case. No other upgrading factors were evaluated.

4.16.5.1 *Conclusion weight of evidence fish intake and RA*

There is evidence from more than two independent and good quality cohort studies on total fish intake (VKM identified six studies, and one meta-analysis including a dose-response analysis). There is evidence from the meta-analysis that total fish intake is associated with reduced risk of RA. This conclusion is based on studies with a case-control or cohort design, but the result is not statistically significant for cohort studies only. Similarly, VKM's summary RR for cohort studies is not statistically significant. Evidence was too limited to conclude on the association between intake of sub-types of fish and RA risk or on the intake of fish and sub-types of RA. In conclusion, the evidence is graded "limited, suggestive" for a protective effect of fish consumption on the risk of RA.

4.17 Introduction to fish intake and anthropometric measures in children and adults

This chapter is an introduction to the weight of evidence analysis chapters for the included anthropometric outcomes related to weight changes in children and adults (Chapters 4.18-4.20).

Overview of studies summarized according to anthropometric outcomes

These chapters (Chapters 4.18-4.20) review a sparse number of papers on heterogeneous outcomes related to weight, weight change, growth, and other anthropometric measures, both among adults and children, and related to own and maternal fish consumption. Due to the low number of papers, and the disparate ways to express the outcomes, a detailed comparison of studies is difficult. The anthropometric measures include not only weight or body mass index (BMI) per se, but also measures of circumferences (waist, hip), and skinfold thickness. As the protocol specifies the general healthy population, papers on dieting or on energy restricted diets that included fish, have been excluded. Some of the studies also include other outcomes such as metabolic factors (blood pressure, blood glucose parameters, blood lipids), risk scores combining metabolic and anthropometric factors, or adipokines, but these were considered more intermediate endpoints and not included. Studies with birth weight as an outcome can be found in Chapter 4.22-4.27. All the included studies are prospective cohort studies. An overview of the population groups and outcomes in the included studies can be found in Figure 4.17-1. These chapters include results from analyses based on subjectively (self-or parent-reported) and objectively reported (measured) anthropometric measures.

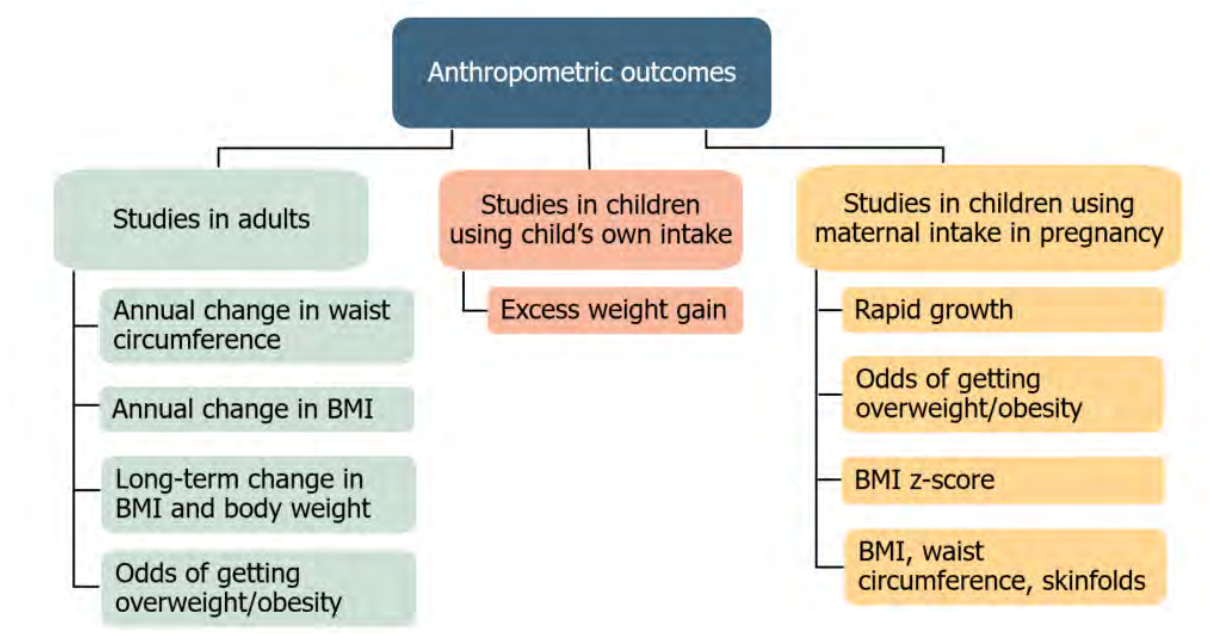


Figure 4.17-1 Overview of the included studies and their major endpoints. BMI=body mass index.

Mechanisms

There are several hypotheses on how fish consumption may prevent obesity, but none of them are backed by strong evidence. Most of the theories are related to the high content of LC n-3 FA in fish, see Chapter 5.2 for more details about mechanisms for LC n-3 FA.

4.18 Fish intake and body weight in adults

4.18.1 VKM's search for published systematic reviews and meta-analyses on fish intake and body weight

4.18.1.1 *Description of the identified publications*

VKM's search for systematic reviews and meta-analyses (see Chapter 3.1.2.2) identified one publication on the association between fish intake and body weight that fulfilled the inclusion criteria and were included.

In this paper, fish was one of the food groups covered in a broader systematic review and meta-analysis on food groups and risk of overweight, obesity, and weight gain in adults (Schlesinger et al., 2019). For overweight/obesity, and weight gain, the quality of evidence was very low, as assessed by NutriGrade, and for abdominal obesity, the quality of the evidence was graded low (Schlesinger et al., 2019; Schwingshackl et al., 2016).

4.18.1.2 *Results from the meta-analysis*

The review found one study on risk of getting overweight/obesity that showed no association with fish intake (Jakobsen et al., 2013), and two studies on abdominal obesity (measured by waist circumference) that found a protective association with fish intake, summarized in Table 4.18.1.2-1 (Schlesinger et al., 2019).

Table 4.18.1.2-1 Summary of results from the systematic review and meta-analysis on total fish intake and overweight, obesity, and weight gain in adults.

Author, year	Type of studies included	No of studies	No of cases	Comparison	Summary RR (95 % CI)	Heterogeneity	Overall
Schlesinger et al., 2019	Prospective cohort studies on abdominal obesity in adults ¹	2	2 364	Highest vs lowest	0.75 (0.62, 0.89)	0%	Higher total fish intake is related to sig. reduced abdominal obesity
				Per 100 g/day	0.83 (0.71, 0.97)		

¹Only the results for abdominal adiposity are shown since that outcome was the only outcome covered in more than one study.

4.18.2 VKM's systematic review of primary studies on fish intake and body weight in adults

4.18.2.1 *Included studies from search*

We evaluated four publications graded A or B with body weight measures in adults as outcome (Figure 4.17-1) (Huang et al., 2019a; Huang et al., 2019b; Jakobsen et al., 2013; Jakobsen et al., 2012).

The two studies by Huang both looked at genotypes and genes associated with weight gain (Huang et al., 2019a; Huang et al., 2019b), and one was excluded as it did not provide estimates for the fish intake per se, but how fish intake attenuated the association between genes related to obesity and long-term weight gain (Huang et al., 2019b).

A description of the studies (study name, design, time period, size and age of the study population, and dietary assessment method) can be found in Table 4.18.2.1-1.

Table 4.18.2.1-1 Overview of primary studies included in weight of evidence analysis of fish intake and body weight in adults.

Author, year	Study name	Design	Inclusion year(s), end, follow-up time	Study size, participant age	Dietary assessment method	Dietary assessment period
Huang, 2019a	Nurses' Health Study (NHS) and the Health Professionals Follow-up Study (HPFS), replication in the Women's Health Initiative (WHI) and the Singapore Chinese Health Study (SCHS)	Prospective cohort studies	1990 (baseline) with 10 yrs follow-up (NHS, HPFS), 1994-1998 (baseline) with 6 yrs follow-up (WHI), and 1998 (baseline) to 2004 (SCHS)	11 323 female nurses and 6833 male health professionals with genetic data, replication in 6254 postmenopausal women (WHI) and 5264 Chinese Singaporeans. Mean age 57 yrs (NHS, HPFS), 68 yrs (WHI), or 56 yrs (SCHS)	FFQ	Not stated
Jakobsen, 2013	European Prospective Investigation into Cancer and Nutrition (EPIC)/PANACEA project	Prospective cohort studies	Enrolment took place between 1992 and 2000, follow-up information on weight was collected 2.1–10.2 years (median 5.0) after enrolment	344 757 (final population, total fish) adults, women and men. Median age 51 yrs in females, 53 yrs in males	Mainly sub-cohort specific FFQ, some interview-based, some combinations of questionnaires and food diaries	Generally last year/usual intake
Jakobsen, 2012	European Prospective Investigation into Cancer and Nutrition (EPIC)/DiOGenes project	Prospective cohort studies	Enrolment took place between 1993 and 1998, Follow-up information on waist circumference was collected 3.7–9.9 years (median 5.5 years) after enrolment	146 543 adults, women and men. Median age 54 yrs in both genders	Country-specific self-administered FFQ	Previous year

4.18.2.2 *Overlapping publications*

There were two publications from the EPIC study (the two papers from Jakobsen), but they had different outcomes (body weight and waist circumference) and were thus both included in the evaluation.

4.18.2.3 *Studies by design and geographic region*

All studies were prospective cohort studies, the publications from EPIC are based on up to ten European countries, the publication from Huang is mostly based on US participants, but the replication of some of the genetic work is partly done in a study of Singapore Chinese, and partly done in a study with American participants.

4.18.2.4 *Studies by sex, potential effect modification, and other sub-groups*

Huang et al. (2019a) presented results on fish intake and BMI stratified by carriers and non-carriers of the T-allele in SNPs near the fatty acid desaturase gene (FADS) cluster (T-carrying is common in Inuit, rare in Europeans and Asians) (Huang et al., 2019a). Jakobsen et al. (2013) presented weight gain among men and women separately, although the gender difference seems not to have been formally tested (Jakobsen et al., 2013).

4.18.2.5 *Studies by fish exposure*

All three studies assessed total fish. Both the papers from Jakobsen additionally assessed lean and fatty fish intake in sub-groups (Huang et al., 2019a; Jakobsen et al., 2013; Jakobsen et al., 2012).

4.18.2.6 *Studies assessing potential non-linearity*

None of the included studies reported a dose-response relationship.

4.18.3 Results from the included primary studies on fish intake and body weight in adults

4.18.3.1 *Studies of total fish intake and body weight in the general adult population*

We included three publications with seven estimates of the association between total fish intake and body weight in adults in the weight of evidence analysis. The exposure levels and results (high-low relative risk, and overall) are included in Table 4.18.3.1-1.

Table 4.18.3.1-1 Results from studies included in the weight of evidence analysis of total fish intake and body weight in adults in the general population.

Author, year, country	Study design	Fish exposure, sex	Intake unit	High-low intake	Total cases	Effect measure with estimate of precision (95% CI or +/- SE)	Overall result
Huang, 2019a, US/Singapore	Prospective cohort study	Total fish, M/W	Serving/d	≥1/d vs ≤1/wk	29 674	Mean 10-yrs change in BMI units for intake ≥1/d vs ≤1/wk (pooled, 2 main and 2 replication cohorts) was 1.11 kg/m ² +/-0.16 vs 0.38 kg/m ² +/-0.07 (T carriers) and 0.81 kg/m ² +/-0.08 vs 0.50 kg/m ² +/-0.03 (non-carriers)	Frequent fish eaters increase their BMI more than infrequent fish eaters; association much more pronounced in T-carriers than non-T carriers (non-T carriers more frequent among Europeans)
	Prospective cohort study	Total fish, M/W		Per serving	18 156	Mean 10-yrs change in BMI units per serving/day (pooled NHS & HPFS cohorts) was $\beta \pm SE = 0.64 \text{ kg/m}^2 \pm 0.16$ (T-carriers) and $\beta \pm SE = 0.18 \text{ kg/m}^2 \pm 0.08$ (non-T carriers)	Frequent fish eaters increase their BMI more than infrequent fish eaters; association much more pronounced in T-carriers than non-T carriers (non-T carriers more frequent among Europeans)
Jakobsen, 2013, 10 European countries	Prospective cohort study	Total fish, W	g/d	Continuous per 10 g/day	249 558	Annual weight change was 5.70 g/10 g higher total fish consumption per d (4.35, 7.06)	Fish consumption has no appreciable association with body weight gain
		Total fish, M	g/d	Continuous per 10 g/day	95 199	Annual weight change per 10 g higher total fish consumption per d: $\beta = -1.81$ (-3.96, 0.33)	No significant association
		Total fish, W	g/d	Continuous per 10 g/day	150 808	OR=1.02 (1.01, 1.02) for getting overweight	Slightly higher odds of getting overweight/obesity with higher fish consumption
		Total fish, M	g/d	Continuous per 10 g/day	33 663	OR=0.99 (0.98-1.00) for getting overweight	No significant association between fish consumption and odds for getting overweight/obesity
Jakobsen 2012, 5 European countries	Prospective cohort study	Total fish, M/W	g/d	Continuous per 10 g/day	89 432	Association between fish consumption and 1-yr change in waist circumference $\beta = -0.01$ (-0.01, 0.00)	No association between fish intake and waist circumference

4.18.3.2 Studies of fatty and lean fish intake and body weight in the general adult population

There were two publications with five estimates for lean and fatty fish consumption (Tables 4.18.3.2-1 and 4.18.3.2-2).

Table 4.18.3.2-1 Results from studies included in the weight of evidence analysis of lean fish intake and body weight in adults in the general population.

Author, year, country	Study design	Fish exposure, sex	Intake unit	High-low intake	Total cases	Effect measure with estimate of precision (95% CI)	Overall result
Jakobsen, 2013, ten European countries	Prospective cohort study	Lean fish, W	g/d	Continuous per 10 g/day	222 609	2.23 g higher annual weight gain/10 g higher lean fish consumption per d (0.16, 4.31)	Slightly higher annual weight gain with higher lean fish consumption
		Lean fish, M	g/d	Continuous per 10 g/day	77 219	No difference in annual weight gain β -coefficient -0.73 (-4.17, 2.71)	No significant difference in annual weight change
		Lean fish, W	g/d	Continuous per 10 g/day	137 457	OR for getting obesity/overweight =1.01/10 g higher lean fish consumption per d (1.00, 1.02)	Slightly higher odds of getting overweight/obese with higher lean fish consumption
		Lean fish, M	g/d	Continuous per 10 g/day	28 296	OR for getting obesity/overweight =0.98 (0.96, 1.00) per 10 g higher lean fish consumption per day	No significant association between lean fish consumption and odds for getting overweight/obese
Jakobsen 2012, five European countries	Prospective cohort study	Lean fish, M/W	g/d	Continuous per 10 g/day	73 125	Average annual waist circumference change per 10 g higher lean fish consumption β -coefficient =0.00 (-0.01, 0.00)	No association between lean fish intake and waist circumference

Table 4.18.3.2-2 Results from studies included in the weight of evidence analysis of fatty fish intake and body weight in adults in the general population.

Author, year, country	Study design	Fish exposure, sex	Intake unit	High-low intake	Total cases	Risk estimate with estimate of precision (95% CI)	Overall result
Jakobsen, 2013, 10 European countries	Prospective cohort study	Fatty fish, W	g/d	Continuous per 10 g/day	222 609	Annual weight change 11.12 g higher/10 g higher fatty fish consumption per d (8.17, 14.08)	Slightly higher weight gain with higher fatty fish consumption
		Fatty fish, M	g/d	Continuous per 10 g/day	77 219	β -coefficient for annual weight change -0.25 (-4.10, 3.60)	No significant difference
		Fatty fish, W	g/d	Continuous per 10 g/day	137 457	OR for getting overweight/obesity = 1.02/10 g higher fatty fish consumption per d (1.01, 1.04)	Slightly higher odds of getting overweight/obese with higher fatty fish consumption
		Fatty fish, M	g/d	Continuous per 10 g/day	28 296	OR for getting overweight/obesity = 1.00 (0.97, 1.02) per 10 g higher fatty fish consumption per day	No significant association
Jakobsen 2012, 5 European countries	Prospective cohort study	Fatty fish, M/W	g/d	Continuous per 10 g/day	73 125	β -coefficient for change in waist circumference -0.01 (-0.02, -0.01)	Weak negative association between fatty fish consumption and subsequent change in waist circumference

4.18.3.3 *Summary relative risks (RR) based on VKM's inclusion of primary studies*

Due to few studies and heterogenous presentation of results, no summary RR was calculated based on the included primary studies.

4.18.3.4 *VKM's search compared to previous meta-analyses on adult body weight*

The systematic review by Schlesinger et al. (2019) identified four papers, three of which were not identified by VKM's search (Jakobsen et al., 2013; Baik, Abbott, Curb, & Shin, 2010; Y. S. Kim et al., 2016; Schulz et al., 2002). There was no association in the study looking at food groups, with separate estimates for fish intake and weight gain (Schulz et al., 2002). This paper is based on one of the EPIC sub-cohorts and would have been captured by Jakobsen et al. (2013). Baik et al. (2010), and Kim et al. (2016) had metabolic syndrome as main outcome and was therefore excluded during VKM's screening process. VKM additionally identified two papers by Huang published after the systematic review (Huang et al., 2019a; Huang et al., 2019b). As described above, only one of them was kept.

Based on VKM's search, which only identified one paper for abdominal obesity (Jakobsen et al., 2012), there was no association between fish intake and abdominal obesity, while Schlesinger found a protective association. However, the number of participants in the study by Jakobsen et al. (2012) is 30 times larger than the number of participants in the studies included in Schlesinger combined (Schlesinger et al., 2019). Including the two small studies identified from the review would not have changed VKM's conclusion.

4.18.4 Heterogeneity fish intake and body weight in adults

Not assessed due to the low number of papers and variable ways to express the results.

4.18.5 Dose-response relationship fish intake and body weight in adults

Schlesinger et al. (2019) performed a meta dose-response analysis for abdominal obesity, based on two studies and reported that abdominal obesity was significantly reduced with higher fish consumption (Table 4.18.1.2-1).

4.18.6 Weight of evidence for fish intake and adult body weight

In this section, the evidence of the association between fish intake and body weight in adults is weighed according to the WCRF criteria presented in Chapter 3.1.6 (Box 2). The following criteria are used; the published evidence of fish intake and some mechanical evidence.

Published evidence of fish intake and body weight in adults

There are few studies, they do not report the same endpoints, and the results show weak or no associations between fish consumption and weight gain, general or abdominal obesity. However, these studies included a large number of participants.

Heterogeneity

Not assessed.

Mechanisms/biological plausibility

Several hypotheses regarding fish intake and body weight (anthropometry) exist, but they are not backed by solid mechanistic evidence.

Upgrading factors

No upgrading factors were evaluated.

4.18.6.1 *Conclusion weight of evidence fish intake and body weight in adults*

Due to the low number of papers, and disparate ways to measure the outcome, the current body of evidence for an association between fish intake and adult body weight is graded "limited, no conclusion".

4.19 Fish intake and body weight/anthropometric outcomes in children

4.19.1 VKM's search for published systematic reviews and meta-analyses on fish intake and body weight in children, based on their own fish intake

VKM's search for systematic reviews and meta-analyses (see Chapter 3.1.2.2) identified no publications with data on fish intake and body weight in children, measured by the children's own fish intake.

4.19.2 VKM's systematic review of primary studies on fish and body weight in children, based on their own fish intake

4.19.2.1 *Included studies from search on body weight in children*

We evaluated one publication graded A with body weight measures in children as outcome (Dong et al., 2015). A description of the study (study name, design, time period, size and age of the study population, and dietary assessment method) can be found in Table 4.19.2.1-1.

Table 4.19.2.1-1 Overview of primary study included in weight of evidence analysis of fish intake and childhood/adolescent body weight.

Author, year, country	Study name	Design	Inclusion year(s), end, follow-up time	Study size, participant age	Dietary assessment method	Dietary assessment period
Dong, 2015, UK	Avon Longitudinal Study of Parents and Children in the United Kingdom (ALSPAC)	Birth cohort	Children born 1991-1992, follow-up at ages 7, 10, 13 yrs	4 646 boys and girls, 7-13 yrs	3-day food diary, parent assisted, repeated at ages 7, 10, 13 yrs Standard serving sizes	3 days

4.19.2.2 *Studies by design and geographic region*

This ALSPAC study is a birth cohort from the area around Bristol in the UK.

4.19.2.3 *Studies by sex, potential effect modification, and other sub-groups*

Main analyses were presented for boys and girls of all ages together. Separate analysis for boys and girls were considered exploratory and not extracted (Dong et al., 2015). The study used repeated measurements in childhood to early adolescence.

4.19.2.4 *Studies by fish exposure*

Results were presented for coated (breaded or battered) fish (coated and fried white fish and shellfish – e.g., fish and chips) and uncoated fish (white fish and shellfish without coating, oily fish). Uncoated fish was defined as fish that was neither breaded nor battered.

4.19.2.5 *Studies assessing potential non-linearity*

Only linear models were used.

4.19.3 Results from the included primary studies of fish intake and body weight in children using children's own intake

4.19.3.1 *Studies of coated (breaded or battered) and uncoated fish intake and body weight in children*

We included one publication with two estimates of the association between coated and uncoated fish intake and body weight in children, based on children's own intake in the weight of evidence analysis. The exposure levels and results (high-low relative risk, and overall) are included in Table 4.19.3.1-1.

Table 4.19.3.1-1 Results from study included in the weight of evidence analysis of coated and uncoated¹ fish intake and body weight in children using the children's own intake.

Author, year, country	Study design	Fish exposure, sex ²	Intake unit	High-low intake	Total cases	Effect measure with estimate of precision ²	Overall result
Dong, 2015, UK	Birth cohort	Coated fish, boys/girls	g/day	100 g increase in daily intake	4 646	290 g excess weight gain (over 3 yrs) per 100 g/d increase in intake ($P<0.05$, no CI provided)	Sig. association ($P<0.05$) of higher coated fish intake with excess weight gain
		Un-coated fish, boys/girls	g/day	100 g increase in daily intake	4 646	140 g excess weight gain (over 3 yrs) per 100 g/d increase in intake (non-sig, no CI provided)	Non-sig. association of higher uncoated fish intake with excess weight gain

¹Coated fish is deep-fried (typically fish and chips) fish. ²Delta estimates from multivariate linear regression with repeated measurement where change in consumption of other foods and beverages in the period was accounted for.

The repeated measurements were analysed with two different models, the change-change model that is presented in Table 4.19.3.1-1, and the change-level model. The conclusions were the same, but the association was stronger with the change-level model (665 g excess weight gain over 3 years per 100 g/day higher daily intake of coated fish, no significant results for uncoated fish) (Dong et al., 2015). The change-change model is more commonly reported in the literature and is therefore presented here. The change-change model considers dietary change independent of the underlying level of consumption, while the change-level model considers the underlying intake levels of the food, food group or nutrient of interest. (Dong et al., 2015).

4.19.3.2 *Conclusion weight of evidence fish intake and body weight in children using children's own intake*

Due to only one paper, the weight of evidence conclusion is "limited, no conclusion". However, this paper highlights the importance of preparation methods, and that not only the fish itself, but also how it is being processed or cooked might impact the association with health.

4.20 Maternal fish intake and body weight/anthropometric outcomes in children

4.20.1 VKM's search for published systematic reviews and meta-analyses on maternal intake and body weight in children

VKM's search for systematic reviews and meta-analyses (see Chapter 3.1.2.2) identified no publications with data on fish intake and body weight in children, measured by maternal intake in pregnancy.

4.20.2 VKM's systematic review of primary studies on fish and body weight in children, based on maternal consumption in pregnancy

4.20.2.1 *Included studies from search*

We evaluated four publications graded A or B with body weight measures in children as outcome (Figure 4.17-1); (Maslova et al., 2018; Stratakis et al., 2016; van den Berg et al., 2016). A description of the studies (study name, design, time period, size and age of the study population, and dietary assessment method) can be found in Table 4.20.2.1-1. The updated search yielded one additional paper

Table 4.20.2.1-1 Overview of primary studies included in weight of evidence analysis of maternal fish intake and childhood/adolescent body weight.

Author, year, country	Study name	Study design	Inclusion year(s), end, follow-up time	Study size, participant age	Dietary assessment method	Dietary assessment period
Maslova, 2018, US	Project Viva	Birth cohort	1999-2002, followed until offspring median age 7.7 yrs	1 418, mother-offspring pairs, followed from pregnancy to median age 7.7 yrs	FFQ, semi quantitative	Three months, measured in second trimester
Papadopoulou 2021, Norway	MoBa	Birth cohort	Inclusion 2002-2008, followed until 8 years of age	51 952 mother-child pairs in seafood analyses, 2277 blood samples from mothers included in mercury analyses	FFQ, semi-quantitative	The first 4-5 months of pregnancy
Stratakis, 2016, Europe/US		Birth cohorts, pooled	Inclusion from 1996 to 2011, followed up at 2-year intervals until the age of 6 years	26 184 pregnant women and their children. Mothers were predominantly older than 29 yrs	FFQ or other questionnaire (study specific)	Not stated
van den Berg, 2016, the Netherlands	PIAMA	Birth cohort	Children born in 1996–1997	3 684 followed from birth to 14 yrs	Short FFQ	Last month

4.20.2.2 *Overlapping publications*

The paper by Stratakis et al. (2016) is a pooled analysis that includes both Project Viva (Maslova et al., 2018) and PIAMA (van den Berg et al., 2016). Therefore, we only extracted data from the paper by Maslova et al. (2018) for outcomes not covered in Stratakis et al. (2016). Incidentally, more Project Viva participants are included in the paper by Stratakis et al. (2016) than in the original Project Viva paper since the latter partly relies on biomarker data. The opposite was the case for the Piama study, the paper by van den Berg includes more participants and has longer follow-up than the pooled analysis by Stratakis, and the Piama results were excluded from the categorical analyses in the pooled analysis by Stratakis et al. (2016), hence data from both papers were extracted.

4.20.2.3 *Studies by design and geographic region*

The studies are birth cohorts mainly from Europe, but Project Viva is from USA, and was also included in the pooled analysis from Stratakis (Maslova et al., 2018; Papadopoulou et al., 2021; Stratakis et al., 2016; van den Berg et al., 2016).

4.20.2.4 *Studies by sex, potential effect modification, and other sub-groups*

Stratakis et al. (2016) looked at interactions with sex, while van den Berg et al. (2016) looked at interactions with age at measurement, and Papadopoulou et al. (2021) looked at both age and gender. In addition, they found an interaction between maternal fatty fish intake and prenatal mercury exposure (Papadopoulou et al., 2021).

4.20.2.5 *Studies by fish exposure*

Maslova et al. (2018) and van den Berg et al. (2016) present results for total fish consumption, while Stratakis and Papadopoulou also include results for lean and fatty fish (Maslova et al., 2018; Papadopoulou et al., 2021; Stratakis et al., 2016; van den Berg et al., 2016).

4.20.2.6 *Studies assessing potential non-linearity*

No studies with dose-response curves were found.

4.20.3 Results from the included primary studies on maternal fish intake and body weight in children

4.20.3.1 *Studies of total maternal fish intake and body weight in children*

We included four publications with 33 estimates of the association between total fish intake and body weight in children, based on pregnancy intake, in the weight of evidence analysis. The exposure levels and results (high-low relative risk, and overall) are included in Table 4.20.3.1-1.

Table 4.20.3.1-1 Results from birth cohort studies included in the weight of evidence analysis of maternal total fish intake and body weight in children (boys and girls combined).

Author, year, country	Intake unit	High-low intake	Total cases	Child age	Outcome measure	Effect measure with estimate of precision (95 %CI)	Overall result
Maslova, 2018, US	Portions/wk, continuous		1 418	Median 3.2 yrs	Waist circumference	$\beta = -0.04$ (-0.18, 0.11)	No sig. assoc. between fish intake (continuous) and waist circumference in early childhood
	Portions/wk	≥ 3 portions/wk vs 0	1 418	Median 3.2 yrs	Waist circumference	$\beta = -0.21$ (-1.04, 0.62)	No sig. assoc. between fish intake (categorical) and waist circumference in early childhood
	Portions/wk, continuous		1 418	Median 7.7 yrs	Waist circumference	$\beta = 0.20$ (-0.14, 0.53)	No sig. assoc. between fish intake (continuous) and waist circumference in mid-childhood
	Portions/wk	≥ 3 portions/wk vs 0	1 418	Median 7.7 yrs	Waist circumference	$\beta = -0.12$ (-2.06, 1.81)	No sig. assoc. between fish intake (categorical) and waist circumference in mid-childhood
	Portions/wk, continuous		1 418	Median 7.7 yrs	Higher skinfold ratio ³	$\beta = 0.95$ (0.14, 1.77)	Higher fish intake (continuous) associated with higher skinfold ratio
	Portions/wk	≥ 3 portions/wk vs 0	1 418	Median 7.7 yrs	Higher skinfold ratio ³	$\beta = 2.70$ (-2.11, 7.50)	No sig. assoc. between fish intake (categorical) and skinfold ratio
Papadopoulou, 2021, Norway ¹	Servings/wk	>3 vs 0-1 serving/wk	51952	1 mo-8 yrs	BMI	$\beta = -0.0028$ (-0.032, 0.026) kg/m ²	No sig. change in BMI at any ages (children 1 mo to 8 yrs)
Stratakis, 2016*, Europe/US	Times/wk, continuous		25 625	2 yrs	BMI z-score	$\beta = 0.009$ (0.003, 0.0016)	Slightly higher z-scores for BMI at age 2 per frequency of fish intake per week
	Times/wk, continuous		25 355	4 yrs	BMI z-score	$\beta = 0.009$ (0.001, 0.0016)	Slightly higher z-scores for BMI at age 4 per frequency of fish intake per week
	Times/wk, continuous		22 668	6 yrs	BMI z-score	$\beta = 0.010$ (0.001, 0.019)	Slightly higher z-scores for BMI at age 6 per frequency of fish intake per week
	Times/wk	>3 times/wk vs ≤ 1	2 709	2 yrs	BMI z-score	$\beta = 0.050$ (0.004, 0.096)	Slightly higher z-scores for BMI in the highest category of fish intake per week, at age 2

Author, year, country	Intake unit	High-low intake	Total cases	Child age	Outcome measure	Effect measure with estimate of precision (95 % CI)	Overall result
	Times/wk	>3 times/wk vs ≤1	2 709	4 yrs	BMI z-score	$\beta=0.050$ (0.001, 0.100)	Slightly higher z-scores for BMI in the highest category of fish intake per week, at age 4
	Times/wk	>3 times/wk vs ≤1	1 469	6 yrs	BMI z-score	$\beta=0.039$ (-0.033, 0.111)	No difference in z-scores for BMI in across categories of fish intake per week, at age 6
	Times/wk, continuous		26 184	0-2 yrs	Rapid growth	OR=1.02 (0.99, 1.04)	No sig. assoc. between fish intake and rapid growth birth to 2 yrs
	Times/wk, continuous		25 355	4 yrs	Getting overweight/obesity	OR=1.02 (0.99, 1.04)	No sig. assoc. between fish intake and childhood overweight/obesity at age 4
	Times/wk, continuous		22 668	6 yrs	Getting overweight/obesity	OR=1.02 (0.99, 1.05)	No sig. assoc. between fish intake and childhood overweight/obesity at age 6
	Times/wk	>3 times/wk	2 739	0-2 yrs	Rapid growth	OR=1.22 (1.05, 1.42)	Highest intake category (total fish) associated with higher odds for rapid growth birth to 2 yrs
	Times/wk	>3 times/wk	2 709	4 yrs	Getting overweight/obesity	OR=1.14 (0.99, 1.32)	No sig. assoc. between categories of fish intake and childhood overweight/obesity at age 4
	Times/wk	>3 times/wk	1 469	6 yrs	Getting overweight/obesity	OR=1.22 (1.01, 1.47)	Higher odds of childhood overweight/obesity in highest category of fish intake at age 6
Van den Berg, 2016, the Netherlands	Times/wk	≥1 time/wk vs never	Range 2000-3684, depending on age	0-15.5 yrs	BMI z-score	delta mean BMI z-score=-0.027 (-0.112, 0.057)	Fish consumption associated with lower tendency of overweight/obesity in crude analyses, but not in adjusted analyses

*Pooled analysis of birth cohorts, ¹Total seafood as exposure. ²OR (95% CI), beta coefficient (95% CI) or delta coefficient (95% CI). ³Based on subscapular (SS) and triceps (TR) skinfold thickness (SS:TRx100).

4.20.3.2 *Studies of lean and fatty fish intake and body weight in children/adolescents based on maternal fish intake during pregnancy*

Results for lean and fatty fish, twenty estimates from two studies are shown in Tables 4.20.3.2-1 and 4.20.3.2-2.

Table 4.20.3.2-1 Results from birth cohort studies included in the weight of evidence analysis of maternal lean fish intake and body weight in children (boys and girls combined).

Author, year, country	Intake unit	High-low intake	Total cases	Child age	Outcome measure	Effect measure with estimate of precision (95 % CI)	Overall result
Papadopoulou, 2021, Norway	Servings/wk	>3 vs 0-1 serving/wk	51952	1 mo	BMI change	$\beta=0.022$ (-0.0069, 0.050) kg/m ²	No sig. assoc. with change in child BMI at 1 month
	Servings/wk	>3 vs 0-1 serving/wk		2 mo	BMI change	$\beta=0.022$ (-0.0064, 0.050) kg/m ²	No sig. assoc. with change in child BMI at 2 months
	Servings/wk	>3 vs 0-1 serving/wk		3 mo	BMI change	$\beta=0.022$ (-0.0059, 0.051) kg/m ²	No sig. assoc. with change in child BMI at 3 months
	Servings/wk	>3 vs 0-1 serving/wk		6 mo	BMI change	$\beta=0.023$ (-0.0044, 0.051) kg/m ²	No sig. assoc. with change in child BMI at 6 months
	Servings/wk	>3 vs 0-1 serving/wk		9 mo	BMI change	$\beta=0.025$ (-0.0031, 0.052) kg/m ²	No sig. assoc. with change in child BMI at 9 months
	Servings/wk	>3 vs 0-1 serving/wk		12 mo	BMI change	$\beta=0.026$ (-0.0018, 0.053) kg/m ²	No sig. assoc. with change in child BMI at 12 months
	Servings/wk	>3 vs 0-1 serving/wk		18 mo	BMI change	$\beta=0.028$ (0.00051, 0.055) kg/m ²	Small positive assoc. with change in child BMI at 18 months
	Servings/wk	>3 vs 0-1 serving/wk		2 yrs	BMI change	$\beta=0.030$ (0.0025, 0.057) kg/m ²	Small positive assoc. with change in child BMI at 2 years
	Servings/wk	>3 vs 0-1 serving/wk		3 yrs	BMI change	$\beta=0.034$ (0.0056, 0.063) kg/m ²	Small positive assoc. with change in child BMI at 3 years
	Servings/wk	>3 vs 0-1 serving/wk		4 yrs	BMI change	$\beta=0.039$ (0.0075, 0.070) kg/m ²	Small positive assoc. with change in child BMI at 4 years

Author, year, country	Intake unit	High-low intake	Total cases	Child age	Outcome measure	Effect measure with estimate of precision (95 % CI)	Overall result
	Servings/wk	>3 vs 0-1 serving/wk		5 yrs	BMI change	$\beta=0.043$ (0.0086, 0.077) kg/m ²	Small positive assoc. with change in child BMI at 5 years
	Servings/wk	>3 vs 0-1 serving/wk		6 yrs	BMI change	$\beta=0.047$ (0.0091, 0.085) kg/m ²	Small positive assoc. with change in child BMI at 6 years
	Servings/wk	>3 vs 0-1 serving/wk		7 yrs	BMI change	$\beta=0.051$ (0.0090, 0.094) kg/m ²	Small positive assoc. with change in child BMI at 7 years
	Servings/wk	>3 vs 0-1 serving/wk		8 yrs	BMI change	$\beta=0.056$ (0.0086, 0.10) kg/m ²	Small positive assoc. with change in child BMI at 8 years
Stratakis, 2016*, Europe/USA	Times/wk, continuous		9 615	2 yrs	BMI z-score	$\beta=0.016$ (-0.003, 0.034)	No difference in z-scores for BMI per occasion of lean fish intake per week age 2
	Times/wk, continuous		9 345	4 yrs	BMI z-score	$\beta=0.010$ (-0.012, 0.031)	No difference in z-scores for BMI per occasion of lean fish intake per week age 4
	Times/wk, continuous		6 657	6 yrs	BMI z-score	$\beta=-0.002$ (-0.034, 0.029)	No difference in z-scores for BMI per occasion of lean fish intake per week age 6
	Times/wk, continuous		10 107	0-2 yrs	Rapid growth	OR=1.03 (0.99, 1.08)	No sig. assoc. between lean fish intake per week and rapid growth birth to 2 yrs
	Times/wk, continuous		9 345	4 yrs	Getting overweight/obesity	OR=1.01 (0.96, 1.07)	No sig. assoc. between lean fish intake per week and overweight/obesity at 4 yrs
	Times/wk, continuous		6 657	6 yrs	Getting overweight/obesity	OR=0.91 (0.81, 1.03)	No sig. assoc. between lean fish intake per week and overweight/obesity at 6 yrs

*Pooled analysis of birth cohorts. ²OR (95% CI), or beta coefficient (95% CI).

Table 4.20.3.2-2 Results from birth cohort studies included in the weight of evidence analysis of maternal fatty fish intake and body weight in children (boys and girls combined).

Author, year, country	Intake unit	High-low intake	Total cases	Child age	Outcome measure	Effect measure with estimate of precision (95 %CI)	Overall result
Papadopoulou, 2021, Norway	Servings/wk	0-1 servings/wk vs >3	51952	1 mo-8 yrs	BMI	$\beta=0.0083$ (-0.019, 0.035) kg/m ²	No sig. change in BMI at any age (children 1 mo to 8 yrs)
Stratakis, 2016*, Europe/US	Times/wk, continuous		11 196	2 yrs	BMI z-score	$\beta=0.003$ (-0.014, 0.020)	No difference in z-scores for BMI per occasion of fatty fish intake per week at 2 yrs
	Times/wk, continuous		10 926	4 yrs	BMI z-score	$\beta=0.004$ (-0.016, 0.024)	No difference in z-scores for BMI per occasion of fatty fish intake per week at 4 yrs
	Times/wk, continuous		8 238	6 yrs	BMI z-score	$\beta=0.015$ (-0.010, 0.040)	No difference in z-scores for BMI per occasion of fatty fish intake per week at 6 yrs
	Times/wk, continuous		11 689	0-2 yrs	Rapid growth	OR=1.01 (0.96, 1.06)	Sig. assoc. between fatty fish intake per week and rapid growth birth to 2 yrs
	Times/wk, continuous		10 926	4 yrs	Overweight/obesity	OR=1.02 (0.97, 1.07)	No sig. assoc. between fatty fish intake per week and overweight/obesity at 4 yrs
	Times/wk, continuous		8 238	6 yrs	Overweight/obesity	OR=1.02 (0.95, 1.09)	No sig. assoc. between fatty fish intake per week and overweight/obesity at 6 yrs

*Pooled analysis of birth cohorts.

4.20.3.3 *Summary relative risks (RR) based on VKM's inclusion of primary studies*

None of the studies have expressed their data on the same form, hence no summary RR could be calculated from included primary studies.

4.20.4 Weight of evidence for maternal fish intake and child body weight

In this section, the evidence of the association between maternal fish intake and body weight in children/adolescents is weighted according to the WCRF criteria presented in the Chapter 3.1.6 (Box 2). The following criteria are used; the published evidence of fish intake and the sparse mechanistic evidence.

Published evidence of fish intake and body weight in children

As for adult exposures, we have few reports of the same endpoint for childhood exposures, making it difficult to draw conclusions. Several of the studies highlight confounding factors: eating fish during pregnancy is associated with higher socioeconomic status and a healthier lifestyle (e.g., lower pre-pregnancy BMI). There is no clear picture suggesting an association between maternal fish intake in pregnancy and anthropometric measures in the offspring. The childhood studies generally have many repeated measurements (up to 11). This allows for better characterization of long-term effects and trajectories. Maslova et al. (2018) comments that their results (stronger with n-3 PUFA than fish) are limited and possibly transient, as they tend to weaken with time. Stratakis et al., 2016 modelled BMI percentile trajectories of children up to 6 years and found that children of mothers that consumed fish more than three times per week had a higher risk of rapid growth and childhood overweight/obesity. Similarly, Papadopoulou et al., 2021 modelled BMI change trajectories from 1 month to 8 years of age and found that maternal lean fish intake during pregnancy was positively, but weakly associated with BMI growth trajectory. However, they argue that their findings are most likely not causal.

Heterogeneity

Not assessed due to the low number of papers and variable ways to express the results.

Mechanisms/biological plausibility

Several hypotheses regarding fish intake and body weight/weight gain exist, but they are not backed by solid mechanistic evidence.

Upgrading factors

No upgrading factors have been evaluated.

4.20.4.1 *Conclusion weight of evidence maternal fish intake and child body weight*

Due to the low number of papers, the current body of evidence is graded “limited, no conclusion”.

4.21 Fish intake and bone health (hip fracture)

The current chapter summarizes the epidemiological evidence for a role of fish intake in bone health. Different indicators for bone health such as hip fracture and changes in bone mineral density (BMD) were identified in VKM's literature search. BMD was considered an intermediate endpoint for fractures and not summarized as a separate outcome but results on BMD are commented on in relation to fracture in studies that assessed both BMD and fractures in the same individuals.

Hip fractures are a major public health burden in Western societies, and Scandinavians have the world's highest incidence rates. Numbers are likely to increase with an aging population. Most fractures in the elderly happen due to decreased bone mass combined with a fall, and hip fractures are associated with increased mortality.

Mechanisms

Fish is a source of various nutrients that may affect bone health, including vitamin D, omega-3 fatty acids and proteins. Vitamin D is important for calcium homeostasis and could possibly also affect the risk of falling (Lamberg-Allardt et al., 2013). N-3 fatty acids might positively influence bone metabolism (Farina et al., 2011), and dietary proteins may have a beneficial effect on both bone and muscles. Although there has been a concern that a high protein intake can cause calcium loss, summarized evidence conclude that a high protein intake is not associated with increased fracture risk (Dawson-Hughes et al., 2017).

4.21.1 VKM's search for previous systematic reviews and meta-analyses of fish intake and hip fracture

4.21.1.1 *Description of the identified publications*

VKM's search for systematic reviews and meta-analyses (see Chapter 3.1.2.2) identified two publications on the association between fish intake and hip fracture that fulfilled the inclusion criteria and were read as full papers. One paper was excluded, see Table 4.21.1.1-1 for reason for exclusions.

Table 4.21.1.1-1 Reasons for exclusion of identified papers from the search of systematic reviews and meta-analysis of fish intake and hip fracture 2016-2021.

Included papers	Excluded paper and reasons for exclusions
Sadeghi et al., 2019	Perna et al., 2017: AMSTAR quality C. Unclear methods. No report of risk of bias assessment of primary studies

The systematic literature review and meta-analysis by Sadeghi et al. (2019) is described in more detail below; first a main description of the methods used and then selected results from each analysis are provided (see Table 4.21.1.3-1).

4.21.1.2 *Description of the identified publications*

Sadeghi et al. (2019) is a systematic review and meta-analysis including observational studies investigating the association of n-3 polyunsaturated fatty acids and fish intake with fractures. The authors performed a systematic literature search in PubMed, ISI, Web of Science, Scopus, ProQuest, Science Direct and Embase databases until August 2017. The quality of the eligible papers included in the meta-analysis was assessed by The Newcastle-Ottawa Scale criteria for prospective studies. Six studies (four prospective cohorts and two case control studies) were included in the meta-analysis of fish consumption and risk of hip fractures. The quality of all the included papers was overall high.

4.21.1.3 *Results from the systematic review and meta-analyses*

Below is a summary table for fish intake and hip fracture (Table 4.21.1.3-1) based on the identified meta-analysis (Sadeghi et al., 2019). For hip fracture, four prospective cohort studies (including one study of all any fractures) and two case-control studies, with a total of 154 362 participants and 4167 cases were included in the high-low meta-analyses of fish intake.

Table 4.21.1.3-1 Summary of results from meta-analysis on total fish intake and risk of hip fractures.

Author, year	Type of studies included	Total no studies	No of cases	Comparison	Summary RR (95 % CI)	Heterogeneity	Overall conclusion
Sadeghi, 2019	Prospective cohort studies of fish intake and fracture incidence (hip or all osteoporotic fractures)	4	3337	Highest vs lowest	0.91 (0.82, 1.02), $P=0.11$	$I^2=58.1\%$, $P=0.049$, fixed-effects	Combining 5 estimates from 4 prospective studies showed borderline sig protective association
	Case control studies which evaluate the association between fish intake and hip fracture incidence	2	830	Highest vs lowest	0.56 (0.37, 0.98), $P=0.004$	$I^2=57.9\%$, $P=0.02$, fixed-effects	Combining 3 estimates from 2 case control studies showed significant protective association

The summary RR (risk ratio) for cohort studies became non-significant after the exclusion of the study on any fractures (RR=1.04, 95% CI: 0.87, 1.24).

4.21.2 VKM's systematic review of primary studies on fish intake and bone health

4.21.2.1 *Included studies from search*

We evaluated eight publications graded A or B with bone health as outcome (Benetou et al., 2011; Chan et al., 2011; Fan et al., 2013; Farina et al., 2011a; Farina et al., 2011b; Rosendahl-Riise et al., 2018; Virtanen et al., 2010; Virtanen et al., 2012). Of these, six studies examined hip fracture, three studies examined bone mineral density (BMD) in the femoral neck (Chan et al., 2011; Farina et al., 2011b; Virtanen et al., 2010), or in the total hip (Chan et al., 2011; Virtanen et al., 2010) as % change or absolute change (g/cm²) over time, or at one time point after fish intake was measured.

A description of the studies (study name, design, time period, size and age of the study population, and dietary assessment method) can be found in Table 4.21.2.1-1.

Table 4.21.2.1-1. Overview of primary studies included in weight of evidence analysis of fish intake and bone health.

Author, year, country	Study name	Study design	Inclusion year(s), end, follow-up time	Population characteristics	Dietary assessment method	Dietary assessment period
Benetou, 2011, Europe (5 countries: Italy, the NL, Greece, Germany, Sweden)	European Prospective Investigation into Cancer and nutrition - Elderly Network on Ageing and Health (EPIC-Elderly-NAH)	Prospective cohort, multi-center	NA (inclusion, end), 8 yrs follow-up (median)	29122 elderly (10538 men and 18584 women), 60-86 yrs, mean age 64.3 yrs	FFQ, self-administered or interview-administered, country or center specific	Previous yr, at enrolment
Chan, 2011, Hong-Kong/China	Cohort of Hong-Hong Chinese elderly (no name given)	Prospective cohort	2001-2003 to 2005-2007, 4 yrs follow-up	2217 elderly (1225 men and 992 women), ≥ 65 yrs, mean age 72 yrs	FFQ by interview	12 months prior to interview
Fan, 2013, China	Case-control study of middle-aged/elderly from Guangdong Province in China (no name given)	Case-control	June 2009 to June 2012	581 hip fracture patients and 581 controls (398 community and 183 hospital controls), cases: 55 to 80 years and age-matched controls (+/- 3 years)	FFQ by interview	12 months prior to interview
Farina, 2011a, USA	Framingham Osteoporosis Study	Prospective cohort	1988-89, follow-up throughout 2005	Final sample size (n = 904) comprised 552 women and 352 men. Mean age, baseline: 72.2 years	FFQ, self-administered	Previous year
Farina, 2011b, USA	Framingham Osteoporosis Study	Prospective cohort	1988-89 and re-examination four years later	Final sample size at baseline n = 854 (530 women and 324 men). Final sample size longitudinal analyses n=622 (397 women and 225 men). Mean age, baseline: 75.2 years	FFQ, self-administered	Previous year
Rosendahl-Riise, 2018, Norway	Hordaland Health Study (HUSK)	Prospective cohort	1997-99	Out of 3327 eligible participants, information regarding food intake and hip fractures was available for 2865 (86%), 71-74 years at baseline	FFQ, self-administered	Previous year

Author, year, country	Study name	Study design	Inclusion year(s), end, follow-up time	Population characteristics	Dietary assessment method	Dietary assessment period
Virtanen, 2010, USA	Cardiovascular Health Study (CHS)	Prospective cohort	1989-90. Hip fracture follow-up until 30 June 2003 (average 11.1 years). BMD measured in a subsample in 1994-95	After exclusion of individuals with missing information on fish or EPA+DHA consumption, 5045 of 5201 were included in the hip fracture analyses and 1305 in the BMD analyses, Mean 72.8 years, minmax 65 to 100 years	Picture-sort version of the National Cancer Institute food frequency questionnaire (FFQ), validated	Previous year
Virtanen, 2012, USA	Nurses' Health Study (NHS) and Health Professionals Follow-up Study (HPFS)	Prospective cohorts	NHS: Inclusion 1984, followed to 1 June 2006; HPFS: Inclusion 1986, followed until January 2008.	81757 women (NHS) and 49934 men (HPFS), Women: 63 years; men 65 years	Repeated FFQ (self-reported), validated	Previous year

4.21.2.2 *Overlapping publications*

There were no overlapping publications. The two publications from the Framingham Osteoporosis Study were on different outcomes, hip fracture (Farina et al., 2011a) and BMD (Farina et al., 2011b).

4.21.2.3 *Studies by design and geographic region*

All studies were based on prospective cohorts, except one case-control study (Fan 2013). One study (Benetou et al., 2011) was a multicentre study (European Prospective Investigation into Cancer and nutrition -Elderly Network on Ageing and Health; EPIC-Elderly-NAH) combining data from 5 European countries, and one study combined data from two US cohort restricted to health professionals; the Nurses' Health Study (NHS) and Health Professionals Follow-up Study (HPFS). Overall, the geographic distribution was relatively even with three studies from the US, two from Europe, and two from Asia.

4.21.2.4 *Studies by sex, potential effect modification, and other sub-groups*

All studies included both men and women, but most cases were female. Gender could be potential effect modifier but estimates for women and men combined were emphasized when available, due to small case numbers in most studies. The largest study (Virtanen et al., 2012) also pooled their sex-specific estimates from the Nurses' Health Study (NHS) and Health Professionals Follow-up Study (HPFS) in the final analysis.

4.21.2.5 *Studies by fish exposure*

Of the six studies on hip fracture, five included a measure of total fish intake (sum of all fish, unspecified fish, or fish including shellfish and/or fish products). Sub-classifications of fish were unique to each study (saltwater/freshwater fish, fried and other fish, fatty fish with and without tuna) and could not be summarized. Of the three studies on BMD, two included total fish intake.

4.21.2.6 *Studies assessing potential non-linearity*

One study in Norwegian men and women from the Hordaland Health Study presented a dose-response figure of total fish and risk of hip fracture that explored potential non-linearity using a restricted cubic spline model (Rosendahl-Riise et al., 2018).

4.21.2.7 *Studies with converted risk estimates*

Risk estimates reported as low versus high (Rosendahl-Riise 2018) were converted to high versus low. One continuous estimate per quintile (Benetou et al., 2011) was also converted to the estimate for the highest versus lowest quintile.

4.21.3 Results from the included primary studies fish intake and bone health

4.21.3.1 *Studies of total fish intake and hip fracture in the general population*

We included five publications with six estimates of the association between total fish intake and hip fracture in the weight of evidence analysis. The exposure levels and results (high-low relative risk, and overall) are included in Table 4.21.3.1-1.

Table 4.21.3.1-1 Results from studies included in the weight of evidence analysis of total fish intake and hip fracture in the general population.

Author, yr, country	Study design	Fish exposure, sex	Intake unit	High-low intake	Total cases	RR high-low or continuous (95% CI)	Overall result
Benetou, 2011, Europe (5 countries: Italy, the NL, Greece, Germany, Sweden)	Prospective cohort, multicentre	Fish, incl shellfish, M/W	Frequency, quintiles (sex and country-specific)	Quintile 5 vs 1, limits not specified	275, (222 W and 53 M)	HR 0.75 (0.52, 1.08), reported as HR (continuous) per increasing quintile =0.93 (0.85, 1.02)	Borderline protective assoc.
Fan, 2013, China	Case-control	Fish, incl shellfish, M/W	g/d, energy adjusted quartiles	Quartile 4 vs 1, 70.15/73.42 vs 9.75/7.88 (M/W)	581	OR 0.47 (0.28, 0.79)	Sig. protective assoc.
Farina, 2011a, USA	Prospective cohort	Fish, incl shellfish, M/W	servings/wk	≥3 vs <1 serving/wk	98 (78 W and 20 M)	HR 0.66 (0.38, 1.17)	No sig. assoc.
Rosendahl-Riise, 2018, Norway	Prospective cohort	Fish, M	g/d, energy adjusted quartiles	Quartile 4 vs 1, >65 vs ≤33 g/1000 kcal	72	HR 0.57 (0.29, 1.14), reported as 1.75 (0.88, 3.47) for low vs high	No sig. assoc., <i>P</i> -trend 0.12
		Fish, W	g/d, energy adjusted quartiles	Quartile 4 vs 1, >57 g vs ≤28 vs g/1000 kcal	154	HR 0.68 (0.41, 1.14), reported as 1.47 (0.88, 2.44) for low vs high	No sig. assoc., <i>P</i> -trend 0.50
Virtanen, 2012, USA	Prospective cohorts	Fish, M/W	Servings/mo or wk, 5 cat (cumulative average)	≥5 servings/wk vs <1 serving/mo	1580	HR 0.83 (0.56, 1.22)	No sig. assoc.

4.21.3.2 *Studies on hip fracture in relation to bone mineral density*

In two studies, results could be compared for hip fracture and BMD. The Framingham Osteoporosis Study presented results (separate publications) on total fish intake in relation to hip fractures (Farina et al., 2011a) and 4-year changes in bone mineral density in the femoral neck (Farina et al., 2011b). None of the results reached statistical significance. The Cardiovascular Health Study (CHS) was the only study on fried and non-fried fish intake and presented results on hip fracture and BMD in the femoral neck or total hip at one time point around five years after the dietary assessment (Virtanen et al., 2010). Similar to the Framingham Osteoporosis Study, none of the results reached statistical significance (results not shown for BMD in any of the two studies).

4.21.3.3 *Summary relative risks (RR) based on VKM's inclusion of primary studies*

VKM calculated a summary RR for incident hip fracture in relation to the highest versus lowest intake of total fish, based on four prospective studies (Table 4.21.3.1-1) reporting HRs (Benetou et al., 2011; Farina et al., 2011a; Rosendahl-Riise et al., 2018; Virtanen et al., 2012). The summary RR suggested a protective association for the highest intake that was statistically significant (RR=0.70, 95% CI: 0.55, 0.88) without significant heterogeneity ($p_{\text{heterogeneity}}=0.93$). One case-control study that was excluded from the summary RR (Fan 2013) reported a statistically significant protective association for the highest intake, supporting the summary RR.

Compared with the previous meta-analysis by Sadeghi et al. (2019), the summary estimate from cohort studies (Table 4.21.1.3-1) was closer to unity than VKM's estimate and only borderline statistically significant, with moderate between-study heterogeneity ($I^2=58.1\%$, $P=0.049$).

Differences in results could be related to the selection of studies (as described below).

4.21.3.4 *VKM's search compared to previous meta-analyses on hip fracture*

Sadeghi et al. (2019) identified seven primary studies in their systematic review of fish intake and fractures. All studies were identified by VKM, but Sadeghi et al. (2019) and VKM differed in the selection of studies into the high-low meta-analysis. Sadeghi et al. (2019) included two studies that were excluded by VKM during abstract screening (Appleby et al. 2007 in vegetarians and non-vegetarians; and a Japanese case-control study by Suzuki et al. 1997). VKM also excluded one study from the summary RR because the exposure was not total fish (Virtanen et al., 2010). One study of total fish (Benetou et al., 2011) reporting a continuous risk estimate was excluded by Sadeghi et al. (2019) but included by VKM after estimate conversion. Further, VKM included a primary study from Norway (Rosendahl-Riise et al., 2018) published after the last search date in Sadeghi et al. (2019).

4.21.4 Heterogeneity fish intake and hip fracture

Sadeghi et al. (2019) reported significant between-study heterogeneity in the analysis of fish intake and hip fractures (cohort and case-control studies combined). Heterogeneity was explored by dividing five studies into sub-groups by gender, follow-up duration (less than 10 years and 10 years or above), sample size (less than 10000 people and 10000 people or more), methods used to assess bone fractures (self-reported, or medical records), and adjustment for body mass index. Several sub-groups contained no more than one or two studies which is insufficient for a representative estimate of heterogeneity. However, studies that adjusted for BMI (n=4) and large studies (10000 people or more, n=3) had low heterogeneity, and the sub-group RRs showed protective associations of similar magnitude that were statistically significant. Results in primary studies were consistently protective or on the protective side, except in one study that assessed intake of fried fish and tuna/other fish (Virtanen et al., 2010). This study was not included in VKM's summary RR for total fish, which may explain the lower and non-significant heterogeneity.

4.21.5 Dose-response relationship fish intake and hip fracture

No meta dose-response analysis was performed in Sadeghi et al. (2019). Among the primary studies identified by VKM there were few reports of test for trend across categories of fish intake (Table 4.21.3.1-1). The non-linear dose-response analysis by Rosendahl-Riise et al. (2018) from the Hordaland study had wide confidence limits (95%) and did not show a statistically significant relationship in any segment of the curve.

4.21.6 Weight of evidence for fish intake and hip fracture

Published evidence of fish intake and hip fractures

VKM's high-low summary RR based on four prospective studies, suggests that a high intake of fish may lower the relative risk of hip fractures. The summary RR reported in the previous meta-analysis by Sadeghi et al. (2019), also based on four prospective studies (two studies overlapping with VKM) was on the protective side, but closer to unity and only borderline significant. Results from case-control studies identified by VKM and Sadeghi et al. (2019) support a protective association.

Heterogeneity

No significant heterogeneity was observed between prospective studies included by VKM, whereas Sadeghi et al. (2019) reported borderline statistically significant heterogeneity between prospective studies. The conflicting results on heterogeneity may arise from differences in study selection. Estimates were consistently protective or on the protective side except in one study included by Sadeghi et al. (2019).

Mechanisms/biological plausibility

There is evidence for several plausible mechanisms operating in humans (presented above in section on mechanisms).

Upgrading factors

Dose-response was not found to be an upgrading factor in this case. No other upgrading factors were evaluated.

4.21.6.1 *Conclusion weight of evidence fish intake and bone health*

In conclusion, the evidence that high fish consumption may lower the risk of hip fractures is graded "limited, suggestive". No conclusion could be drawn for fatty fish or lean fish due to one study only.

4.22 Introduction to maternal fish intake and birth outcomes

This chapter is an introduction to the weight of evidence analysis chapters for birth outcomes related to maternal fish intake (Chapters 4.23-4.27). Systematic reviews and meta-analyses and also the inclusion and exclusion of the primary studies and their study characteristics, are presented here in the introductory chapter. The results from the primary studies are presented in the chapters below for the specific birth outcomes. Additionally, in this introductory chapter we show an overlap table between VKM's included primary studies, and the those included in the one meta-analysis found for birth outcomes and a pooled analysis of several European birth cohort studies. We have chosen to present the overlap table here, as it is valid for all the birth outcomes in Chapters 4.23-4.27.

Overview of birth outcomes

The following sections summarizes the epidemiological evidence for associations of maternal fish intake (during or prior to pregnancy) with different measures of newborn growth and maturity (referred to as birth outcomes). In the identified studies, these measures include birth weight, birth length, the ponderal index (birth weight divided by the birth length cubed), head circumference, and gestational age (Figure 4.22-1). Birth weight and gestational age have been analysed on a continuous scale and/or dichotomized according to clinical cut-off values. Low birth weight (LBW) is usually defined as birth weight < 2500 g, and some studies additionally reported on high birth weight (> 4000 g). Preterm birth (PTB) is defined as length of gestation < 37 weeks. Newborns can also be classified as small for gestational age (SGA) or large for gestational age (LGA) based on birth weight (or height or head circumference) that is lower or higher than a certain percentile of the reference distribution (usually bottom or top 10% or 5% for a given gestational age and sex). The percentile cut-off and reference distribution used may differ between countries and studies. Some studies of SGA include all infants, whereas others exclude preterm births.

The dichotomized measure PTB is summarized first (Chapter 4.23) because of the established clinical relevance, followed by SGA/LGA (Chapter 4.24) and LBW (Chapter 4.25). Although LBW is a commonly used indicator, the biological interpretation is challenging. Low birth weight can be a consequence of being born too small or too early, but small babies are not necessarily premature. Therefore, analyses of LBW often include gestational age to aid the interpretation. Continuous measures of growth are summarized last (birth weight in Chapter 4.26 and other anthropometric birth outcomes in Chapter 4.27). They may capture associations of smaller magnitude than dichotomized outcomes, but the clinical relevance may be more uncertain.

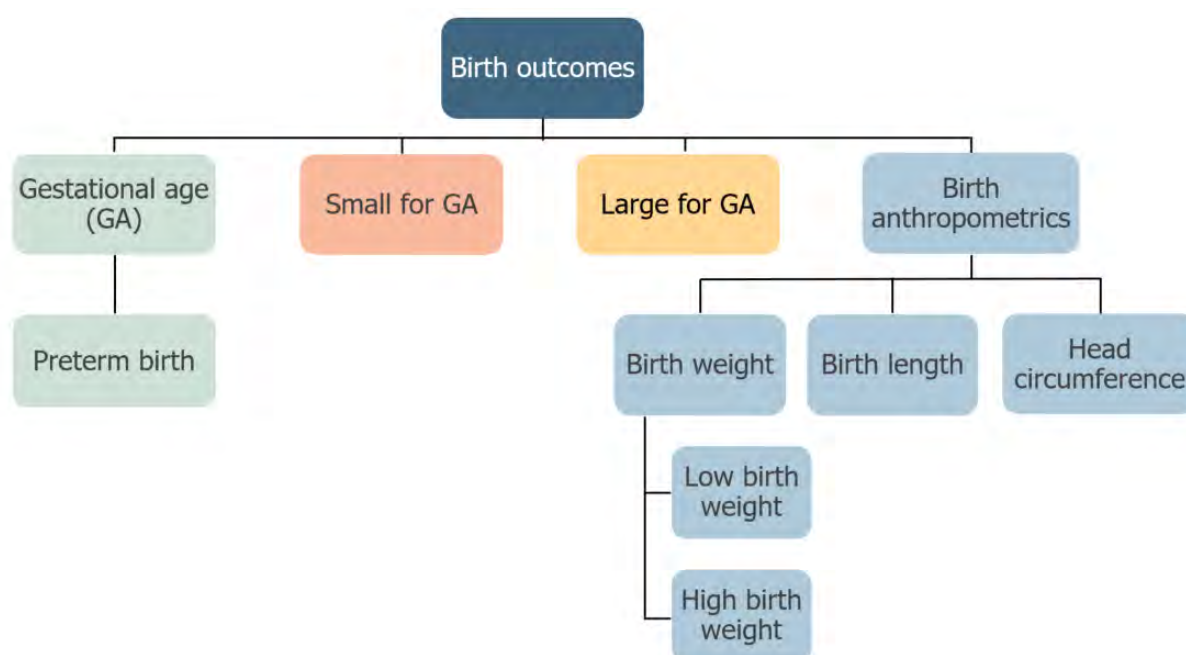


Figure 4.22-1 Overview over evaluated birth outcomes.

We extracted data from two studies including ponderal index, but no evaluation or weight of evidence analysis has been conducted for this birth outcome measure; both birth weight and length are already included.

Mechanisms

Based on the observations that birth weights in the Faroes average 200 g more than in Denmark, it has been suggested that a maternal diet rich in marine n-3 fatty acids could prolong gestation by interfering with endogenous production of prostaglandins (eicosanoids derived from n-3 fatty acids), and/or increase the fetal growth rate by increasing the ratio of prostacyclins to thromboxane, resulting in an improved placental blood flow. As described in the literature review of nutrients (Chapter 5.2), LC n-3 FA have been found to reduce the risk of early preterm birth in supplementations studies (Yelland et al., 2016).

Low concentration of 25-hydroxyvitamin D has been related to low birth weight in observational studies. Vitamin D might be of importance to placental development via effects on the expression of human chorionic gonadotropin and the synthesis of placental sex steroids (Shin et al., 2010).

Maternal contaminant intake from fish could also affect birth outcomes. Effects of methyl mercury on birth outcomes were summarized by EFSA in 2012 (EFSA, 2012). The studies on effects of methylmercury on anthropometric birth outcomes were inconclusive according to EFSA (2012), and a recent systematic search came to the same conclusion (Dack et al., 2021). Another review concluded that mercury exposure was consistently associated with lower birth weight, although based on only four included studies (Saavedra et al., 2022). The mechanisms for adverse effects of mercury on birth weight are unknown. A study from

Norway reported higher birth weight with increasing seafood consumption, but at the same time, lower birth weight with higher methylmercury intake in strata of seafood intake (Vejrur et al., 2014). It has been suggested that mercury toxicity may be mitigated by nutrients in fish in the maternal diet.

Effects on birth outcomes of PCDD/Fs and DL-PCBs were summarized by EFSA (EFSA, 2018) and it was concluded that the available studies on birth weight and other birth outcomes were inconclusive.

Regarding PFASs, EFSA concluded that “there may well be a causal association between PFOS and PFOA and birth weight”. EFSA also stated that “the decrease in birth weight after adjusting for confounders is not large and the potential longer-term consequences of this decrease are unclear” (EFSA, 2020). EFSA concluded that the evidence for an association between PFASs and preterm delivery is still limited and there is little evidence for an increase in the proportion of children with low birth weight. The mode of action of a decrease in birth weight by PFASs is not known but may be linked to hormonal effects (sex hormones, thyroid hormones), disturbances in energy balance or effects on the placenta (EFSA, 2018; Blake et al., 2020).

Sexual maturation, ovulation, conception, pregnancy, and birth all require a normally functioning endocrine system, involving among others the hypothalamic-pituitary-ovarian axis, the female reproductive tract, and the semen parameters. Endocrine disrupting compounds (EDCs) may interfere with all these aspects through multiple pathways and mechanisms, causing miscarriage, preterm birth, or reduced foetal growth (Bergman et al., 2013; Gore et al., 2015). Exact mechanisms for these effects are not known, but in the comprehensive Second Scientific statement on Endocrine-Disrupting Chemicals from the Endocrine Society (EDC-2), it is suggested that some EDCs such as phthalates and pesticides may cause disturbance of placental function, whereas others (e.g. TCDD) may cause increased sensitivity to inflammation, leading to adverse birth outcomes (Gore et al., 2015).

Fatty acids, fat-soluble vitamins, and contaminants are stored in different tissues of the body. Thus, maternal nutrient and contaminant levels throughout pregnancy may be a result of dietary intake prior to conception. Thus, studies of maternal intake both prior to or during pregnancy, were considered relevant in relation to birth outcomes, but the different exposure periods were evaluated separately.

4.22.1 VKM’s search for published meta-analyses and systematic reviews on fish intake and birth outcomes

4.22.1.1 *Description of the identified publications*

VKM’s search for systematic reviews and meta-analyses (see Chapter 3.1.2.2) identified two publications on the association between fish intake and birth outcomes that were assumed to fulfill the inclusion criteria and were read as full papers. One paper was excluded, see Table 4.22.1.1-1 for reason for exclusion.

Table 4.22.1.1-1 Reasons for exclusion of identified papers from the search of systematic reviews and meta-analyses of fish intake and birth outcomes 2016-2021.

Included papers	Excluded paper and reasons for exclusions
Zhao et al., 2020	Gete et al, 2020: Narrative analysis, only qualitative summaries

The systematic review/ meta-analysis is described below; first, main descriptions of the methods used and then main results from the meta-analysis.

Zhao et al. (2020) is a meta-analysis of observational studies including both cohorts, nested case-control and case-control studies, investigating the association between maternal fish intake and adverse birth outcomes. The authors performed a systematic literature search in PubMed, Web of Science, Embase, and Cochrane Library databases until October 2019. The quality of the eligible papers included in the meta-analysis was assessed by the Newcastle-Ottawa Scale criteria (Stang et al., 2010). Twenty-one studies looking into maternal fish intake and preterm birth, low birth weight and small for gestational age were included. No clear distinction was made between maternal intake prior to pregnancy or during pregnancy. The quality of all the papers included in the meta-analysis were overall 13 high-quality articles and 6 medium-quality articles.

4.22.1.2 *Results from the meta-analysis*

The quantitative meta-analysis of fish intake and birth outcomes by Zhao et al. (2020) is summarized below (Table 4.22.1.2-1). The meta-analysis included 571 641 participants and 65 360 cases with adverse birth outcomes. The high-low analysis of seafood intake included 11 studies of LBW, 11 studies of PTB and 9 studies of SGA. In the linear dose-response meta-analyses for per 45 g/day increment of maternal seafood consumption, 13 studies were included all together, seven studies for each outcome (PTB, SGA and LBW).

Table 4.22.1.2-1 Summary of results from meta-analysis of maternal total seafood intake and birth outcomes (Zhao et al., 2020).

Outcome	Total no. of studies	No. of cases	Comparison	Summary OR (95 % CI)	Heterogeneity	Overall result
Low birth weight (LBW)	11	26823	Highest vs lowest	0.78 (0.61, 1.00)	$I^2=50.80\%$, $P=0.03$	Protective assoc., borderline statistically sig.
	7	869	Dose-response, increment 45 g/day	0.65 (0.47, 0.90)	$I^2=0.00\%$, $P=0.51$	A 45 g/day increment in seafood consumption was associated with a 35% lower risk of LBW. No evidence of departure from linearity.
Preterm birth (PTB)	11	36 391	Highest vs lowest	0.90 (0.72, 1.14)	$I^2=76.70\%$, $P<0.001$	No sig. assoc.
	7	4675	Dose-response, increment 45 g/day	0.84 (0.70, 1.01)	$I^2=44.60\%$, $P=0.09$	A 45 g/day increment in seafood consumption was associated with a borderline significant 16% lower risk of PTB. Significant non-linear dose-response relationship, no further benefit observed for intake above 45 g/day
Small for gestational age (SGA) for birth weight	9	2146	Highest vs lowest	0.79 (0.59, 1.06)	$I^2=73.20\%$, $P<0.001$	No sig. assoc.
	7	1360	Dose-response, increment 45 g/day	0.84 (0.71, 0.98)	$I^2=53.70\%$, $P=0.04$	A 45 g/day increment in seafood consumption was associated with a 16% lower risk of SGA. No evidence of departure from linearity

In the highest versus lowest intake analysis, there were no statistically significant association with LBW (borderline protective), PTB, or SGA. The linear dose-response analyses (45 g/day increment) based on fewer studies showed protective associations (statistically significant or borderline) for all outcomes. The magnitude was strongest for LBW, slightly lower but similar for PTB and SGA for birth weight. Non-linear dose-response analyses (restricted cubic splines with 3 knots at fixed percentiles; 10th, 50th, and 90th) did not reveal departure from linearity for LBW, or SGA for birth weight, but for PTB there appeared to be a threshold with no further benefit of intake above 45 g/day.

Zhao et al. (2020) explored the heterogeneity by study design (cohort, case-control), geographic location (Europe, America, Asia, and Oceania), sample size (<3000 and ≥3000), study quality (<7 and ≥7, Newcastle Ottawa scale), methods of dietary assessment (self-administered FFQ and in-person interview), and adjustments for various confounders (maternal age, pre-pregnancy body mass index (BMI)/pre-pregnant weight, maternal smoking status, maternal alcohol consumption, energy intake, and use of fish oil).

Similar analyses were conducted for lean fish and fatty fish (Table 4.22.1.2-2) but based on few studies. In the linear dose-response analysis an increment of 45 g/day of lean fish intake was associated with a higher risk of LBW (OR: 3.51, 95% CI: 1.16 to 10.66) based on two studies only, and the result should be interpreted with caution.

A significant non-linear association was found between maternal fatty fish intake and the risk of PTB ($P_{\text{non-linearity}}=0.01$). The risk of PTB decreased from 0 to 30 g of fatty fish per day but began to increase when intake was above 30 g/day.

Table 4.22.1.2-2 Summary of results from meta-analysis on lean and fatty fish intake and birth outcomes (Zhao et al., 2020).

Outcome	Total no. of studies	Comparison	Summary OR (95 % CI)	Heterogeneity	Overall result
Lean fish					
Low birth weight (LBW)	2	Highest vs lowest	1.71 (0.95, 3.08)	$I^2=34.4\%$, $P=0.22$	No sig. assoc.
	2	Dose-response, increment 45 g/day	3.51 (1.16, 10.66)	$I^2=0.0\%$, $P=0.43$	An increment of 45 g/day lean fish positively related to LBW risk. However, the authors outlined that the results, which only included two studies, should be interpreted with caution
Preterm birth (PTB)	4	Highest vs lowest	1.05 (0.67, 1.66)	$I^2=66.3\%$, $P=0.03$	No sig. assoc.
	3	Dose-response, increment 45 g/day	0.98 (0.46, 2.08)	$I^2=63.6\%$, $P=0.06$	No sig. assoc.
Small for gestational age (SGA) for birth weight	4	Highest vs lowest	0.91 (0.73, 1.15)	$I^2=29.5\%$, $P=0.24$	No sig. assoc.
	2	Dose-response, increment 45 g/day	0.64 (0.22, 1.86)	$I^2=37.8\%$, $P=0.21$	No sig. assoc.
Fatty fish					
LBW	4	Highest vs lowest	0.84 (0.57, 1.23)	$I^2=0.0\%$, $P=0.79$	No sig. assoc.
	4	Dose-response, increment 45 g/day	0.92 (0.46, 1.82)	$I^2=0.0\%$, $P=0.69$	No sig. assoc.
PTB	5	Highest vs lowest	0.85 (0.65, 1.11)	$I^2=28.1\%$, $P=0.23$	No sig. assoc.
	4	Dose-response, increment 45 g/day	0.70 (0.42, 1.17)	$I^2=55.8\%$; $P=0.08$	A significant non-linear association was found between maternal fatty fish intake and the risk of PTB ($p_{\text{non-linearity}}=0.01$). The risk of PTB decreased from 0 to 30 g of fatty fish per day, but began to increase when intake was above 30 g/day

Outcome	Total no. of studies	Comparison	Summary OR (95 % CI)	Heterogeneity	Overall result
SGA for birth weight	5	Highest vs lowest	0.96 (0.68, 1.36)	$I^2=67.0\%$, $P=0.02$	No sig. assoc.
	3	Dose-response, increment 45 g/day	0.62 (0.35, 1.10)	$I^2=36.3\%$, $P=0.21$	No sig. assoc.

4.22.2 VKM's systematic review of primary studies on fish intake and birth outcomes

4.22.2.1 *Included studies*

We evaluated 26 studies graded A or B including 25 single studies (Amezcu-Prieto et al., 2018; Benjamin et al., 2019; Brantsaeter et al., 2012; Brantsaeter et al., 2017; Drouillet et al., 2009; Guldner et al., 2007; Halldorsson et al., 2007; Haugen et al., 2008, Heppe et al., 2011, Mendez et al., 2010; Mitchell et al., 2004; Mohanty et al., 2015; Mohanty et al., 2016; Muthayya et al., 2009; Nykjaer et al., 2019; Olsen et al., 1990; Olsen et al., 2002; Petridou et al., 1998; Ramon et al., 2009; Ricci et al., 2010, Rogers et al., 2004; Smid et al., 2019; Thorsdottir et al., 2004; Wang et al., 2021) and one large, pooled analysis (Leventakou et al., 2014) with one or more of the following newborn growth outcomes; preterm birth, small or large for gestational age (by weight, or length, or head circumference), and low or high birth weight (all binary outcomes), as well as gestational length, birth weight, birth length, head circumference, and ponderal index (continuous outcomes) (Figure 4.22-1).

Haugen et al. (2008) was excluded (replaced by Leventakou et al. 2014 and Brantsaeter et al. 2017). One recent publication was limited to a high-risk group (Smid et al., 2019) and excluded from the current summary of general population studies. A description of the remaining 23 studies (study name, design, time period, size of the study population, and dietary assessment method) can be found in Table 4.22.2.1-1).

Table 4.22.2.1-1 Overview of 23 studies that were evaluated for inclusion in weight of evidence analysis of birth outcomes.

Author, year country	Study name	Study design	Inclusion year(s) (enrolment)	Study size	Population characteristics	Dietary assessment method	Dietary assessment period
Amezcu-Prieto 2018, Spail		Case-control	2012-2015	553 cases		FFQ semi-quant, validated	Usual diet during pregnancy
Benjamin 2019, USA	National Birth Defects Prevention Study (NBDPS)	Secondary analysis of controls in NBDPS	1997–2011 (deliveries) to 1997-2013 (maternal interviews), 6-24 months delay	10 919 mother-infant pairs, singleton pregnancies	7.8% PTB and 7.7% SGA 18% smoking during pregnancy	FFQ, semi-quant, shortened version of Willett (Nurses' Health Study) FFQ, full version validated	Usual diet one year prior to pregnancy
Brantsaeter 2012, Norway	Norwegian Mother and Child Cohort Study (MoBa)	Birth cohort	2002-2008 (pregnancies)	62 099 mother infant pairs, singleton pregnancies	0.7% LBW. Mean BW: 3590 (SD 540) g	FFQ semi-quant, validated	Habitual intake, incl supplements, first 4–5 months of pregnancy, reported around 22nd week of gestation
Brantsaeter 2017, Norway	Norwegian Mother and Child Cohort Study (MoBa)	Birth cohort	2002-2008 (pregnancies)	67 007 mother-infant pairs, singleton pregnancies	5.4% PTB	FFQ semi-quant, validated	Habitual intake, incl supplements, first 4–5 months of pregnancy, reported around 22nd week of gestation
Drouillet 2009, France	EDEN mother-child cohort	Birth cohort	2003-2005 (pregnancies)	1805 mother-infant pairs	Mean birth weight (SE) 3284.0 (507.6) g, 26% smoking during pregnancy 26.2% overweight women	Two times FFQ semi-quant, validated	Usual diet the year before pregnancy and during the last three months of pregnancy (results only reported for diet before pregnancy)

Author, year country	Study name	Study design	Inclusion year(s) (enrolment)	Study size	Population characteristics	Dietary assessment method	Dietary assessment period
Guldner 2007*, France	PELAGIE mother-child cohort	Birth cohort	2002-2005 (pregnancies)	2 353 mother-infant pairs	4.2% PTB, 5.3% SGA, 3.1% LBW Mean birth weight (SD) girls 3324 g (SD 484) and boys 3448 g (498) 11.3% smoking at inclusion 11.8% were overweight women	FFQ semi-quant	Usual diet one year prior to pregnancy
Halldorsson 2007, Denmark	DNBC, Danish National Birth Cohort	Birth cohort	1996-2002 (pregnancies)	44 824 mother-infant pairs		Modified version of the FFQ used by the Danish Cancer Registry, validated	Frequency and type of fish during pregnancy
Heppe 2011, The Netherlands	Generation R Study	Birth cohort	2002-2006 (deliveries)	3 380 mother-infant pairs	Mean birth weight (SD) 3489 (556) g 4.7 % PTB 6.1 % SGA 4.0 % LBW	Modified version of the validated semi-quantitative FFQ of Klipstein-Grobusch et al.	Habitual intake over the prior 3 months, i.e. intake in the first trimester
Leventakou 2014, Europe	19 European cohort studies	Birth cohorts, pooled	1996-2011	151 880 mother-infant pairs	Range PTB 2.8% to 10.5%, Range LBW 1.7% to 6.4%	FFQs and questionnaires	Intake during pregnancy, except EDEN study (year before pregnancy)
Mendez 2010*, Spain	INMA, Sabadell	Birth cohort	2004-2006 (pregnancies)	592 mother-infant pairs	7.8% SGA	FFQ semi-quant, validated	Usual intakes since the start of pregnancy
Mitchell 2004, USA		Case-control study	1995-1997 (deliveries)	1 691		FFQ semi-quant based on the Life in New Zealand survey	Diet at about time of conception and in the last month of pregnancy

Author, year country	Study name	Study design	Inclusion year(s) (enrolment)	Study size	Population characteristics	Dietary assessment method	Dietary assessment period
Mohanty 2015, USA	Omega Study	Birth cohort	1996-2008 (pregnancies)	3 141 mother-infant pairs	Mean birth weight (SD) 3460.4 (534.6) g 5% smoking	FFQ semi-quant, validated	Usual periconceptional diet. Seafood Intake Scale (SIS) FFQ to determine usual intake of 35 types of seafood during the prior three-month period
Mohanty 2016, USA	Omega Study	Birth cohort	1996-2008 (pregnancies)	3 279 mother-infant pairs	8% PTB	FFQ semi-quant, validated	Usual periconceptional diet. Seafood Intake Scale (SIS) FFQ to determine usual intake of 35 types of seafood during the prior three-month period
Muthayya 2009, India		Birth cohort	2002-2006 (pregnancies)	676 mother-infant pairs	Mean birth weight all newborns 2.82±0.46 kg and LBW babies 2.18±0.30 kg	FFQ semi-quant, validated	Habitual dietary intake for the preceding 3 months of each trimester
Nykjaer 2019, UK	CARE Study	Birth cohort	2003-2006 (pregnancies)	1 208 mother-infant pairs	4% PTB 13% SGA 4% LBW Mean BW (SD) 3446 (537) g 16% smoking during pregnancy	FFQ semi-quant + 2x24 hour recall, FFQ validated for caffeine intake	Three surveys covering 1 st , 2 nd and 3 rd trimester
Olsen 1990, Denmark	Healthy Habits for Two	Retrospective cohort	1984-1987 (pregnancies)	11 980 mother-infant pairs	PTB 3.4% LBW 2.7% Mean BW (SD) 3577 (531) g	Questionnaire	Intake during the past month
Olsen 2002, Denmark	NA, Aarhus cohort	Retrospective cohort	1992-1996?	8 729 mother-infant pairs		Questionnaire	Habitual intake in pregnancy

Author, year country	Study name	Study design	Inclusion year(s) (enrolment)	Study size	Population characteristics	Dietary assessment method	Dietary assessment period
Petridou 1998, Greece	NA, Athens maternity clinics	Retrospective cohort	1995	400 mother infant pairs		FFQ semi-quant, validated	Habitual intake in pregnancy
Ramon 2009, Spain	INMA Valencia	Birth cohort	2004-2006 (deliveries)	554 mother-infant pairs	10.5% SGA for weight and 5.4% SGA for length Mean birth weight ($\pm$ SD) 3273 $\pm$ 487g 22% smoked during pregnancy BMI 23.9 $\pm$ 4.6 Mothers from other countries of origin accounted for 11.3% of the study population	2xFFQ semi-quant, modified version of the validated Harvard questionnaire adapted for Spanish population	FFQ1 covered habitual intakes since last menstrual period. FFQ2 covered habitual intake after FFQ1
Ricci 2010, Italy		Case-control study	1989-1999 (deliveries)	555 cases and 1 966 controls,	143 births were preterm SGA and 412 full-term SGA	FFQ, repeated in 400 women, validated	Weekly consumption in the period immediately before becoming pregnant and in the last month of pregnancy. (Results only given for food late in pregnancy)
Rogers 2004, UK	ALSPAC	Birth cohort	1991-1992 (deliveries)	11 585 mother-infant pairs		FFQ semi-quant	During pregnancy, "nowadays", distributed 32 week's gestation
Thorsdottir 2004, Iceland		Retrospective cohort study	1998	491	Mean birth weight (SD) 3 790 (506) g 16% smoked during pregnancy	FFQ semi-quant, validated. The amount eaten at each fish meal overestimated by 15%	Frequency during pregnancy

Author, year country	Study name	Study design	Inclusion year(s) (enrolment)	Study size	Population characteristics	Dietary assessment method	Dietary assessment period
Wang 2021, China	No name,	No name, Gansu Provincial Maternity and Child Care Hospital study	2010-2012 (deliveries)	10 179 mother- infant pairs	1019 PTBs in sample	FFQ by interview, 1- 3 days after delivery in most women	Habitual intake in pregnancy

The European pooled analysis by Leventakou et al. (2014) is a large study of 19 cohorts and 151 880 liveborn singleton births from 1996-2011. The studies, including both the national Norwegian (MoBa study) and Danish (DNBC) birth cohorts, were among twenty-nine invited cohorts identified from the European inventory of birth cohorts or from individual websites or published articles (assessed until June 2011). Seven cohorts did not reply, and three cohorts declined participation for reasons not related to the objective of the article. Information about fish consumption during pregnancy, gestational age and weight at birth were the minimum requirements for inclusion. The number of cohorts contributing to the different analyses varied according to data availability. Most birth outcomes were analyzed in relation to maternal intake in two ways; as a continuous variable (time per week) for all cohorts with outcome data, and for fish intake as a categorical variable with 3 levels (≥ 3 times/week, > 1 but < 3 times/week, and ≤ 1 time/week) excluding six cohort studies with insufficient data in each intake category ($< 5\%$ of sample). When available, VKM has mainly emphasized results from the categorical analysis and the highest versus lowest intake level for comparison with other studies and previous meta-analyses.

The main difference between the meta-analysis by Zhao et al. (2020), the pooled analysis by Leventakou et al. (2014), and VKM's inclusion of studies, is that Zhao et al. (2020) is based on a systematic literature review, whereas Leventakou et al. (2014) is an older pooled analysis of primary data from a European research collaboration. VKM treated Leventakou et al. (2014) as a multicenter study included among other primary studies, whereas Zhao et al. (2020) performed a systematic literature review independently of Leventakou et al. (2014). The results from the European cohorts in Leventakou et al. (2014) were only included in Zhao et al. (2020) if found elsewhere as a separate publication.

4.22.2.2 VKM's search compared to the previous meta-analysis and pooled analysis

Table 4.22.2.2-1 presents overlap between VKM's included primary studies, the one meta-analysis by Zhao et al. (2020) and the pooled analysis by Leventakou et al. (2014).

This table covers overlap for all the included birth outcomes preterm birth (PTB), small for gestational age (SGA), low birth weight (LBW), birth weight (BW), birth length (BL), and head circumference (HC).

Table 4.22.2.2-1 Overlap by sub-group of birth outcomes between VKM's included primary studies, one meta-analysis and one pooled analysis.

Author, year country (study)	Included by VKM	Included in Zhao, 2020	European cohorts included in pooled analysis, Leventakou, 2014
Amezcu-Prieto 2018, Spain	SGA	SGA	
Benjamin 2019, USA	PTB, SGA		
Brantsaeter, 2012, Norway (MoBa study)	BW, LBW, BL, HC	LBW (only for high vs low analysis, excluded for dose-response meta-analysis)	BW, LBW

Author, year country (study)	Included by VKM	Included in Zhao, 2020	European cohorts included in pooled analysis, Leventakou, 2014
Brantsaeter, 2017, Norway (MoBa study)	PTB	PTB	MoBa included for the analyses of early and late PTB
Burch, 2014, USA		PTB, LBW (only for high vs low analysis, excluded for dose-response meta- analysis)	
Canda, 2011, Tyrkia		SGA, LBW (only for high vs low analysis, excluded for dose-response meta- analysis)	
Drouillet, 2009, France (EDEN)	BW		SGA, BW
Guldner, 2007, France (PELAGIE)	PTB, BW, LBW	PTB, SGA, LBW	BW
Halldorsen, 2007, Denmark (DNBC)	SGA, BW, BL, HC	SGA (only for high vs low analysis, excluded for dose- response meta-analysis)	BW
Heppe, 2011, The Nedtherlands (Generation R)	PTB, SGA, BW, LBW, BL, HC	PTB, SGA, LBW	LBW
Klebanoff, 2011, USA		PTB (only for high vs low analysis, excluded for dose- response meta-analysis)	
Le Donne, 2016, Italy		PTB (only for high vs low analysis, excluded for dose- response meta-analysis)	
Mendez, 2010 (INMA, Sabadell)	SGA, BW	SGA	SGA, BW
Mitchell, 2004, USA	SGA	SGA	
Mohanty, 2015, USA	BW, LBW, BL, HC	LBW	
Mohanty, 2016, USA	PTB	PTB	
Muthayya, 2009, India	LBW	LBW (only for high vs low analysis, excluded for dose- response meta-analysis)	
Nykjaer, 2019, UK (CARE)	PTB, SGA, BW, LBW	PTB, SGA, LBW	
Olsen, 1990, Denmark	BW, BL, HC		
Olsen, 2002, Denmark	PTB, LBW	PTB, LBW	
Petridou, 1998, Greece	BW		
Ramon 2009, Spain (INMA, Valencia)	BW, BL, HC		SGA, BW
Ricci, 2010, Italy	SGA	SGA	
Rogers 2004, UK (ALSPAC)	PTB, LBW	PTB, LBW	
Rylander, 1996,		LBW	
Smid, 2019,		PTB (only for high vs low analysis, excluded for dose- response meta-analysis)	
Thorsdottir, 2004, Iceland	BW, BL, HC		
Wang, 2021	PTB		
Leventakou, 2014, Europe (pooled analysis)	PTB, SGA, BW, LBW, HBW, gestational days		For PTB: 13 cohorts not included in VKM or Zhao

Author, year country (study)	Included by VKM	Included in Zhao, 2020	European cohorts included in pooled analysis, Leventakou, 2014
			For SGA: 11 cohorts not included in VKM or Zhao

PTB=preterm birth, SGA=small for gestational age, LBW=low birth weight, BW=birth weight, BL=birth length, HC=head circumference.

4.23 Fish intake and preterm birth

4.23.1 VKM's search for published systematic reviews and meta-analyses on fish intake and preterm birth

See Chapter 4.22.1.

4.23.2 VKM's systematic review of maternal fish intake and preterm birth

4.23.2.1 *Included studies from search*

Eleven studies, ten single studies (Benjamin et al., 2019; Brantsaeter et al., 2017; Guldner et al., 2007; Haugen et al., 2008; Heppe et al., 2011; Mohanty et al., 2016; Nykjaer et al., 2019; Olsen et al., 2002; Rogers et al., 2004; Wang et al., 2021) and the pooled analysis by Leventakou (2014), reported results on maternal fish intake and preterm birth. In all studies, preterm birth was defined as birth before 37 weeks of gestation. Four of the studies also reported results on gestational length as a continuous outcome (Guldner et al., 2007; Leventakou et al., 2014; Mohanty et al., 2016; Olsen et al., 1990) which is described in brief under results.

4.23.2.2 *Overlapping publications*

Leventakou et al. (2014) included estimates of preterm birth from 13 unique European birth cohorts in relation to categories of fish intake. Other publications were checked for overlap. The Norwegian Mother, Father and Child Cohort Study (MoBa) was found to contribute data (all inclusion years) to the pooled analysis. In addition, there were two separate MoBa publications on PTB. The oldest (Haugen et al., 2008) did not cover all inclusion years and was excluded (replaced by Leventakou et al. 2014). The most recent publication (Brantsaeter et al., 2017) was also excluded from the main summary to not count the MoBa study twice but results not covered by Leventakou et al. (2014) on sub-categories of PTB and also on high-low intake of lean and fatty fish were included. The cohorts Generation R (the Netherlands) and Pelagie (France) were included in Leventakou et al (2014) but excluded from the analysis of fish intake categories due to data harmonization difficulties. Therefore,

the separate publications from these cohorts by Guldner et al. (2007) (Pelagie) and Heppe et al. (2011) (Generation R) were kept.

4.23.2.3 *Studies by design and geographic region*

The body of evidence on preterm birth (eight single studies and one pooled analysis, counting the MoBa study once) had a skewed geographic distribution between Europe (six studies in addition to Leventakou et al. (2014) with 12 European birth cohorts), and other continents with two studies from USA and one from China.

All studies from Europe were birth cohorts, whereas one study from USA was based on the control sample from a case-control study (Benjamin et al., 2019), and the other was a birth cohort (Mohanty et al., 2016). The study from China had a retrospective design based on pregnant women admitted to a childcare hospital for delivery, with dietary assessment after delivery.

4.23.2.4 *Studies by sub-groups and potential effect modification*

Maternal smoking, pre-pregnancy weight or BMI, parity and other factors did not modify the association between fish intake and preterm birth. Therefore, overall effect measure estimates were emphasized.

Three studies included additional sub-group analyses of preterm births classified as late, moderate or early preterm births (Benjamin et al., 2019; Brantsaeter et al., 2017). Wang et al. (2021) also included extremely preterm births (< 28 weeks). Brantsaeter et al. (2017) and Wang et al. (2021) also classified preterm births spontaneous or iatrogenic preterm births.

4.23.2.5 *Studies by fish exposure (type and timing)*

All studies, except Nykjaer et al. (2019), included total fish or seafood exposure (sum of all fish, unspecified fish, or fish including shellfish and/or fish products). Four studies (Brantsaeter et al., 2017; Heppe et al., 2011; Leventakou et al., 2014; Mohanty et al., 2016) presented sub-classification of fish intake as lean and fatty fish, and one study reported only fatty fish (Nykjaer et al., 2019). As the only study, Wang et al. (2021) included sub-types by aquatic environment (saltwater, freshwater) which was not summarized.

Regarding timing of maternal fish intake, Guldner et al. (2007) and Benjamin et al. (2019) investigated intake prior to pregnancy only, and Nykjaer et al. (2019) investigated 4 weeks prior to pregnancy in addition to during pregnancy. The remaining publications (Brantsaeter et al., 2017; Heppe et al., 2011; Leventakou et al., 2014; Mohanty et al., 2016; Olsen et al., 2002; Rogers et al., 2004; Wang et al., 2021) assessed habitual fish intake during pregnancy in one or more trimesters.

VKM used the results for categories of fish intake (high versus low intakes) for comparisons with other studies.

4.23.2.6 *Studies assessing potential non-linearity*

None of the included primary studies of maternal fish intake and risk of preterm birth presented a dose-response figure or dose-response information that could not be conveyed in the result tables.

4.23.3 Results from the included primary studies on preterm birth

4.23.3.1 *Studies of total fish intake and preterm birth*

We included eight publications with estimates of the association between total fish or total seafood intake prior to or during pregnancy and preterm birth. The pooled estimate in Leventakou et al. (2014) was based on 13 unique European cohorts. The exposure levels and results for all studies are included in Table 4.23.3.1-1.

Table 4.23.3.1-1 Results from studies included in the weight of evidence analysis of maternal total fish intake prior to or during pregnancy and preterm birth (PTB).

Author, year, country	Study design	Fish exposure	Intake unit	High-low intake	Total cases	RR high-low or continuous (95 % CI)	Overall result
Prior to pregnancy							
Benjamin, 2019, USA	Controls from case-control	Fish probably incl shellfish	Servings, 5 cat	$\geq 1/d$ vs $< 1/mo$	851	OR=0.7 (0.3, 1.6)	No sig. assoc. (no <i>P</i> -trend value)
Guldner, 2007, France	Birth cohort	Fish only	Frequency, 3 cat	$\geq 2/wk$ vs $< 1/mo$	94	OR=0.71 (0.35, 1.46)	No sig. assoc., <i>P</i> -trend 0.2
During pregnancy							
Heppe, 2011, The Netherlands	Birth cohort	Fish only	g/wk, 5 cat	> 210 vs 0 g/wk	159	OR=1.21 (0.61, 2.38)	No sig. assoc., <i>P</i> -trend 0.82
Leventakou, 2014, Europe	Pooled analysis of 13 birth cohorts	Fish only	Times/wk, 3 cat	≥ 3 vs ≤ 1 time/wk	Range preterm births 2.8 to 10.5%, sample 140 337	Pooled RR=0.89 (0.84, 0.96), $I^2 \leq 25\%$, $P_{\text{heterogeneity}}=0.55$. Fixed-effect meta-analysis	Similar protective associations for intake > 1 but < 3 times/wk and ≥ 3 times/week, vs ≤ 1 time/wk
	Pooled analysis of 19 birth cohorts		Times/wk, Continuous, per 1-time/wk increase		Range preterm births 2.8 to 10.5%, sample 151 880	Pooled RR=1.00 (0.97, 1.03), $I^2 > 25\%$, $P_{\text{heterogeneity}}=0.008$. Random effects meta-analysis	No sig. assoc., with sig. between study heterogeneity
Mohanty, 2016, USA	Birth cohort	Seafood	Servings/mo or wk, 4 cat	$> 1/$ wk vs $< 0.2/$ month	259, preterm births 8%	RR (Poisson)=1.76 (1.00, 3.09)	Sig. or borderline sig. adverse assoc. in all intake categories above reference, <i>P</i> -trend 0.37
Olsen, 2002, Denmark	Birth cohort	Fish	Frequency hot meals and sandwiches containing fish, 4 cat	$\geq 1/wk$ vs 0	299	OR=0.3 (0.1, 0.9) originally reported as low vs high OR 3.60 (1.15, 11.20)	Protective association, <i>P</i> -trend 0.003

Author, year, country	Study design	Fish exposure	Intake unit	High-low intake	Total cases	RR high-low or continuous (95 % CI)	Overall result
Rogers, 2004, UK	Birth cohort	Fish only	Portions, 4 cat	4.44 portions/wk vs 0	434	OR=1.32 (0.88, 1.92), originally reported as low vs high OR 0.76 (0.52, 1.13)	No sig. assoc., <i>P</i> -trend 0.827
Wang, 2021, China	Retrospective study	Fish, incl shellfish	g/wk, quartiles	>176 g/wk vs <14 g/wk	1019	OR=0.54 (0.43, 0.67)	Protective assoc., <i>P</i> -trend 0.003

Of the six studies that investigated maternal fish intake during pregnancy and preterm births, three studies reported significant lower risks in the highest fish consumption categories as compared to the lowest categories (Leventakou et al., 2014; Olsen et al., 2002; Wang et al., 2021). The other studies found no association (Heppe et al., 2011, Rogers et al., 2004) or significantly higher risk (Mohanty et al., 2016). The Norwegian MoBa study was included in the pooled analysis of preterm birth by Leventakou et al. (2014). Two studies of maternal intake prior to pregnancy (Benjamin et al., 2019; Guldner et al., 2007) reported associations on the protective side, but not statistically significant.

4.23.3.2 *Studies of total fish intake and sub-categories of preterm birth*

The MoBa study (Brantsaeter et al., 2017) reported estimates of similar magnitude for total seafood intake in relation to risk of late (35 to <37 weeks), moderate (32 to <34 weeks) and early (22 to <32 weeks) onset of preterm delivery (although only statistically significant for late onset). Estimates were also similar for spontaneous and iatrogenic preterm deliveries. Benjamin et al. (2019) observed no association between fish consumption prior to pregnancy and risk of all preterm births, or when restricted to early onset of preterm birth (<32 weeks and <35 weeks). Wang 2021 reported a protective association of fish and shellfish intake with preterm birth that was stronger for very preterm (28-< 32 weeks) than moderate preterm births. Estimates for spontaneous and medically indicated preterm births were similar and differed little from the overall estimate.

4.23.3.3 *Studies of lean and fatty fish intake and preterm birth*

We included four publications (all prospective, observational studies) on preterm birth in the weight of evidence analysis for an association with intake of lean fish, and five publications on intake of fatty fish (six estimates). The pooled analysis by Leventakou et al. (2014) presented estimates for intake on a continuous scale (times per week) based on 10 unique European cohorts for lean fish, and 11 unique European cohorts for fatty fish. Estimates for the highest versus lowest intake were available in other studies. One study of fatty fish (Nykjaer et al., 2019) presented results by different time points (prior to pregnancy and by trimester).

The exposure levels and results (high-low odds ratio, and overall results) are included in Table 4.23.3.3-1.

Table 4.23.3.3-1 Results from birth cohort studies included in the weight of evidence analysis of maternal lean and fatty fish intake prior to or during pregnancy and preterm birth (PTB).

Author, year, country	Intake unit	High-low intake, or continuous	Total cases	RR high-low or continuous (95 % CI)	Overall result
Lean fish					
Brantsaeter, 2017, Norway	g/d (servings/wk), 5 cat	>60 g/d vs ≤5 g/d (≥3/wk vs ≤never/rarely)	3 630	HR=0.91 (0.67, 1.23)	Sig. protective assoc. for >5 to 60 g/d, but not highest category, <i>P</i> -trend 0.005
Heppe, 2011, The Netherlands	g/wk, 4 cat	>70 vs 0 g/wk	159	OR=0.61 (0.33, 1.10)	No sig. assoc., <i>P</i> -trend 0.15
Leventakou, 2014, Europe*	Times/wk	Continuous, per 1-time/wk increase	Range PTB 2.8 to 10.5%, sample 129,886	Pooled RR=1.00 (0.96, 1.05), <i>P</i> _{heterogeneity} =0.03. Random effects meta-analysis	No sig. assoc., with sig between study heterogeneity
Mohanty, 2016, USA	Servings/mo or wk, 4 cat	>1/ wk vs <0.2/ mo	258, occurrence of PTB 8%	RR (Poisson) 1.59 (1.06, 2.37)	Adverse assoc. for highest intake, <i>P</i> -trend 0.04
Fatty fish					
Brantsaeter, 2017, Norway	g/d (servings/wk), 5 cat	>60 g/d vs ≤5 g/d (≥3/wk vs ≤never/rarely)	3 630	HR=1.02 (0.80, 1.31)	Sig. or borderline sig protective assoc. for intake >5 to 40 g/d but not higher, <i>P</i> -trend 0.41
Heppe, 2011, The Netherlands	g/wk, 4 cat	>70 vs 0 g/wk	159	OR=0.87 (0.54, 1.42)	No sig. assoc., <i>P</i> -trend 0.15
Leventakou, 2014, Europe*	Times/wk	Continuous, per 1-time/wk increase	131 651, range PTB 2.8 to 10.5%	Pooled RR=1.04 (0.98, 1.09) (95% CI), <i>P</i> _{heterogeneity} =0.02. Random effects meta-analysis	No sig. assoc., with sig between study heterogeneity
Mohanty, 2016, USA	Servings/mo or wk, 4 cat	>1/ wk vs <0.2/mo	Occurrence of PTB 8%	RR 0.76 (0.50, 1.15), poisson	No sig. assoc., <i>P</i> -trend 0.13
Nykjaer, 2019, UK	Portions, 3 cat	>2/wk vs 0	43 (first trimester)	OR 0.3 (0.1, 1.3)	No sig. assoc., <i>P</i> -trend 0.3
	Portions, 3 cat	>2/wk vs 0	35 (second trimester)	OR 1.1 (0.4, 3.6)	No sig. assoc., <i>P</i> -trend 0.2
	Portions, 3 cat	>2/wk vs 0	26 (third trimester)	OR 0.7 (0.2, 2.8)	No sig. assoc., <i>P</i> -trend 0.6

Author, year, country	Intake unit	High-low intake, or continuous	Total cases	RR high-low or continuous (95% CI)	Overall result
	Portions, 3 cat, prior to pregnancy	>2/wk vs 0	44 (4 weeks prior to pregnancy)	OR 0.4 (0.1, 1.5) (95% CI)	No sig. assoc., <i>P</i> -trend 0.4

*Pooled analysis of 12 birth cohorts

For lean fish, none of the studies reported a statistically significant association for the highest versus lowest intake category, except in Mohanty 2016 where the association was adverse. Brantsaeter 2017 (MoBa study) reported protective association for intake below the highest category (< 60g/day). However, the MoBa study was also included in Leventakou 2014 where the overall result on lean fish (continuous, per 1-time/wk increase) was non-significant.

For fatty fish, there were no reports of statistically significant associations for the highest versus lowest intake. Brantsaeter 2017 reported a protective association for fatty fish intake below 40 g/day, but the overall result in Leventakou 2014 (continuous, per 1-time/week increase), including the MoBa study, was non-significant.

4.23.3.4 *Studies of fish intake and gestational age*

Four studies reported results on fish intake in relation to gestational length as a continuous outcome (Guldner et al., 2007; Leventakou et al., 2014; Mohanty et al., 2016; Olsen et al., 1990). In the pooled analysis by Leventakou et al. (2014) (protective association with PTB), intake ≥ 3 times/week was associated with a higher gestational age of 0.2 days (95% CI: 0.1, 0.4 d) and intake >1 but < 3 times/week of 0.4 days (95% CI: 0.3, 0.6 d) versus the reference (≤ 1 time/week) on average. Mohanty et al. (2016) (significant adverse association with PTB) and Olsen et al. (1990) (significant protective association with PTB) did not report significant differences in gestational age (weeks or days) for the highest versus lowest intake. Guldner et al. (2007) (no significant association of maternal intake prior to pregnancy with PTB), found a small increase in gestational age with higher intakes.

4.23.3.5 *Summary relative risk (RR) based on VKM's inclusion of primary studies*

VKM calculated a summary RR for PTB in relation to the highest versus lowest intake of total fish (or seafood in one study) during pregnancy (Table 4.23.3.1-1). Four cohort studies, three European (Heppe et al., 2011; Olsen et al., 2002; Rogers et al., 2004) and one US birth cohort (Mohanty et al., 2016) were added to the 13 cohort-specific estimates in Leventakou et al. (2014). The pooled RR (high-low) in Leventakou et al. (2014) (RR=0.89, 95% CI: 0.84, 0.96, $p_{\text{heterogeneity}}=0.55$), was attenuated and lost significance when VKM added these four studies (RR=0.96, 95% CI: 0.84, 1.09, $p_{\text{heterogeneity}}=0.08$).

In addition to the four prospective cohort studies, there was one retrospective study reporting a statistically significant protective association (Wang 2021, China), and two additional studies of intake prior to pregnancy with estimates on the protective side (Benjamin et al., 2019; Guldner et al., 2007). Adding all studies (five of intake in pregnancy, including the retrospective study, and two of intake prior to pregnancy) to Leventakou et al. (2014) made VKM's high-low summary estimate similar to Leventakou et al. (2014) in magnitude and borderline statistically significant, but with significant heterogeneity (RR=0.89, 95% CI: 0.77, 1.04, $p_{\text{heterogeneity}} = 0.001$).

The high-low estimate in the meta-analysis by Zhao et al. (2020) was not statistically significant (11 studies, RR=0.90, 95% CI 0.72, 1.14, $p_{\text{heterogeneity}} < 0.001$) whereas the linear dose-response analysis (per 45 g/day increment) was borderline protective with less heterogeneity (7 studies, RR=0.84, 95% CI 0.70, 1.01, $p_{\text{heterogeneity}}=0.09$). There was significant departure from linearity (Table 4.22.1.2-1).

VKM's high-low summary estimates for maternal intake of lean fish based on three cohort studies (RR=0.83, 95% CI 0.64, 1.08, $p_{\text{heterogeneity}}=0.44$) and fatty fish based on four cohort studies (RR=0.89, 95% CI 0.70, 1.13, $p_{\text{heterogeneity}}=0.29$) were not statistically significant, and without significant heterogeneity.

In comparison, Leventakou et al. (2014) did not find an association between maternal intake of lean fish on a continuous scale (per 1-time/week increase) and PTB (no cohort specific estimates provided), nor did Zhao et al. (2020) (4 studies in the high-low analysis, and 3 studies in the dose-response analysis). Conclusions were similar for fatty fish: Leventakou et al. (2014) did not find an association between maternal intake of fatty fish on a continuous scale (per 1-time/week increase) and PTB (no cohort specific estimates provided). Zhao et al. (2020) (5 studies in the high-low analysis, and 4 studies in the dose-response analysis) reported a slight decrease in risk of PTB from 0 to 30 g of fatty fish intake per day in the non-linear dose-response analysis. The dose-response analyses by Zhao et al. (2020) were based on studies that also met VKM's eligibility criteria but results from Leventakou et al. (2014) were not considered by Zhao et al. (2020).

4.23.3.6 VKM's search compared to the previous meta-analysis and pooled analysis on preterm birth

An overview of overlapping studies from VKM's search on birth outcomes, including the pooled analysis of European birth cohorts (Leventakou et al., 2014), and the systematic review and meta-analysis by Zhao et al. (2020), is given Table 4.22.2.2-1.

VKM identified two recent primary studies of PTB (Benjamin et al., 2019; Wang et al., 2021) not included in the meta-analysis by Zhao et al. (2020). Among the 11 studies in Zhao et al. (2020), all were identified by VKM, but four were excluded after quality assessment (Le Donne et al., 2016), or because studies were limited to high-risk populations (Klebanoff et al., 2011 and Smid et al., 2019), or used proxies (geographic location) for dietary exposure (Burch et al., 2014). In addition, VKM included the pooled analysis by Leventakou et al. (2014), which was mentioned by Zhao et al. (2020) but not included among the primary studies.

4.23.4 Heterogeneity maternal fish intake and preterm birth

In Leventakou et al. (2014), heterogeneity was high in the analysis of fish intake on a continuous scale using the maximum number of studies (19 studies, $I^2 > 25\%$, $p_{\text{heterogeneity}}=0.008$), but not statistically significant in the high-low analysis emphasized by VKM (13 studies, $I^2=0\%$, $p_{\text{heterogeneity}}=0.55$). VKM's high-low summary estimate indicated

borderline significant heterogeneity ($P=0.08$) when new studies were added to the analysis of harmonized exposure categories in Leventakou et al. (2014).

Zhao et al. (2020) reported significant heterogeneity in the high-low analysis. This analysis included studies excluded by VKM for reasons described above. In the linear dose-response analysis based on 7 studies with adequate data, heterogeneity was lower (I-squared 44.6%, P -value 0.09 compared with I-squared 76.7%, P -value <0.001 in the high-low analysis) and estimates were generally consistent in the protective direction, except in one US study with a significant adverse association (Mohanty et al., 2016). This study was included by both Zhao et al. (2020) and by VKM, but not Leventakou et al. (2014) (European studies only).

4.23.5 Dose-response relationship maternal fish intake and preterm birth

Leventakou et al. (2014) meta-analyzed fish intake on a continuous scale and as categories (high-low of ≥ 3 times/gweek vs ≤ 1 time/week, and mid-range of > 1 but < 3 times/week vs ≤ 1 time/week). There was no association on the continuous scale, and the protective association in the categorical analysis was of similar magnitude for each category. Thus, there was no evidence of a linear gradient in the association of maternal seafood intake with risk of PTB, but results are compatible with a potential threshold effect.

Zhao et al. (2020) found an average protective association of maternal seafood intake with risk of PTB in their linear meta dose-response analysis (16% reduced risk of PTB with each 45 g/day increase of seafood consumption, borderline significant) but with significant departure from linearity. The non-linear dose-response analysis (for intake up to 80 g/day) suggested a threshold with little or no further risk reduction for intakes above 45 g/day. The dose-response analysis was based on cohort studies of maternal intake of seafood prior to (one study) or during pregnancy, including one study of fatty fish. For fatty fish only, the linear analysis showed no significant association overall, but in the non-linear analysis the risk of PTB decreased slightly from 0 to 30 g of fatty fish intake per day. For higher intakes, the confidence limits of the curve were too wide to determine a dose-response relationship. Zhao et al. (2020) included some studies that VKM excluded due to quality issues, but these studies did not have sufficient dose-response data and did therefore not affect the meta dose-response analysis.

4.23.6 Weight of evidence for maternal fish intake and preterm birth

Published evidence on maternal fish intake prior to or during pregnancy and PTB

The association of maternal fish intake with risk of PTB has been examined in a large number of birth cohorts from Europe, with less evidence from other populations and other study designs. The results depend on the methods of analysis. Leventakou et al. (2014) (pooled analysis) found no association in the analysis of fish intake on a continuous scale (19 studies, 151 880 participants), but when using harmonized intake categories (13 of 19 studies, 140 337 participants) there was a protective association for each intake level relative

to the reference (high-low of ≥ 3 times/week vs ≤ 1 time/week, and mid-range of > 1 but < 3 times/week vs ≤ 1 time/week). This analysis excluded six studies due to data harmonization issues, but the exclusion did not reduce the study sample by more than 8%. The magnitude of the protective estimate did not depend on a specific study in the influence (leave-one-out) analysis but became borderline significant without the national Norwegian birth cohort (MoBa, 56% relative weight). Similar to VKM, Zhao et al. (2020) did not find a statistically significant association in the high-low analysis, but there was a protective association in the linear and non-linear dose-response analyses (7 studies, 87 625 participants).

In general, analyses based on the maximum number of studies (pooled analysis of fish on a continuous scale, or high-low meta-analysis by VKM and by Zhao et al., 2020) do not show statistically significant associations for total fish intake, whereas analyses restricted to studies with similar intake categories, or sufficient data for dose-response meta-analysis, show protective associations. The association could be non-linear as suggested by the dose-response meta-analysis of seven studies. Results on fatty and lean fish are based on fewer studies and do not show statistically significant associations, except in one dose-response meta-analysis of fatty fish intake (four studies).

In three studies of sub-categories of PTB, there was little difference or gradient in results for late versus moderate or early onset PTB. Four studies had results on both PTB and gestational age in days. The pooled analysis by Leventakou et al. (2014) showed a small average increase of less than half a day (upper 95% CI of 0.6 days) for intake in the high- or mid category. In the remaining studies, results on gestational age did not clearly reflect results on PTB.

Heterogeneity

Between-study heterogeneity is significant ($P_{\text{heterogeneity}} < 0.05$) or borderline significant in the high-low meta-analyses performed by VKM and Zhao et al. (2020) that do not show statistically significant associations. In analyses restricted to studies with similar intake categories, or with sufficient data for dose-response meta-analysis, associations are more consistent in the protective direction, with lower heterogeneity.

Mechanisms/biological plausibility

LC n-3 fatty acids may reduce the risk of early preterm birth (see Chapter 5.2 on mechanisms). The current summary of the evidence did not reflect a stronger association with fatty fish than with lean fish, but the evidence was more limited than for total fish and seafood.

Upgrading factors

A dose-response relation was the only upgrading factor identified.

There is evidence of a biological gradient with a potential threshold from two studies. Zhao et al. (2020) (non-linear dose-response meta-analysis) found reduced risk of PTB for

maternal intake of seafood up to 80 g/day, but with no further reductions after 45 g/day (relative to no intake). Leventakou et al. (2014) (pooled categorical analysis) found similar risk in women eating fish > 1 times/week and ≥3 times/week (relative to vs ≤1 time/week).

4.23.6.1 *Conclusion weight of evidence maternal fish intake and preterm birth*

There is evidence from more than two independent and good quality cohort studies on maternal intake of total fish or seafood (VKM included eight publications of which one was a large, pooled analysis, and one previous meta-analysis of seafood intake with a dose-response analysis).

VKM's summary RR for the highest versus lowest intake of total fish during pregnancy, does not show an overall association with risk of PTB, whereas a dose-response meta-analysis (seven cohort studies) indicates that intake of fish/seafood protects against PTB. The dose-response meta-analysis utilizes more data than high-low analysis, but from fewer studies because the data reporting requirements are higher. The dose-response meta-analysis has been given more weight by VKM. Because there is evidence for biological plausibility and a dose-response relationship, the evidence is graded "probable" for a protective effect of maternal fish consumption during pregnancy on risk of PTB.

No conclusion could be drawn for fatty and lean fish due to inconsistent evidence. VKM's summary RRs for the highest versus lowest intake of lean fish and fatty fish were not statistically significant but on the protective side for risk of PTB. The pooled analysis (13 European cohort studies) reported no overall associations for fatty and lean fish intake analyzed using a continuous scale. The dose-response meta-analysis (four studies) reported decreasing risk of PTB for intakes of fatty fish up to 30 g per day, and no association for lean fish.

4.24 Maternal fish intake and small or large for gestational age

4.24.1 VKM's search for previous systematic reviews and meta-analyses

See Chapter 4.22.1.

4.24.2 VKM's systematic review of primary studies of maternal fish intake and small or large for gestational age

4.24.2.1 *Included studies from search*

A total of 12 publications, 11 single studies (Amezcu-Prieto et al., 2018; Benjamin et al., 2019; Drouillet et al., 2009; Guldner et al., 2007; Halldorsson et al., 2007; Heppe et al., 2011; Mendez et al., 2010; Mitchell et al., 2004; Nykjaer et al., 2019; Ramon et al., 2009;

Ricci et al., 2010) and one pooled analysis (Leventakou et al., 2014) reported results for fish intake and SGA for weight. Two studies additionally reported SGA for birth length and SGA for head circumference (Halldorsen et al., 2007; Leventakou et al., 2014). One publication was excluded from all analysis of SGA (Drouillet et al., 2009) due to study overlap (described below) leaving 11 publications for further analysis. The excluded study (Drouillet et al., 2009) was the only that investigated large for gestational age (LGA), which was considered insufficient for a conclusion.

In Leventakou et al. (2014) (pooled analysis), neonatal weights were defined as SGA if they were below the 10th percentile of the cohort-specific growth curves stratified by gestational length and sex. The same method was used to define SGA for length and head circumference. All single studies except one (Heppe et al., 2011) also defined SGA as gestational age and sex-adjusted birth anthropometrics below the 10th percentile for the infants' gestational age, according to either national standard curves or within the cohort. In Heppe 2011, SGA was defined as gestational age and sex-adjusted birth weight below the 5th percentile in the study cohort.

4.24.2.2 *Overlapping publications*

Leventakou et al. (2014) reported a pooled estimate for maternal fish intake (categorized) and SGA for weight from 11 unique European birth cohorts. Two studies (EDEN, France and INMA, Spain) were found to contribute data to the pooled analysis, and separate publications from these studies (Drouillet et al., 2009; Mendez et al., 2010; Ramon et al., 2009) were excluded from main summaries to not count the same studies twice. However, two publications from INMA (Mendez et al., 2010; Ramon et al., 2009) were kept for results on contaminants (described separately). Although GenerationR (the Netherlands), Pelagie (France), and the Danish National Birth Cohort (DNBC) were included in Leventakou et al. (2014), these cohorts did not contribute to the analysis of SGA due to difficulties in harmonizing categories of fish intake, or missing data on SGA (DNBC). Therefore, the separate publications from these cohorts by Guldner et al. (2007) (Pelagie), Halldorssen et al. (2007) (DMBC), and Heppe et al. (2011) (GenerationR) were kept.

4.24.2.3 *Studies by design and geographic region*

The body of evidence (8 single studies and 1 pooled analysis) on SGA had a skewed geographic distribution between Europe (6 studies in addition to Leventakou et al., 2014 with 11 cohorts), USA (1 study), and New Zealand (1 study). Studies from Europe were mainly birth cohorts, except 2 case-control studies (from Italy and Spain). The 2 studies from USA and New Zealand were both case-control studies. In total 4 of 9 studies were based on a case-control design.

4.24.2.4 *Studies by sub-groups and potential effect modification*

There were no reports of significant effect modification by maternal smoking, pre-pregnancy weight or BMI, parity and other factors tested for among the included studies of fish intake with SGA. Therefore, overall estimates were emphasized.

Two studies presented results on SGA for the sub-group of full-term infants in addition to all infants (Benjamin et al., 2019; Ricci et al., 2010), and two studies limited all results on SGA to term infants (Halldorsson et al., 2007, Mitchell et al., 2004).

4.24.2.5 *Studies by fish exposure (type and timing)*

All studies, except Nykjaer et al. (2019), included total fish or seafood exposure (sum of all fish, unspecified fish, or fish including shellfish and/or fish products). Four studies, three single studies (Amezcu-Prieto et al., 2018; Halldorsson et al., 2007; Heppe et al., 2011) and the pooled analysis (Leventakou et al., 2014) reported on lean fish. Five studies reported on fatty fish (all studies of lean fish, and Nykjaer et al., 2019 on fatty fish only). Two publications that focused on contaminants (INMA multi-center study), presented results on canned tuna (Mendez et al., 2010; Ramon et al., 2009).

With regard to timing of intake, Benjamin et al. (2019) and Guldner et al. (2007) investigated overall fish intake prior to pregnancy only. Nykjaer et al. (2019) surveyed fatty fish consumption 4 weeks prior to pregnancy in addition to during pregnancy. Remaining studies investigated habitual fish intake during pregnancy in one or more (Mitchell et al., 2004) trimesters.

VKM used the results for categories of fish intake high versus low intakes for comparisons with other studies.

4.24.2.6 *Studies assessing potential non-linearity*

None of the included primary studies presented a dose-response figure of the relation between maternal fish intake and risk of SGA.

4.24.2.7 *Studies with converted risk estimates*

Most studies presented risks estimates for SGA for categories of fish intake, with the lowest category as the reference. When the reference was the highest (Mitchell et al., 2004) we recalculated the estimate by setting the lowest intake category as reference to facilitate comparisons with other studies, and with high-low forest plots in the meta-analysis.

4.24.3 Results from the included primary studies maternal fish intake and SGA

4.24.3.1 *Studies of maternal total fish intake and SGA*

We included 8 publications with estimates of the association between total fish or total seafood intake and SGA in the weight of evidence analysis (Amezcu-Prieto et al., 2018; Benjamin et al., 2019; Guldner et al., 2007; Halldorsson et al., 2007; Heppe et al., 2011, Leventakou et al., 2014; Mitchell et al., 2004; Ricci et al., 2010). The pooled analysis by Leventakou et al. (2014) presented overall estimate for 11 unique European birth cohorts, including the Norwegian MoBa study. The exposure levels and results (high-low odds ratio, and overall results) are included in Table 4.24.3.1-1.

Table 4.24.3.1-1 Results from studies included in the weight of evidence analysis of maternal total fish intake prior to or during pregnancy and small for gestational age (SGA).

Author, year, country	Study design	Intake unit	High-low intake	SGA for weight, length or HC	Total cases	RR high-low (95 % CI)	Overall result
Prior to pregnancy							
Benjamin, 2019, USA*	Case-control	Servings, 5 cat	≥1/day vs <1/month	For weight	824	All, OR 2.1 (1.2, 3.4)	Adverse assoc. for the highest intake
			≥1/day vs <1/month	For weight	778	Full-term OR 2.2 (1.3, 3.6)	Adverse assoc. for the highest intake
Guldner, 2007, France	Birth cohort	Frequency, 3 cat	≥2/wk vs <1/month	For weight	120	All, OR 0.57 (0.31, 1.05)	No sig. assoc., <i>P</i> -trend 0.2
During pregnancy							
Amezcu-Prieto, 2018 Spain**	Case-control	Quintiles, 5 cat	>121 vs ≤56 g/day	For weight	518	All, OR 0.63 (0.41, 0.98)	Reduced risk with higher intakes, <i>P</i> -trend 0.025
Halldorsson, 2007, Denmark	Birth cohort	g/d, 5 cat	>60 vs ≤5 g/d	For weight	NA	Full-term, OR 1.24 (1.03, 1.49)	Increased risk with higher intakes, <i>P</i> -trend 0.08
			>60 vs ≤5 g/d	For length	NA	Full-term, OR 1.20 (1.00, 1.45)	Increased risk with higher intakes, <i>P</i> -trend 0.07
			>60 vs ≤5 g/d	For HC	NA	Full-term, OR 1.21 (1.01, 1.43)	Increased risk for highest intake, <i>P</i> -trend 0.20
Heppe, 2011, the Netherlands	Birth cohort	g/wk, 5 cat	>210 vs 0 g/wk	For weight	205	All, OR 0.67 (0.34, 1.30)	No sig. assoc., <i>P</i> -trend 0.19
Leventakou, 2014***, Europe	Pooled analysis of cohorts	Times/wk, 3 cat	≥3 vs ≤1 time/wk	For weight, 11 studies	Range SGA 3.2% to 12.3% (total sample 81 754)	All, pooled RR 0.95 (0.89, 1.02)	No sig. assoc., <i>P</i> _{heterogeneity} =0.56, <i>I</i> ² =0%. Fixed effects meta-analysis
			≥3 vs ≤1 time/wk	For length, 6 studies	NA (total sample 66 725)	All, pooled RR 0.97 (0.90, 1.06)	No sig. assoc., <i>P</i> _{heterogeneity} =0.47, <i>I</i> ² =0%. Fixed effects meta-analysis

Author, year, country	Study design	Intake unit	High-low intake	SGA for weight, length or HC	Total cases	RR high-low (95 % CI)	Overall result
			≥3 vs ≤1 time/wk	For HC, 5 studies	NA (total sample 66 838)	All, pooled RR 1.06 (0.97, 1.16)	No sig. assoc., $I^2_{\text{heterogeneity}}=0.78$, $P=0\%$. Fixed effects meta-analysis
Mitchell, 2004, New Zealand*	Case-control	Frequencies, 3 cat	>1/wk vs 0, at time of conception	For weight	529	Full term, OR 0.59 (0.37, 0.93) originally reported as low vs high OR 1.69 (1.07, 2.69)	Protective assoc. for highest intake
			>1/wk vs 0, last mo of pregnancy	For weight	529	Full term, OR 0.77 (0.50, 1.20) originally reported as low vs high OR 1.29 (0.83 to 1.99)	No sig. assoc.
Ricci, 2010, Italy	Case-control	Frequencies, 3 cat	≥2/ wk vs 0	For weight	555	All, OR 0.8 (0.6, 1.0)	Borderline protective assoc. for higher intake, $P_{\text{trend}}=0.045$, chi-square, trend 4.0
			≥2/ wk vs 0	For weight	412	Full-term deliveries, OR 0.7 (0.4, 1.1)	No sig assoc., $P_{\text{trend}} 0.11$
			≥2/ wk vs 0	For weight	143	Preterm deliveries, OR 0.8 (0.6, 1.1)	No sig assoc., $P_{\text{trend}} 0.09$

*Including shellfish. **Total seafood. ***Pooled analysis of 12 birth cohorts.

Of the five single studies that investigated the association between maternal fish consumption during pregnancy and SGA for weight, one study reported significant higher risk for SGA in the highest fish consumption category (Halldorsson et al., 2007), three studies reported reduced risk (Amezcu-Prieto et al., 2018; Mitchell et al., 2004; Ricci et al., 2010) and one study no association (Heppe et al., 2011) compared to the lowest category. Ricci et al. (2010) reported results for fish consumption late in pregnancy. Mitchell et al. (2004) reported results for fish consumption at time of conception and late in pregnancy and found no effect from fish consumption late in pregnancy. The pooled analysis of 11 European cohorts found no association between fish consumption (categories of intake) and SGA. Two studies investigated the association between maternal fish consumption prior to pregnancy (Benjamin et al., 2019; Guldner et al., 2007). Benjamin et al. (2019) reported significantly higher risk of SGA, whereas Guldner et al. (2007) found a borderline reduced risk of SGA (high-low analysis).

In two studies that reported on SGA for all three measures (weight, length, and head circumference), results were consistent within studies, but not between studies. Leventakou et al. (2014) found no significant associations, but Halldorsson et al. (2007) reported adverse associations for all measures (high-low analysis).

4.24.3.2 *Studies of lean and fatty fish and SGA*

We included four publications on SGA in the weight of evidence analysis for an association with intake of lean fish, and five publications on intake of fatty fish. Nykjaer et al. (2019) reported results for fatty fish only, both prior to pregnancy and by trimester. The pooled analysis by Leventakou et al. (2014) presented overall estimate for 10 unique European cohorts for lean fish, and 11 unique European cohorts for fatty fish. The exposure levels and results (high-low relative risk, and overall results) are included in Table 4.24.3.2-1.

Table 4.24.3.2-1 Results from studies included in the weight of evidence analysis of maternal lean and fatty fish intake and small for gestational age (SGA).

Author, year, country	Study design	Intake unit	High-low intake	Total cases	SGA for weight, length or HC	RR high-low or continuous (95 % CI)	Overall result
Lean fish							
Amezcu-Prieto, 2018	Case-control	g/week	100-150 g portions >1/wk vs never	518	Not specified	OR 0.53 (0.28 to 1.01)	Borderline protective assoc. for the highest intake
Halldorsson, 2007, Denmark	Birth cohort	g/d, 5 cat	>60 vs ≤5 g/d	NA	For weight	OR 0.96 (0.86, 1.06)	No sig. assoc., <i>P</i> -trend 0.51
		g/d, 5 cat	>60 vs ≤5 g/d	NA	For length	OR 1.10 (0.99, 1.22)	Borderline adverse assoc. for higher intakes, <i>P</i> -trend 0.05
		g/d, 5 cat	>60 vs ≤5 g/d	NA	For HC	OR 0.9 (0.89, 1.08)	No sig. assoc., <i>P</i> -trend 0.66
Heppe, 2011, the Netherlands	Birth cohort	g/wk, 5 cat	>70 vs 0 g/wk	205	For weight	OR 1.15 (0.73, 1.79)	No sig. assoc., <i>P</i> -trend 0.92
Leventakou, 2014*, Europe	Pooled analysis of cohorts	Times/wk, Continuous, per 1-time/wk increase		71 303	For weight	RR 1.02 (0.98, 1.06)	No sig. assoc., <i>P</i> _{heterogeneity} =0.21, <i>I</i> ² =26%. Random effects meta-analysis
				65 758	For length	RR 1.00 (0.97, 1.02)	No sig. assoc., <i>P</i> _{heterogeneity} =0.27, <i>I</i> ² =21%. Fixed effects meta-analysis
				65 758	For HC	RR 1.01 (0.98, 1.03)	No sig. assoc., <i>P</i> _{heterogeneity} =0.66, <i>I</i> ² =0%. Fixed effects meta-analysis
Fatty fish							
Amezcu-Prieto, 2018	Case-control	g/wk	130 g portions >1/wk vs never	518	Not specified	OR 0.69 (0.40 to 1.20)	No sig. assoc.
Halldorsson, 2007, Denmark	Birth cohort	g/d	>60 vs ≤5 g/d	NA	For weight	OR 1.18 (1.03, 1.35)	Adverse assoc. for higher intakes, <i>P</i> -trend 0.04
			>60 vs ≤5 g/d	NA	For length	OR 1.22 (1.05, 1.40)	Adverse assoc. for higher intakes, <i>P</i> -trend 0.003
			>60 vs ≤5 g/d	NA	For HC	OR 1.10 (0.97, 1.25)	No sig. assoc., <i>P</i> -trend 0.12

Author, year, country	Study design	Intake unit	High-low intake	Total cases	SGA for weight, length or HC	RR high-low or continuous (95 % CI)	Overall result
Heppe, 2011, the Netherlands	Birth cohort	g/wk, 5 cat	>70 vs 0 g/wk	205	For weight	OR 0.71 (0.45, 1.12)	No sig. assoc., <i>P</i> -trend 0.06
Leventakou, 2014*, Europe	Pooled analysis of cohorts	Times/wk, continuous, per 1-time/wk increase		74 077	For weight	RR 0.98 (0.96, 1.01)	Borderline protective assoc., <i>P</i> _{heterogeneity} =0.90, <i>I</i> ² =0%. Fixed effects meta-analysis
				65 758	For length	RR 1.00 (0.97, 1.04)	No sig. assoc., <i>P</i> _{heterogeneity} =0.29, <i>I</i> ² =18%. Fixed effects meta-analysis
				65 0758	For HC	RR 1.02 (0.95, 1.09)	No sig. assoc., <i>P</i> _{heterogeneity} =0.01, <i>I</i> ² =44%. Random effects meta-analysis
Nykjaer, 2019, UK	Birth cohort	Portions, 3 cat	>2/week vs 0, prior to pregnancy	145	Not specified	OR 1.3 (0.8, 2.3)	No sig. assoc., <i>P</i> -trend 0.6
			>2/week vs 0, 1st trimester	144	Not specified	OR 0.7 (0.4, 1.5)	No sig. assoc., <i>P</i> -trend 0.4
			>2/week vs 0, 2nd trimester	126	Not specified	OR 1.5 (0.7, 3.0)	No sig. assoc., <i>P</i> -trend 0.6
			>2/week vs 0, 3rd trimester	39	Not specified	OR 1.2 (0.6, 2.5)	No sig. assoc., <i>P</i> -trend 0.8

*Pooled analysis of 12 birth cohorts.

For lean fish (high-low analysis), there were no reports of statistically significant association with SGA except in Halldorson et al. (2007) where there was a borderline significant increased risk of SGA for length with higher intake. For fatty fish (high-low analysis), Halldorsson et al. (2007) found increased risk of SGA for weight and length, else there were no statistically significant associations.

4.24.3.3 *Summary relative risks (RR) based on VKM's inclusion of primary studies*

VKM calculated summary RRs for risk of SGA (birth weight) in relation to the highest versus lowest maternal intake of total fish or seafood during pregnancy. In addition to the pooled analysis by Leventakou et al. (2014) (11 cohorts) there were two cohort studies and three case-control studies of intake during pregnancy. Two studies (one cohort and one case-control study) assessed intake prior to pregnancy. Most studies assessed SGA in all infants, but some studies restricted the study sample to full-term infants. In studies that presented both (all and full-term), results did not differ in a consistent manner (Benjamin et al., 2019; Ricci et al., 2010). Therefore, when calculating summary RRs VKM used estimates for all infants when available, similar to Leventakou et al. (2014), else term infants were used. When results were presented for multiple time points in pregnancy (Mitchell 2et al., 004), the first was selected a priori.

The pooled RR (high-low) for total fish and SGA in Leventakou et al. (2014) (RR 0.95, 95% CI: 0.89, 1.02, $P_{\text{heterogeneity}}=0.56$) was attenuated and close to unity after the results from two European birth cohort studies of intake during pregnancy (Halldorsson et al., 2007; Heppe et al., 2011) were added (RR=1.02, 95% CI: 0.80, 1.30, $P_{\text{heterogeneity}}=0.02$). The attenuation could be somewhat exaggerated. Because study-specific estimates were not provided for SGA (only for PTB) in Leventakou et al. (2014), all studies were entered as a single estimate in the SGA analysis. The experience from the analysis of PTB was that the relative weight of Leventakou et al. (2014) was much smaller when entered as one estimate rather than as all the study specific estimates. VKM's summary RR (high-low) for three case-control studies of intake prior to pregnancy showed a statistically significant protective association (RR=0.73, 95% CI: 0.61, 0.88, $P_{\text{heterogeneity}}=0.38$).

The high-low estimate in the meta-analysis by Zhao et al. (2020) (based on both cohort and case-control studies) was not statistically significant and had high between-study heterogeneity (11 studies, RR=0.79, 95% CI 0.59, 1.06, $P_{\text{heterogeneity}}<0.001$). The dose-response analysis (per 45 g/day increment) of SGA was significantly protective (7 studies, RR=0.84, 95% CI 0.71, 0.98, $P_{\text{heterogeneity}}=0.04$). The magnitude of the protective association was similar to PTB, but with a narrower confidence interval.

VKM did not calculate summary RRs for SGA in relation to maternal intake of lean or fatty fish due to heterogeneous reporting among prospective studies but relied on the pooled analysis by Leventakou et al. (2014) and the more recent meta-analysis by Zhao et al. (2020). Leventakou et al. (2014) did not find an association between maternal intake of lean fish (continuous scale, per 1-time/week increase) and SGA for weight (10 studies), SGA for

length (7 studies) or SGA for head circumference (7 studies). Conclusions were similar for fatty fish (per 1-time/week increase) and SGA for weight (11 studies, borderline protective), SGA for length (7 studies) or SGA for head circumference (7 studies). Similarly, lean fish was not associated with SGA for weight in Zhao et al. (2020) (4 studies in high-low analysis, and 2 studies in dose-response analysis) or fatty fish (5 studies in high-low analysis, and 3 studies in dose-response analysis).

4.24.3.4 VKM's search compared to the previous meta-analysis and pooled analysis on SGA

An overview of overlapping studies in the included meta-analysis (Zhao et al., 2020), the European pooled analysis (Leventakou et al., 2014) and VKM's included single studies for birth outcomes, including SGA, is given in Table 4.22.2.2-1.

VKM's literature search identified one recent publication on SGA (Benjamin et al., 2019) not included in the meta-analysis by Zhao et al. (2020). Among the nine studies of SGA in Zhao et al. (2020) (high-low analysis of maternal seafood intake), all studies were identified by VKM, but one was excluded due to study quality (Canda et al., 2011). In addition, VKM included the pooled analysis by Leventakou et al. (2014). The results from the European cohorts were only included in Zhao et al. (2020) if published elsewhere.

4.24.4 Heterogeneity maternal fish intake and SGA

In Leventakou et al. (2014), heterogeneity was low and non-significant in all analyses of SGA for weight (continuous scale using the maximum number of studies, or categorical scale), but became significant ($P_{\text{heterogeneity}}=0.02$) when VKM added more studies to the analysis of harmonized exposure categories in Leventakou et al. (2014).

Zhao et al. (2020) reported significant heterogeneity in the high-low analysis of nine studies ($I^2=73.2\%$, $P_{\text{heterogeneity}}<0.001$). Heterogeneity was lower ($I^2=53.7\%$, $P=0.04$) among seven studies with adequate data for a dose-response analysis. These studies also met VKM's eligibility criteria. VKM included all studies from the dose-response analysis in Zhao 2020 except one (Mendez et al., 2010, Spanish INMA cohort) which was excluded due to overlap with Leventakou et al. (2014).

Studies in Zhao et al. (2020) were generally consistent in the protective direction but with differences in magnitude. This could be related to study design, as Zhao et al. (2020) combined estimates from cohort and case-control studies. In the heterogeneity analysis (high-low estimates) by Zhao, statistically significant reduced risk of SGA was limited to studies with a case-control design, studies with lower quality score (<7), and a small number of participants (<3000), and studies that did not adjust for maternal age, BMI, energy intake, or fish oil. However, none of the confounding factors were found to be significant contributors to the observed heterogeneity in meta-regression analyses (all test $P>0.05$).

4.24.5 Dose-response relationship maternal fish intake and SGA

Leventakou et al. (2014) did not perform a continuous dose-response analysis, but meta-analyzed two intake levels in the categorical analysis: ≥ 3 vs ≤ 1 time/week (high-low) and > 1 but < 3 times/week vs ≤ 1 time/week. Estimates were non-significant and of similar magnitude and did not suggest a linear gradient in the association of maternal fish and seafood intake during pregnancy with risk of SGA.

Zhao et al. (2020) found a protective association of maternal seafood intake with risk of SGA (16% reduced risk of SGA with each 45 g/day increase) without significant departure from linearity (dose-response analysis for intake up to 150 g/day). The dose-response relation was based on both case-control and cohort studies, and studies of maternal intake prior to (one study) and during pregnancy.

4.24.6 Weight of evidence for maternal fish intake and SGA

In this chapter, the evidence of the association between fish intake and SGA is weighted according to the WCRF criteria presented in the Chapter 3.1.6 (Box 2).

Published evidence on maternal fish intake prior to or during pregnancy and SGA

The association of maternal fish or seafood intake with risk of SGA has been examined in a large number of birth cohorts, and there is also evidence from case-control studies (but less than five). Most of the studies are from Europe with some evidence from other Western populations (USA, New Zealand).

Leventakou et al. (2014) (pooled analysis of European birth cohorts) found no association in the analysis of fish intake on a continuous scale (17 studies, 93 297 participants). When using harmonized intake categories (11 of 17 studies, 81 754 participants) the high-low pooled estimate was on the protective side, but only borderline statistically significant. Heterogeneity was non-significant. When two additional birth cohort studies of intake during pregnancy were taken into account, VKM's summary RR (high-low) estimate was close to unity (no association). The summary RR for three case-control studies of intake during pregnancy showed a consistent protective association. VKM did not include studies of fish intake prior to pregnancy in the pooled RR, but results do not seem to differ in a systematic way from studies of intake during pregnancy.

The meta-analysis by Zhao et al. (2020) reported a significant protective association based on a non-linear dose-response analysis (no significant departure from linearity) of seven studies (with cohort or case control design) that assessed maternal intake of total seafood or fatty fish (one study) during or prior to pregnancy. These studies met VKM's eligibility criteria (study objective and quality). The protective association could to some extent be driven by results from case-control studies or studies of lower quality, as reported in the heterogeneity analysis in Zhao et al. (2020).

Results on sub-categories of fish (fatty and lean) did not show statistically significant associations with risk of SGA in the pooled analyses by Leventakou et al. (2014) or meta-analysis by Zhao et al. (2020).

Heterogeneity

Estimates are generally consistent in the protective direction but with reports of adverse associations (statistically significant or on the adverse side). Heterogeneity was high among all studies in Zhao et al. (2020) (high-low analysis) but moderate in the dose-response meta-analysis which was based on studies that also passed VKM's eligibility criteria. Heterogeneity increased when VKM added studies to the pooled analysis by Leventakou et al. (2014) (where heterogeneity was non-significant). Case-control studies tended to report stronger associations than cohort studies, which also contributes to heterogeneity in the magnitude of associations, more than direction. Overall, heterogeneity appears to be moderate.

Mechanisms/biological plausibility

It has been suggested that LC n-3 FA may lead to an increased ratio of prostacyclins to thromboxane, resulting in an improved placental blood flow (Olsen et al., 1990). This could improve the fetal growth rate and protect against SGA and intrauterine growth restriction. Limited evidence on fatty fish and lean fish did not reflect a stronger association with fatty fish.

Upgrading factors

A dose-response relation was the only upgrading factor identified.

The meta-analysis by Zhao et al. (2020) included a linear dose-response analysis of maternal seafood intake and risk of SGA showing a reduction in risk for intake up to 150 g/day.

4.24.6.1 *Conclusion weight of evidence fish intake and SGA*

There is evidence from more than two independent and good quality cohort studies on maternal intake of total fish or seafood (VKM included eight publications of which one was a large, pooled analysis, and one previous meta-analysis of seafood intake with a dose-response analysis).

The pooled analysis (11 European cohort studies) finds a protective association between maternal intake of fish and risk of SGA that is modest in magnitude (weaker than for preterm birth) and borderline statistically significant. The dose-response meta-analysis (seven studies with cohort or case-control design) finds a linear, protective association for intake up to 150 g/day. However, heterogeneity analyses suggest that the protective association to some extent could be driven by results from case-control studies and studies of lower quality. In conclusion, the current evidence that maternal consumption of fish during pregnancy reduces risk of SGA is graded "limited, suggestive". The evidence is not limited in the number of studies but could potentially be limited by methodological weaknesses.

No conclusions could be drawn for the effects of fatty fish or lean fish on SGA due to no statistically significant results in previous pooled analysis or meta-analysis.

4.25 Maternal fish intake and low and high birth weight

4.25.1 VKM's search for previous systematic reviews and meta-analyses

See Chapter 4.22.1.

4.25.2 VKM's systematic review of primary studies of maternal fish intake and birth weight

4.25.2.1 *Included studies from search*

VKM's search identified a total of nine studies, eight single studies (Brantsaeter et al., 2012; Guldner et al., 2007; Heppe et al., 2011; Mohanty et al., 2015; Muthayya et al., 2009; Nykjaer et al., 2019; Olsen et al., 2002; Rogers et al., 2004) and one pooled analysis (Leventakou et al., 2014), with results on maternal fish intake and LBW. Leventakou et al. (2014) also reported results on high birth weight (>4000 g). One publication (Brantsaeter et al., 2012) was excluded due to overlap (described in more detail below), leaving eight for further analysis.

4.25.2.2 *Overlapping publications*

Leventakou et al. (2014) reported overall estimates of LBW in relation to categories of maternal fish intake from 13 European prospective cohorts. The publication by Brantsaeter et al. (2012) was excluded because data from the Norwegian Mother, Father and Child Cohort Study (MoBa) on LBW was used in Leventakou et al. (2014). As previously described, the cohorts Generation R (the Netherlands) and Pelagie (France) were included in Leventakou et al. (2014), but excluded from the categorical analysis, therefore, the separate publications from these cohorts by Guldner et al. (2007) (Pelagie) and Heppe et al. (2011) (GenerationR) were kept.

4.25.2.3 *Studies by design and geographic region*

The body of evidence on LBW (seven single studies and one pooled analysis) was dominated by studies from Europe (five single studies, and one pooled analysis of 13 cohorts), followed by USA (one study) and India (one study). All studies were birth cohorts or prospective cohorts including pregnant women.

4.25.2.4 *Studies by sub-groups and potential effect modification*

There were no reports of significant effect modification by maternal smoking (Guldner et al. (2007); Rogers et al., 2004), pre-pregnancy weight or BMI, parity, or other factors tested for among the included studies of fish intake and LBW. Therefore, overall estimates were emphasized. One study (Mohanty et al., 2015) examined if associations of maternal seafood intake with fetal growth indices differed by infant sex as a secondary analysis. This was considered insufficient for evidence synthesis.

LBW could be a consequence of being born small and/or early. Most studies of LBW with some exceptions (Olsen et al., 2002; Rogers et al., 2004) adjusted for gestational age in the main analysis or sensitivity analysis. The interpretation of the result in these studies will be the effect of fish on LBW not mediated through an effect on gestational age, although there is some concern that adjustment for gestational age as a mediating variable could lead to bias (Wilcox et al., 2011).

4.25.2.5 *Studies by fish exposure (type and timing)*

All studies, except Nykjaer et al. (2019), included total fish or seafood exposure (sum of all fish, unspecified fish, or fish including shellfish and/or fish products). Three studies (Heppe et al., 2011; Leventakou et al., 2014; Mohanty et al., 2015) presented sub-classification of fish intake according to lean and fatty fish. Nykjaer et al. (2019) only reported on fatty fish.

Regarding timing of intake, Guldner et al. (2007) investigated intake prior to pregnancy. Nykjaer et al. (2019) surveyed fatty fish consumption 4 weeks prior to pregnancy in addition to during pregnancy. Remaining studies (Heppe et al., 2011; Leventakou et al., 2014; Mohanty et al., 2015; Muthayya et al., 2009; Olsen et al., 2002; Rogers et al., 2004) investigated habitual fish intake during pregnancy in one or more trimesters.

VKM used the results for high versus low categories of intake for comparisons with other studies where available.

4.25.2.6 *Studies assessing potential non-linearity*

Mohanty et al. (2015) presented a figure of categorical dose-response analysis (four intake categories from <0.2 servings/month to >1 servings/week) of total fish, lean fish, fatty fish, and shellfish in relation to risk of LBW.

4.25.3 Results from the included primary studies on maternal fish intake and low birth weight

4.25.3.1 *Studies of total fish intake and low birth weight*

We included seven publications with estimates of the association between total fish or total seafood intake and risk of LBW in the weight of evidence analysis. The pooled analysis by

Leventakou et al. (2014) presented overall estimate for risk of LBW from 13 European prospective cohorts. The exposure levels and results (high-low coefficient where available, and overall results) are included in Table 4.25.3.1-1.

Table 4.25.3.1-1 Results from birth cohort studies included in the weight of evidence analysis of maternal total fish intake prior to or during pregnancy and low birth weight (LBW).

Author, year, country	Intake unit	High-low intake	Total cases	RR high-low or continuous (95 % CI)	Overall result
Guldner, 2007, France	Frequency, 3 cat	≥2/wk vs <1/month	71	OR=0.65 (0.21, 2.09)	No sig. assoc., <i>P</i> -trend 0.5
Heppe, 2011, The Netherlands	g/wk, 5 cat	>210 vs 0 g/wk	138	OR=0.86 (0.34, 2.17)	No sig. assoc., <i>P</i> -trend 0.89. Sig protective association for intake 1-69 g/week (but not higher): OR 0.47 (0.23, 0.9)
Leventakou, 2014, Europe*	Times/wk	≥3 vs ≤1 time/wk	Range 1.7% to 6.4% LBW of 140 337, all births	RR=0.91 (0.81, 1.02)	No sig. assoc., <i>I</i> ² ≤25%, <i>P</i> _{heterogeneity} =0.44. Fixed-effects meta-analysis.
	Times/wk	≥3 vs ≤1 time/wk	NA/131 831, excluding preterm births	RR=1.00 (0.81, 1.02)	No sig. assoc., <i>I</i> ² =32.4%, <i>P</i> _{heterogeneity} =0.15. Random effects meta-analysis
	Times/wk, Continuous, per 1-time/wk increase			Pooled RR=1.00 (0.96, 1.04), <i>I</i> ² >25% <i>P</i> _{heterogeneity} =0.06. Random effects meta-analysis.	No sig. assoc., with borderline sig between study heterogeneity
Mohanty, 2015, USA**	Frequency, 4 cat	>1/wk vs < 0.2/month	123	RR (Poisson)=2.02 (0.80, 5.05)	No sig. assoc.
Muthayya, 2009, India	g/d, 3 cat (above/below median, null)	Above median vs null	140/675, prev 20.7%, 1 st trimester	OR=0.65 (0.37, 1.14) originally reported as 1.54 (0.88, 2.70) for highest intake as reference	No sig. assoc.
	g/d, 3 cat (above/below median, null)	Above median vs null	NA/419, 3 rd trimester	OR=0.40 (0.19, 0.86) originally reported as 2.49 (1.16, 5.36) for highest intake as reference, <i>P</i> -value 0.019	Sig. protective assoc.
Olsen, 2002, Denmark	Frequency hot meals and sandwiches containing fish, 4 cat	≥1/wk vs 0	232	OR=0.3 (0.1, 0.9)	Sig. protective assoc., <i>P</i> =0.004

Author, year, country	Intake unit	High-low intake	Total cases	RR high-low or continuous (95 % CI)	Overall result
Rogers, 2004, UK	Portions, 4 cat	4.44 portions/wk vs 0	373	OR=0.93 (0.61, 1.45)	No sig. assoc., <i>P</i> -trend 0.492

*Pooled analysis of birth cohorts. **Total seafood.

The largest study (Leventakou et al., 2014) reported a borderline protective association that became null (RR=1) when preterm births were excluded. Two smaller studies reported a significant protective association for the highest fish intake in relation to LBW (Muthayya et al., 2009; Olsen et al., 2002). Muthayya et al. (2009) found an association for fish consumption in the third trimester, but no effect in the first trimester. The other four studies did not report statistically significant results.

4.25.3.2 Studies of lean and fatty fish and low birth weight

We included three publications (all prospective, observational studies) in the weight of evidence analysis for an association of LBW with intake of lean fish, and four with intake of fatty fish (six estimates). The pooled analysis by Leventakou et al. (2014) presented overall estimate for 12 European cohorts for lean fish, and 13 European cohorts for fatty fish. The exposure levels and results (high-low coefficient where available, and overall results) are included in Table 4.25.3.2-1. One study (Nykjaer et al., 2019) reported results prior to pregnancy and by trimester.

Table 4.25.3.2-1 Results from birth cohort studies included in the weight of evidence analysis of maternal lean and fatty fish intake and LBW.

Author, year, country	Intake unit	High-low intake	Total cases	RR high-low or continuous (95% CI)	Overall result
Lean fish					
Heppe 2011, The Netherlands	g/wk, 4 cat	>70 vs 0 g/wk	138	OR=1.22 (0.58, 2.54)	No sig. assoc., P -trend 0.91
Leventakou 2014, Europe*	Times/wk, continuous, per 1-time/wk increase		NA, 129 886, all	RR=1.05 (0.97, 1.13)	No sig. assoc., $P_{\text{heterogeneity}}=0.004$. Random-effects meta-analysis: $P_{\text{heterogeneity}}<0.05$ or $I^2 > 25\%$
			NA, 123 533, excl. preterm birth	RR=1.07 (0.96, 1.19)	No sig. assoc., $P_{\text{heterogeneity}}=0.80$, $I^2=0.0\%$. Random effects meta-analysis. P -value for heterogeneity <0.05 or $I^2 > 25\%$
Mohanty 2015, USA	Frequency, 4 cat	>1/wk vs <0.2/mo	123	RR (Poisson) =2.23 (1.21, 4.09)	Sig. adverse assoc., linear trend P -value across higher intake categories =0.02
Fatty fish					
Heppe 2011, The Netherlands	g/wk, 4 cat	>70 vs 0 g/wk	138	OR=1.00 (0.50, 1.98)	No sig. assoc., P -trend 0.81

Author, year, country	Intake unit	High-low intake	Total cases	RR high-low or continuous (95 % CI)	Overall result
Leventakou, 2014, Europe	Times/wk, continuous, per 1-time/wk increase		NA, 131 651, all	RR=0.98 (0.95, 1.02)	No sig. assoc., $P_{\text{heterogeneity}}=0.31$. Fixed-effects meta-analysis: $P_{\text{heterogeneity}}\geq 0.05$ and $I^2\leq 25\%$
			NA, 125 200, excluding preterm births	RR=1.02 (0.98, 1.06)	No sig. assoc., $P_{\text{heterogeneity}}=0.82$, $I^2=0.0\%$. Fixed effects meta-analysis
Mohanty, 2015, USA	Frequency, 4 cat	>1/wk vs <0.2/ mo	123	RR (Poisson) = 0.65 (0.34, 1.21)	No sig. assoc.
Nykjaer, 2019, UK	Portions, 3 cat	>2/wk vs 0	4 weeks before pregnancy n=46	OR=3.1 (0.8, 12.7)	No sig. assoc., P -trend 0.3
	Portions, 3 cat	>2/wk vs 0	43 (1st trimester)	OR=1.2 (0.2, 7.4)	No sig. assoc., P -trend 0.2
	Portions, 3 cat	>2/wk vs 0	35 (2nd trimester)	OR=1.5 (0.3, 8.1)	No sig. assoc., P -trend 0.2
	Portions, 3 cat	>2/wk vs 0	26 (3rd trimester)	OR=5.5 (0.9, 31.9)	No sig. assoc., P -trend 0.2

*Pooled analysis of birth cohorts.

For lean fish, one study reported significant increased risk of LBW between the highest fish consumption category as compared to the lowest category (Mohanty et al., 2015). There were no other findings of an association with LBW and lean or fatty fish consumption.

4.25.3.3 *Studies of total fish intake and high birth weight*

Leventakou et al. (2014) was the only study reporting on high birth weight (>4000 g). Although one study is insufficient for a weight of evidence assessment, this analysis was based on 13 cohorts and therefore presented here (Table 4.25.3.3-1).

Table 4.25.3.3-1 Results in one pooled analysis of birth cohorts of maternal fish intake (total, lean, and fatty fish) and high birth weight.

Author, year, country	Fish exposure	Intake unit	High-low intake	Total cases	RR high-low or continuous (95 % CI)	Overall result
Leventakou, 2014, Europe	Total fish	Times/wk, 3 cat	≥ 3 vs ≤ 1 time/wk	Range 3.6% to 23.2% high birth weight of 140 337, all births	1.07 (1.03, 1.11)	Highest intake significantly associated with high birth weight. $P_{\text{heterogeneity}}=0.76$, $I^2=0\%$. Fixed effects meta-analysis
	Total fish	Times/wk, continuous, per 1-time/wk increase			1.01 (1.00, 1.01)	Higher intake significantly associated with high birth weight. $P_{\text{heterogeneity}}=0.41$, $I^2=3\%$. Fixed effects meta-analysis
	Lean fish	Times/wk, continuous, per 1-time/wk increase			1.01 (1.00, 1.02)	Higher intake significantly associated with high birth weight. $P_{\text{heterogeneity}}=0.44$, $I^2=0\%$. Fixed effects meta-analysis
	Fatty fish	Times/wk, continuous, per 1-time/wk increase			1.01 (1.00, 1.02)	Higher intake significantly associated with high birth weight. $P_{\text{heterogeneity}}=0.93$, $I^2=0\%$. Fixed effects meta-analysis

4.25.3.4 *Summary relative risks (RR) based on VKM's inclusion of primary studies*

VKM calculated a summary RR for risk of LBW in relation to the highest versus lowest maternal intake of total fish or seafood during pregnancy. Five cohort studies, three European (Heppe et al., 2011; Olsen et al., 2002; Rogers et al., 2004), one from the US (Mohanty et al., 2015), and one from India (Muthayya et al., 2010) were added to Leventakou et al. (2014) (one pooled estimate, no study specific estimates available). In studies that presented results for multiple time-periods during pregnancy (Muthayya et al., 2009), the first period was selected a priori for the summary RR. Among the added studies, one reported a statistically significant protective association, other estimates were non-significant (Table 4.25.3.1-1). The pooled RR (high-low) in Leventakou et al. (2014) (RR=0.91, 95% CI: 0.81, 1.02, $P_{\text{heterogeneity}}=0.44$) shifted slightly in the protective direction when VKM added these studies (RR=0.86, 95% CI: 0.66, 1.13, $P_{\text{heterogeneity}}=0.15$) but heterogeneity and the confidence interval increased. One study of intake prior to pregnancy was not included in the estimate but supported a protective association (Guldner et al., 2007).

The high-low estimate in the meta-analysis by Zhao 2020 was on the protective side and borderline statistically significant (11 studies, RR=0.78, 95% CI 0.61, 1.00, $P_{\text{heterogeneity}}=0.03$) whereas the linear dose-response analysis (per 45 g/day increment) was statistically significant (7 studies, RR=0.65, 95% CI 0.47, 0.90) without departure from linearity.

VKM did not calculate summary RRs for LBW in relation to maternal intake of lean or fatty fish due to heterogeneous reporting among prospective studies.

4.25.3.5 *VKM's search compared to the previous meta-analysis and pooled analysis of low birth weight*

An overview of overlapping studies in the included meta-analysis (Zhao et al., 2020), the European pooled analysis (Leventakou et al., 2014) and VKM's included single studies for birth outcomes, including LBW, is given in Table 4.22.2.2-1.

VKM's literature search identified one recent publication on LBW (Smid et al., 2019) not included in the meta-analysis by Zhao et al. (2020), but the study was excluded by VKM as described previously. Among the 11 studies of LBW in Zhao et al. (2020) (high-low analysis of maternal seafood intake), all studies except one (Rylander et al., 1996, cohort of wives of Swedish fishermen by the Baltic sea) were identified by VKM. Two studies were excluded by VKM due to study quality (Canda et al., 2011 was graded C, and Burch et al., 2014 used proxies for dietary intake) and one study (Brantsaeter et al., 2012) was excluded due to overlap with Leventakou et al. (2014).

VKM included the pooled analysis by Leventakou et al. (2014), whereas the results from the 11 European cohorts were only included in Zhao et al. (2020) if published elsewhere.

4.25.4 Heterogeneity maternal fish intake and low birth weight and high birth weight

In Leventakou et al. (2014), heterogeneity was borderline significant in the analysis of fish intake on a continuous scale and risk of LBW using the maximum number of studies (19 studies, $I^2 > 25\%$, $P_{\text{heterogeneity}} = 0.06$), but not statistically significant in the high-low analysis emphasized by VKM for LBW (13 studies, $I^2 < 25\%$, $P_{\text{heterogeneity}} = 0.44$) or high birth weight ($I^2 = 0\%$, $P_{\text{heterogeneity}} = 0.76$). VKM's high-low summary estimate indicated borderline significant heterogeneity ($P = 0.08$) when five additional cohort studies were added to the analysis of LBW using harmonized exposure categories in Leventakou et al. (2014).

Zhao et al. (2020) reported moderate but significant heterogeneity between all 11 studies of LBW: Heterogeneity analysis of the high-low estimates suggested stronger (more protective) associations in studies that did not adjust for maternal energy intake, alcohol intake, or smoking. Between the seven studies with adequate data for a dose-response analysis, there was no significant heterogeneity ($I^2 = 0\%$, P -value 0.51 compared with $I^2 = 50.8\%$, P -value 0.03 in the high-low analysis) and estimates were generally consistent in the protective direction. These studies adjusted for maternal alcohol intake, smoking and energy intake. With regard to adjustment for maternal energy intake, most studies adjusted for pre-pregnancy BMI or energy intake measured at inclusion. The dose-response analysis included studies with a cohort design (no case-control studies) with one study of maternal intake prior to pregnancy.

Only Leventakou et al. (2014) included high birth weight. Heterogeneity was not statistically significant in the analysis of fish intake on a continuous scale using the maximum number of studies (19 studies, $I^2 = 3\%$, $P_{\text{heterogeneity}} = 0.41$), or in the high-low analysis emphasized by VKM (13 studies, $I^2 = 0\%$, $P_{\text{heterogeneity}} = 0.76$).

4.25.5 Dose-response relationship maternal fish intake and low birth weight

Leventakou et al. (2014) meta-analyzed fish intake on a continuous scale and as categories (high-low of ≥ 3 times/week vs ≤ 1 time/week, and mid-range of > 1 but < 3 times/week vs ≤ 1 time/week) in relation to the relative risk (RR) of LBW. There was no association on the continuous scale. The RRs for a protective association in the categorical analysis were of similar magnitude for each intake category above the reference category, but only statistically significant for the highest category. Thus, there was no evidence of a linear gradient in the association of maternal fish and seafood intake with risk of LBW, but results are compatible with a potential threshold effect.

In contrast, Zhao et al. (2020) found an average protective association of maternal seafood intake with risk of LBW (35% reduced risk of LBW with each 45 g/day increase) with no significant departure from linearity. The dose-response relation (for intake up to 80 g/day) was based on cohort studies, and maternal intake prior to (one study) or during pregnancy.

Studies excluded by VKM due to quality issues did not have sufficient dose-response data and did therefore not affect the meta dose-response analysis by Zhao et al. (2020).

4.25.6 Weight of evidence for fish intake and low birth weight

In this chapter, the evidence of the association between maternal fish intake and LBW is weighed according to the WCRF criteria presented in Chapter 3.1.6 (Box 2).

Published evidence of maternal fish intake and LBW

The evidence-base for an association between maternal fish or seafood intake and risk of LBW largely include the same literature as for PTB (described previously). The association with LBW has been examined in a large number of birth cohorts from Europe, with some evidence from other populations (one US primary study and one from India). As for PTB, the results depend on the methods of analysis making conclusions challenging. Leventakou et al. (2014) (pooled analysis) found no association with LBW in the analysis of fish intake on a continuous scale (19 studies, 151 880 participants) but when using harmonized intake categories (13 of 19 studies, 140 337 participants) there was a borderline protective association for the highest intake level of ≥ 3 times/week relative to the reference of ≤ 1 time/week). This analysis excluded six studies due to data harmonization issues, but these studies only contributed 8% of the study sample. Results on LBW were adjusted for gestational age in multivariable analysis. After excluding preterm births there was no association.

In the high-low meta-analyses of LBW performed by VKM and Zhao et al. (2020), associations were on the protective side but not statistically significant (VKM) or borderline significant (Zhao et al., 2020). However, Zhao et al. (2020) reported a significant protective association based on a dose-response analysis of maternal intake of total seafood or fatty fish (one study) during or prior to pregnancy (seven studies, 87 625 participants). These studies met VKM's eligibility criteria (study objective and quality). The estimate for LBW was stronger (35% risk reduction) than for PTB (around 16% risk reduction) per 45 g/day increase.

Heterogeneity

Heterogeneity increased but remained non-significant when VKM added studies to the pooled analysis by Leventakou et al. (2014). One study reported an association on the adverse side, but not statistically significant. Heterogeneity was moderate among all studies included by Zhao et al. (2020) in the high-low meta-analysis, but lower and non-significant in the dose-response meta-analysis which was based on studies that also passed VKM's eligibility criteria.

Mechanisms/biological plausability

Low birth weight can be a consequence of being born too small or early. Thus, any mechanisms for preterm birth and/or small for gestational age could be relevant to LBW. Several studies of fish intake and LBW suggest that the effect of fish is mediated by an effect

on gestational age. In Leventakou et al. (2014) the results were null/at unity in the analysis of LBW that adjusted for gestational age and additionally excluded preterm births.

Upgrading factors

A dose-response relation was the only upgrading factor identified. The dose-response meta-analysis by Zhao et al. (2020) showed reduced risk of LBW for maternal intake of seafood up to 80 g/day without departure from linearity.

4.25.6.1 *Conclusion weight of evidence maternal fish intake and low birth weight*

There is evidence from more than two independent and good quality cohort studies on maternal intake of total fish or seafood (VKM included seven publications of which one was a large, pooled analysis, and one previous meta-analysis of seafood intake with a dose-response analysis).

VKM's summary RR for primary studies is not statistically significant but suggests lower risk of LBW for the highest versus lowest intake of total fish which is supported by an independent dose-response meta-analysis (seven cohort studies) with low heterogeneity. The dose-response analysis has been given more weight than high-low analyses, as dose-response analyses utilize more of the data (but include fewer studies). Thus, the evidence that maternal consumption of fish or seafood during pregnancy reduces risk of low birth weight is considered "probable". The main effect seems to be through reduced preterm birth because associations with LBW are close to null when gestational age is adjusted for, and preterm births excluded.

4.25.7 Weight of evidence for maternal fish intake and high birth weight

Published evidence of fish intake and birth weight >4000 g

The evidence for an association between maternal fish or seafood intake and risk of high birth weight is more limited than for LBW but has been examined in one pooled analysis of European birth cohorts. Leventakou et al. (2014) found a small increased risk of high birth weight (>4000 g) in the analysis of total fish intake (continuous scale, 19 studies, 151 880 participants) that was stronger when using harmonized intake categories (13 of 19 studies, 140 337 participants). This analysis excluded six studies due to data harmonization issues, but these studies only contributed 8% of the study sample. Results on high birth weight were adjusted for gestational age in multivariable analysis.

Heterogeneity

In Leventakou et al. (2014), heterogeneity was low in the analysis of fish intake on a continuous scale and risk of high birth weight using the maximum number of studies (19

studies, $I^2=3\%$, $P_{\text{heterogeneity}}=0.41$), and in the high-low analysis emphasized by VKM (13 studies, $I^2=0\%$, $P_{\text{heterogeneity}}=0.76$).

Mechanisms/biological plausibility

There is evidence that LC n-3 FA intake during pregnancy may increase birth weight (see Chapter 5.2.14). The mechanisms could be related to increased gestational length and/or fetal growth. The pooled analysis (13 European cohort studies) of total maternal fish intake, found a statistically significant increased risk of high birth weight (and birth weight in grams, see next section) after adjustment for gestational age. This suggests an effect on fetal growth rate. But fish intake may also correlate with total energy intake and maternal weight gain during pregnancy (a predictor of birth weight). Maternal energy intake was not adjusted for in the pooled analysis of high birth weight, only pre-pregnancy BMI (Leventakou et al. 2014). Thus, confounding by maternal energy intake or energy balance during pregnancy cannot be ruled out. Mechanisms for contaminants are less well established and have mostly been related to increased risk of low birth weight.

Upgrading factors

A dose-response relation was the only upgrading factor identified. The small increase in high birth weight (>4000 g) in the analysis of total fish intake on a continuous scale supports a biological gradient.

4.25.7.1 *Conclusion weight of evidence maternal fish intake and high birth weight*

One pooled analysis (Leventakou et al., 2014) finds statistically significant increased risk of high birth weight for the highest versus lowest intake of total fish. Effects of fatty fish and lean fish (continuous scale) were similar, but only borderline statistically significant. VKM did not identify another publication on maternal fish intake and high birth weight that could support the result. The effect of fish intake versus energy intake remains unclear. Thus, the evidence that maternal consumption of fish (total, fatty or lean) during pregnancy increases the risk of high birth weight is graded "limited, suggestive".

4.26 Maternal fish intake and birth weight

4.26.1 **VKM's search for previous systematic reviews and meta-analyses**

See Chapter 4.22.1.

4.26.2 VKM's systematic review of primary studies of maternal fish intake and birth weight

4.26.2.1 *Included studies from search*

Thirteen studies in total, twelve single studies (Brantsaeter et al., 2012; Drouillet et al., 2009; Guldner et al., 2007; Halldorssen et al., 2007; Heppe et al., 2011; Mendez et al., 2010; Mohanty et al., 2015; Nykjaer et al., 2019; Olsen et al., 1990; Petridou et al., 1998; Ramon et al., 2009; Thorsdottir et al., 2004) and one pooled analysis (Leventakou et al., 2014) reported results for fish intake and birth weight in grams as a continuous variable.

4.26.2.2 *Overlapping publications*

Leventakou et al. (2014) included estimates of birth weight from 13 unique European birth cohorts in relation to categories of maternal fish intake. Five of 12 individual studies were found to contribute data to the analysis by Leventakou et al. (2014) and were excluded due to overlap; Brantsaeter et al. (2012) (MoBa study); Drouillet et al. (2009) (EDEN); Halldorsson et al. (2007) (DNBC); Mendez et al. (2010) and Ramon et al. (2009) (both INMA). The cohorts Pelagie (France) and Generation R (the Netherlands) were included in Leventakou et al. (2014) but excluded from the categorical analysis due to data harmonization difficulties. Therefore, the separate publications from these cohorts by Guldner et al. (2007) (Pelagie) and Heppe et al. (2011) (GenerationR) were kept. Thus, seven studies were analyzed in addition to Leventakou et al. (2014) (Guldner et al., 2017; Heppe et al., 2011; Mohanty et al., 2015; Nykjaer et al., 2019; Olsen et al., 1990; Petridou et al., 1998; Thorsdottir et al., 2004).

4.26.2.3 *Studies by design and geographic region*

The body of evidence (seven single studies and one pooled analysis) on birth weight had a skewed geographic distribution between Europe (six studies in addition to Leventakou et al. (2014) with 13 European birth cohorts), and USA (one study) with no studies from other continents.

All studies included in Leventakou were prospective birth cohorts. Among the additional studies, two recruited women after delivery at maternity clinics (Petridou et al., 1998) or based on birth records (Thorsdottir, et al., 2004) and retrospectively assessed maternal intake during pregnancy. These studies were labelled retrospective cohorts.

4.26.2.4 *Studies by sub-groups and potential effect modification*

There were reports of significant effect modification by maternal smoking, and pre-pregnancy BMI among the included studies of fish intake and birth weight in grams. However, VKM used overall estimates for the general population, as stratified analyses to a large extent were exploratory and showed inconsistent results between primary studies.

To illustrate, Leventakou et al. (2014) reported significant effect modification by maternal smoking status (yes, no) in pregnancy (P -interaction=0.01) and by overweight/obesity status in pre-pregnancy (P -interaction=0.03). The overall higher birth weight with higher fish intake was larger in smokers than non-smokers, and among the overweight or obese vs normal weight (BMI<25) in pre-pregnancy. Another study, Olsen et al. (1990), stratified all risk estimates by smoking status (0 vs 1+ cigarettes/day) and found associations with smoking in the opposite direction: lower birth weight (also head circumference, and placental weight but not birth length or gestational age) was only seen in non-smokers. The reason for the divergent results remains unclear but could be related to biological and/or methodological differences. Leventakou et al. (2014) included a large number of cohorts and had high statistical power to study effect modification. Olsen et al. (1990) was a smaller study but adjusted for maternal weight gain during pregnancy, as opposed to only pre-pregnancy BMI (Leventakou et al., 2014). Thus, the result in Olsen et al. (1990) may to a large extent reflect an association controlled for weight gain and energy intake. It was not possible to conclude on the direction or magnitude of the potential effect modification based on the included studies.

Two studies also presented results excluding pre-term infants in sub-group analysis (Leventakou et al., 2014) or limited the study sample to term infants only (Petridou et al., 1998).

4.26.2.5 *Studies by fish exposure (type and timing)*

All studies, except Nykjaer et al. (2019), included total fish or seafood exposure (sum of all fish, unspecified fish, or fish including shellfish and/or fish products). Three studies (Heppe et al., 2011; Leventakou et al., 2014; Mohanty et al., 2015) presented sub-classifications of fish as lean and fatty fish, and Nykjaer et al. (2019) only included fatty fish. All studies investigated habitual fish intake during pregnancy in one or more trimesters, although this was assessed after delivery in the retrospective cohorts. Nykjaer et al. (2019) also surveyed fish consumption prior to pregnancy in addition to all trimesters.

4.26.2.6 *Studies assessing potential non-linearity*

None of the included studies was found to assess potential non-linearity of the associations.

4.26.3 Results from the included primary studies on maternal fish intake and birth weight

4.26.3.1 *Studies of total fish intake and birth weight*

We included seven publications with estimates of the association between maternal fish or seafood intake and birth weight in the weight of evidence analysis. The pooled analysis by Leventakou et al. (2014) presented overall estimate for birth weight from 13 European

prospective cohorts. The exposure levels and results (high-low coefficient where available, and overall results) are included in Table 4.26.3.1-1.

Table 4.26.3.1-1 Results from studies included in the weight of evidence analysis of maternal total fish intake during pregnancy and birth weight. Values are mean change in grams for highest versus lowest level of fish intake, or per reported unit change in intake (β coefficients from linear regression).

Author, year, country	Study design	Fish exposure	Intake unit	High-low or continuous intake	Study sample	β high-low, or continuous (95% CI)	Overall result
Prior to pregnancy							
Guldner, 2007, France	Birth cohort	Fish only	Frequency/wk or mo	Continuous	2278	$\beta = -1.556$ (-5.587, 2.476)	No sig. assoc.
During pregnancy							
Heppe, 2011, the Netherlands	Birth cohort	Fish only	g/wk, 5 cat	>210 vs 0 g/wk	3 367	$\beta = -3.0$ (-70.4, 64.4), high -low	No sig. assoc., P -trend 0.86
Leventakou, 2014, Europe*	Pooled analysis of cohorts	Fish only	Times/wk, 3 cat	≥ 3 vs ≤ 1 time/wk	140 337, all	$\beta = 15.20$ (8.86, 21.54), high-low	Increased weight in the highest vs lowest fish consumption category, $P_{\text{heterogeneity}} = 0.67$, $I^2 = 0.0\%$. Fixed-effects meta-analysis
			Times/wk, 3 cat	≥ 3 vs ≤ 1 time/wk	133 488, excl PTB	$\beta = 14.59$ (8.13 21.05), high-low	Increased weight in the highest vs lowest fish consumption category, $P_{\text{heterogeneity}} = 0.78$, $I^2 = 0.0\%$. Fixed effects meta-analysis
Mohanty, 2015, USA	Birth cohort	Total seafood	Frequency, 4 cat	>1/ wk vs < 0.2/mo	3 141	$\beta = -14.9$ (-82.3, 52.5), high-low	No sig. assoc.
Olsen, 1990 Denmark	Cohort	Fish	Meals past month, 4 cat	(38.4 vs 0 g/d), per 1-level change (0, 1-2, 3-4, 5+)	6 569, non-smoking	$\beta = 15.8$ (-2.3, 33.9), continuous	No sig. assoc., non-smoking mothers
			Meals past month, 4 cat	(38.4 vs 0 g/d), per 1-level change (0, 1-2, 3-4, 5+)	4595, smoking	$\beta = -16.0$ (-37.7, 5.7), continuous	No sig. assoc., smoking mothers
Petridou, 1998, Greece	Retrospective cohort	Fish and fish products	Time/mo or wk	Per 1-time/wk increase	368, term births	β (SE) = 66 (47), P -value=0.16, continuous	No sig. assoc.

Author, year, country	Study design	Fish exposure	Intake unit	High-low or continuous intake	Study sample	β high-low, or continuous (95 % CI)	Overall result
Thorsdottir, 2004, Iceland	Retrospective cohort	Fish as main meal	Frequency, 3 cat	Probably per 1-level change (<4, 4-6, > 6 times/mo)	491	$\beta=50$ (no CI or SE), continuous	No linear assoc. in intake frequency, P -trend=0.098

*Pooled analysis of 12 birth cohorts.

None of the 5 single studies that investigated birth weight reported a significant association between the highest fish consumption categories as compared to the lowest categories. The pooled analysis of 13 European cohorts reported 15.2 g increased birth weight in the highest vs lowest fish consumption category.

4.26.3.2 *Studies of lean and fatty fish and birth weight*

We included three publications (all prospective, observational studies) on birth weight in the weight of evidence analysis for an association with intake of lean fish, and four publications on intake of fatty fish (six estimates). The pooled analysis by Leventakou et al. (2014) presented overall estimate for 10 European cohorts for lean fish, and 11 European cohorts for fatty fish. The exposure levels and results (high-low coefficient where available, and overall results) are included in Table 4.26.3.2-1.

Table 4.26.3.2-1 Results from birth cohort studies included in the weight of evidence analysis of maternal lean and fatty fish intake and birth weight.

Author, year, country	Intake unit	High-low intake	Sample size	β high-low, or continuous (95 % CI)	Overall result
Lean fish					
Heppe, 2011, The Netherlands	g/wk, 4 cat	>70 vs 0 g/wk	3 367	$\beta=-30.2$ (-79.7, 19.3)	No sig. assoc., P -trend 0.84
Leventakou, 2014, Europe*	Times/wk, continuous,		129 886, all births	$\beta=0.76$ (-2.45, 3.98)	No sig. assoc., $P_{\text{heterogeneity}}=0.11$. Random-effects meta-analysis: $P_{\text{heterogeneity}}<0.05$ or $I^2>25\%$

Author, year, country	Intake unit	High-low intake	Sample size	β high-low, or continuous (95 % CI)	Overall result
	per 1-time/wk increase		123 533, excl PTB	$\beta=0.55$ (−2.93, 4.03)	No sig. assoc., $P_{\text{heterogeneity}}=0.08$, $I^2=38.5\%$. Random effects meta-analysis
Mohanty, 2015, USA	Frequency, 4 cat	>1/wk vs <0.2/mo	3 141	$\beta=-47.4$ (−107.8, 13.0)	No sig. assoc.
Fatty fish					
Heppe, 2011, The Netherlands	g/wk, 4 cat	>70 vs 0 g/wk	3 367	$\beta=-8.4$ (−53.3, 36.4)	No sig. assoc., P -trend 0.83
Leventakou, 2014, Europe*	Times/wk	Continuous, per 1-time/wk increase	131 651, all	$\beta=2.38$ (0.51, 4.25)	2.38 g higher birth weight per 1-time/wk increase, $P_{\text{heterogeneity}}=0.97$. Fixed-effects meta-analysis, $P_{\text{heterogeneity}}\geq 0.05$ and $I^2\leq 25\%$
			125 200, excl PTB	$\beta=1.73$ (−0.17, 3.63)	No significant association, $P_{\text{heterogeneity}}=0.97$, $I^2=0.0\%$. Fixed effects meta-analysis: $P_{\text{heterogeneity}}\geq 0.05$ and $I^2\leq 25\%$
Mohanty, 2015, USA	Frequency, 4 cat	>1/wk vs <0.2/mo	3 141	$\beta=21.3$ (−41.1, 83.7)	No sig. assoc.
Nykjaer, 2019, UK	Portions, 3 cat	>2/week vs 0	4 weeks before pregnancy, n=1 029	$\beta=-35.7$ (−115.6, 44.1)	No sig. assoc., P -trend 0.7
			43 (1st trimester) n=1028	$\beta=-64.0$ (−151.1, 23.1)	No sig. assoc., P -trend 0.3
			35 (2nd trimester) n=751	$\beta=-71.4$ (−185.8, 43.13)	No sig. assoc., P -trend 0.3
			26 (3rd trimester) n=387	$\beta=-21.8$ (−169.0, 125.4)	No sig. assoc., P -trend 0.8

*Pooled analysis of 12 birth cohorts.

For lean fish, there were no reports of an association with birth weight. Leventakou et al. (2014) reported a small increase in birth weight (2.4 g increase for each time/week) with consumption of fatty fish, this observation was however no longer significant when repeating the analysis without preterm infants. Summary relative risks (RR) based on VKM's inclusion of primary studies.

VKM did not calculate a high-low summary measure of unstandardized β -coefficients from linear regression due to heterogenous reporting and potentially large range of variation in β -values, which may exaggerate heterogeneity between studies.

4.26.3.3 *VKM's search compared to the previous meta-analysis and pooled analysis of birth weight*

VKM did not identify any previous systematic literature review with a quantitative summary of fish consumption and birth weight in grams (only Zhao et al., 2020 on LBW as described previously) for comparison. VKM's search identified seven studies that did not contribute to the pooled analysis in Leventakou et al. (2014) (either study did not contribute data to categorical analysis or was not included). Among the additional studies, three were published after Leventakou et al. (2014) (Guldner et al., 2017; Mohanty et al., 2015; Nykjaer et al., 2019), and four were older, including the two retrospective cohorts (Petridou et al., 1998; Thorsdottir et al., 2004).

4.26.4 Heterogeneity maternal fish intake and birth weight in grams

In the pooled analysis by Leventakou et al. (2014), heterogeneity was not statistically significant in the high-low analysis emphasized by VKM (13 studies, $I^2=0\%$, $P_{\text{heterogeneity}}=0.67$). The meta-analysis by Zhao et al. (2020) did not include an analysis (high-low or dose-response) of birth weight in grams (only LBW).

4.26.5 Dose-response relationship maternal fish intake and birth weight

Leventakou et al. (2014) meta-analyzed two intake levels in the categorical analysis; ≥ 3 vs ≤ 1 time/week (high-low) and > 1 but < 3 times/week vs ≤ 1 time/week. The estimates suggested increasing birth weight with higher intake (mean difference of 8.9 grams for the mid-range, and 15.2 g for the highest category, relative to the reference). Except for Leventakou, primary studies did not report statistically significant associations or tests for trend.

4.26.6 Weight of evidence for maternal fish intake and birth weight

Published evidence of maternal fish intake and birth weight

The body of evidence for an association between maternal fish and/or seafood intake and birth weight is larger than for high birth weight (previous section). It includes the pooled analysis of European birth cohorts, but also five additional primary studies identified by VKM; four from Europe including two studies with retrospective assessment of diet in pregnancy after birth, and one study from USA. Consistent with results on high birth weight (previous section) Leventakou et al. (2014) (pooled analysis) also found higher birth weight for higher maternal fish intake on a continuous scale (19 studies, 151 880 participants) and for harmonized intake categories (13 of 19 studies, 140 337 participants). Results were adjusted for gestational age, but not maternal energy intake, and suggested a small and statistically significant increase on the continuous scale (1.5 grams per for each time/week) that was slightly larger and statistically significant for fatty fish (2.4 grams per for each time/week), but not lean fish (0.76 grams for each time/week). Among the 5 additional primary studies there were no statistically significant results for total fish, or fatty or lean fish. In the categorical analysis, mean birth weight was 15 grams higher for intake ≥ 3 vs < 1 times/week and 9 grams higher for > 1 but < 3 times/week.

VKM did not calculate a summary RR based on the unstandardized linear regression coefficients due to heterogenous reporting of categorical and continuous fish intake on different scales.

Heterogeneity

Between study heterogeneity in Leventakou et al. (2014) was non-significant for fish intake on a continuous scale (19 studies) or categorical scale (13 studies). Among the 5 additional primary studies included by VKM, results were generally consistent with no significant findings. Coefficients were close to unity or on the positive side (higher birth weight), except in smokers when stratified by smoking status in pregnancy (one study). The coefficient was also on the negative side in one US study that reported increased risk of LBW (previous section) with higher fish intake.

Mechanisms/biological plausibility

Previously described mechanisms relevant to LBW, high birth weight, and gestational length are also relevant for birth weight. However, most studies of birth weight (as LBW) adjusted for gestational age in the main analysis or sensitivity analysis. Thus, any effect of fish in these studies will reflect an effect not mediated by gestational age.

Upgrading factors

One large, pooled analysis (without adjustment for energy intake) suggest a biological gradient where birth weight increases with increasing fish intake. Additional primary studies do not report significant associations or tests for trend in support of a gradient.

A dose-response relation in one pooled analysis (Leventakou et al., 2014) was the only upgrading factor identified.

4.26.6.1 *Conclusion weight of evidence maternal fish intake and birth weight*

Birth weight is related to previously summarized measures of child maturity (preterm birth, gestational age, and small gestational age), and is the basis for studies of low birth weight and high birth weight. Thus, the weight of evidence for an association between maternal fish intake and birth weight as a continuous measure, cannot be evaluated independently of outcomes related to, or based on birth weight.

The pooled analysis by Leventakou et al. (2014) showed higher mean birth weight among women with a higher fish intake on a continuous (19 studies) and categorical (13 studies) scale. In addition to Leventakou et al. (201), VKM identified five primary studies that did not clearly support this result. Results in Leventakou et al. (2014) and most other primary studies were adjusted for gestational age. Thus, the higher birth weight must be explained by other factors than the effect of fish intake on gestational age. These factors are incompletely understood and could be biological and/or methodological. The evidence that maternal fish consumption during pregnancy increases birth weight through other mechanisms than gestational age is therefore graded "limited, suggestive".

4.27 Maternal fish intake and birth length and head circumference

4.27.1 **VKM's search for previous systematic reviews and meta-analyses**

VKM's search for systematic reviews and meta-analyses (see Chapter 3.1.2.2) identified no publications reporting on association between fish consumption and birth length, head circumference or ponderal index.

4.27.2 **VKM's systematic review of primary studies of maternal fish intake and birth length and head circumference**

4.27.2.1 *Included studies from search*

Six studies (Brantsaeter et al., 2012; Halldorssen et al., 2007; Heppe et al., 2011; Mohanty et al., 2015; Olsen et al., 1990; Thorsdottir et al., 2004) reported results on maternal fish

intake in relation to birth length and head circumference, and one study (Ramon et al., 2009) reported on birth length only. The pooled analysis by Leventakou et al. (2014) did not include birth length or head circumference, and therefore national birth cohorts from Norway (MoBa study, Brantsaeter et al., 2012) and Denmark (DNBC, Halldorssen et al., 2007) were included as primary studies. Results on ponderal index in two studies (Mohanty et al., 2015; Thorsdottir et al., 2004) were not summarized.

4.27.2.2 *Overlapping publications*

There were no overlapping publications from the same studies.

4.27.2.3 *Studies by design and geographic region*

The body of evidence on birth length (seven studies) and head circumference (six single studies) had a skewed geographic distribution between Europe (six studies), and USA (one study) with no studies from other continents. Five of the included studies were prospective birth cohorts (Brantsaeter et al., 2012; Halldorssen et al., 2007; Heppe et al., 2011; Mohanty et al., 2015; Ramon et al., 2009) and one study had another prospective observational design (cohort-based on community trial, Olsen et al., 1990). One study was conducted retrospectively in mothers after birth, based on birth records (Thorsdottir et al., 2004).

4.27.2.4 *Studies by sub-groups and potential effect modification*

As for birth weight in grams, VKM primarily used overall estimates, as stratified analyses to a large extent are exploratory. Stratified results are shown when only stratified results were reported (Olsen et al., 1990).

4.27.2.5 *Studies by fish exposure (type and timing)*

All studies, except Ramon et al. (2009), included total fish or seafood exposure (sum of all fish, unspecified fish, or fish including shellfish and/or fish products). Five studies (Brantsaeter et al., 2012; Halldorssen et al., 2007; Heppe et al., 2011; Mohanty et al., 2015; Ramon et al., 2009) presented sub-classification of fish intake as lean and fatty fish, and one study presented on canned tuna (Ramon et al., 2009). All studies investigated habitual fish intake during pregnancy in one or more trimesters. Mohanty et al. (2015) also covered fish consumption prior to pregnancy.

4.27.2.6 *Studies assessing potential non-linearity*

None of the included primary studies on birth length or head circumference presented a dose-response figure or dose-response information that could not be conveyed in tables.

4.27.3 Results from the included primary studies on maternal fish intake and birth length and head circumference

4.27.3.1 *Studies of total fish intake and birth length and head circumference*

We included six publications with estimates of the association between total fish or total seafood intake and infant birth length and head circumference in the weight of evidence analysis. Most studies presented β coefficients for differences in birth length and head circumference for categories of fish intake. In all studies, the lowest fish consumption category was the reference. The exposure levels and results (high-low coefficient where available, and overall results) are included in Table 4.27.3.1-1.

*Table 4.27.3.1-1 Results from studies included in the weight of evidence analysis of maternal total fish intake and birth length and head circumference. Values are mean difference in cm for highest versus lowest level of fish intake.

Author, year, country	Study design	Fish exposure	Intake unit	High-low intake	Study sample	β high-low (95 % CI)	Overall result
Birth length							
Brantsaeter, 2012, Norway	Birth cohort	Total seafood	g/d, 5 cat	>60 vs $\leq$ 5 g/d	62 099	$\beta=0.028$ (–0.052, 0.108)	No sig. assoc., <i>P</i> -trend 0.131
Halldorsson, 2007, Denmark	Birth cohort	Fish only	g/d, 5 cat	>60 vs $\leq$ 5 g/d	44 824	$\beta=-0.08$ (–0.18, 0.02)	No sig. assoc., <i>P</i> -trend 0.04
Heppe, 2011, The Netherlands	Birth cohort	Fish only	g/wk, 5 cat	>210 vs 0 g/wk	2 831	$\beta=-0.2$ (–0.7, 0.2)	No sig. assoc., <i>P</i> -trend 0.26
Mohanty, 2015, USA	Birth cohort	Total seafood	Frequency, 4 cat	>1/wk vs <0.2/mo	3 101	$\beta=-0.2$ (–0.5, 0.2)	No sig. assoc., increase in lower intake category (0.2 (–0.2, 0.7) in 0.5-1/wk vs 0.2/month)
Olsen, 1990 Denmark	Retrospective cohort	Fish	Meals, 4 cat	38.4 vs 0 g/d	6 569, non-smokers 4 594, smokers	$\beta=0.02$ (–0.07, 0.11), non-smokers $\beta=-0.01$ (–0.12, 0.10), smokers	No sig. assoc. in smokers and non-smokers
Thorsdottir, 2004, Iceland	Retrospective cohort	Fish as main meal	Frequency, 3 cat	>6 vs <4 times/mo	491	$\beta=0.35$, <i>P</i> -value 0.007	Increase with increasing intake
Head circumference							
Brantsaeter, 2012, Norway	Birth cohort	Total seafood	g/d, 5 cat	>60 vs $\leq$ 5 g/d	62 099	$\beta=0.102$ (0.042, 0.162)	Minor increase in HC with increasing intake, <i>P</i> -trend <0.001
Halldorsson, 2007, Denmark	Birth cohort	Fish only	g/d, 5 cat	>60 vs $\leq$ 5 g/d	44 824	$\beta=-0.11$ (–0.18, –0.03)	Decreased HC with increasing intake, <i>P</i> -trend 0.005
Heppe, 2011, The Netherlands	Birth cohort	Fish only	g/wk, 5 cat	>210 vs 0 g/wk	2 775	$\beta=0.0$ (–0.4, 0.4)	No sig. assoc., <i>P</i> -trend 0.39
Mohanty, 2015, USA	Birth cohort	Total seafood	Frequency, 4 cat	>1/wk vs <0.2/mo	3 063	$\beta=-0.2$ (–0.4, 0.1)	No sig. assoc.

Author, year, country	Study design	Fish exposure	Intake unit	High-low intake	Study sample	β high-low (95 % CI)	Overall result
Olsen, 1990 Denmark	Retrospective cohort	Fish	Meals, 4 cat	38.4 vs 0 g/d	6 569, non-smokers 4 594, smokers	$\beta=0.080$ (0.016, 0.144), non-smokers $\beta=-0.041$ (-0.122, 0.041), smokers	Minor increase in HC with increasing intake in non-smokers, no sig. assoc. in smokers
Thorsdottir, 2004, Iceland	Retrospective cohort	Fish as main meal	Frequency, 3 cat	>6 vs <4 times/mo	491	$\beta=0.24$, <i>P</i> -value 0.005	Increase with increasing intake

One of six studies of birth length reported significantly increased length in the highest compared to lowest intake category (Thorsdottir et al., 2004), else results were statistically non-significant. Two of six studies on head circumference reported larger (Brantsaeter et al., 2012; Thorsdottir et al., 2004) and one study reported smaller (Halldorsen et al., 2007) circumference in the highest compared to lowest intake category.

4.27.3.2 *Studies of lean and fatty fish and birth length and head circumference*

We included five publications (all prospective, observational studies) on birth length and head circumference in the weight of evidence analysis for an association with intake of lean and fatty fish. The exposure levels and results (high-low coefficient where available, and overall results) are included in Table 4.27.3.2-1 and 4.27.3.22-2 for birth length and head circumference, respectively.

Table 4.27.3.2-1 Results from birth cohort studies included in the weight of evidence analysis of maternal lean and fatty fish intake and birth length. Values are mean difference in cm for highest versus lowest level of fish intake.

Author, year, country	Intake unit	High-low or intake	Study sample	β high-low, or continuous (95 % CI)	Overall result
Lean fish					
Brantsaeter, 2012, Norway	g/day, continuous		61387	$\beta=0.001$ (0.000, 0.002)	Minor, borderline sig. increase with increasing intake
Halldorsson, 2007, Denmark	Frequency, 4 cat	≥ 4 vs 0 meals/mo	44 824	$\beta=-0.05$ (-0.11, 0.01)	No sig. assoc., P -trend 0.06
Heppe, 2011, The Netherlands	g/wk, 4 cat	>70 vs 0 g/wk	2 831	$\beta=-1.5$ (-4.7, 1.8)	No sig. assoc., P -trend 0.56
Mohanty, 2015, USA	Frequency, 4 cat	$>1/$ wk vs <0.2 /mo	3 101	$\beta=-0.2$ (-0.5, 0.2)	No sig. assoc.
Ramon, 2009, Spain	Portion/wk or mo, 4 cat	≥ 2 portions/wk vs <1 portion/mo	543	$\beta=0.25$ (-0.24, 0.75)	No sig. assoc., $P=0.71$, P -trend 0.43
Fatty fish					
Brantsaeter, 2012	g/d, continuous		61387	$\beta=0.000$ (-0.001, 0.001)	No sig. assoc.
Halldorsson, 2007, Denmark	Frequency, 4 cat	≥ 4 vs 0 meals/mo	44 824	$\beta=-0.10$ (-0.18, -0.03)	Minor decrease in birth length, P -trend 0.03
Heppe, 2011, The Netherlands	g/wk, 4 cat	>70 vs 0 g/wk	2 831	$\beta=-0.1$ (-0.4, 0.2)	No sig. assoc., P -trend 0.29
Mohanty, 2015, USA	Frequency, 4 cat	$>1/$ wk vs <0.2 /mo	3 101	$\beta=0.3$ (-0.1, 0.7)	No sig. assoc.
Ramon, 2009, Spain	Portion/wk or mo, 4 cat	≥ 2 portions/wk vs <1 portion/mo	543	$\beta=-0.40$ (-1.01, 0.21)	No sig. assoc., $P=0.39$, P -trend 0.19

Table 4.27.3.2-2 Results from birth cohort studies included in the weight of evidence analysis of maternal lean and fatty fish intake and head circumference. Values are mean difference in cm for highest versus lowest level of fish intake.

Author, year, country	Intake unit	High-low intake	Study sample	β high-low (95 %CI)	Overall result
Lean fish					
Brantsaeter, 2012, Norway	g/day, continuous		62 099	$\beta=0.002$ (0.001, 0.003)	Small increase in HC with increasing intake
Halldorsson, 2007, Denmark	Frequency, 4 cat	≥ 4 vs 0 meals/mo	44 824	$\beta=-0.01$ (-0.05, 0.03)	No sig. assoc., P -trend 0.16
Heppe, 2011, The Netherlands	g/wk, 4 cat	>70 vs 0 g/wk	2 831	$\beta=-0.1$ (-0.3, 0.2)	No sig. assoc., P -trend 0.76
Mohanty, 2015, USA	Frequency, 4 cat	>1 /wk vs <0.2 /mo	3 101	$\beta=0.1$ (-0.2, 0.3)	No sig. assoc.
Fatty fish					
Brantsaeter, 2012, Norway	g/d, continuous		62 099	$\beta=0.000$ (-0.001, 0.001)	No sig. assoc.
Halldorsson, 2007, Denmark	Frequency, 4 cat	≥ 4 vs 0 meals/mo	44 824	$\beta=-0.11$ (-0.16, -0.03)	Decrease in HC with increased intake, P -trend <0.001
Heppe, 2011, The Netherlands	g/wk, 4 cat	>70 vs 0 g/wk	2 831	$\beta=-0.1$ (-0.3, 0.2)	No sig. assoc., P -trend 0.43
Mohanty, 2015, USA	Frequency, 4 cat	>1 /wk vs <0.2 / mo	3 101	$\beta=-0.1$ (-0.4, 0.2)	No sig. assoc.

For lean fish, there were no reports of an association with birth length or head circumference. For fatty fish, one study (Halldorsen et al., 2007) reported a minor negative association between the highest fish consumption categories as compared to the lowest categories for birth length and head circumference.

4.27.3.3 *Summary estimates **based on VKM's** inclusion of primary studies*

VKM did not calculate a high-low summary measure of unstandardized β -coefficients from linear regression.

4.27.3.4 ***VKM's search** compared to the previous meta-analysis and pooled analysis on birth outcome*

VKM did not identify any studies for comparison. Birth length and head circumference as continuous measures were not included in the pooled analysis by Leventakou 2014 or the meta-analysis by Zhao et al. (2020).

4.27.4 Heterogeneity maternal fish intake and birth length and head circumference

Heterogeneity in birth length and head circumference results was evaluated from primary studies. The national birth cohort studies from Norway (MoBa study) and Denmark (DNBC) were the two largest studies with sample sizes of around 62 000 and 45 000 mother-child pairs, respectively. In the high-low analysis of length, both studies reported no significant association which was consistent with the remaining studies, except one with a retrospective design. Overall, heterogeneity was considered to be low or moderate for studies of birth length. In the high-low analysis of head circumference, the Norwegian and Danish birth cohorts reported statistically significant estimates in opposite directions (minor increase with increasing intake of seafood in MoBa, and minor decrease with increasing intake of fish in DNBC). Among the remaining four studies, all except one with a retrospective design, reported no significant association. Overall, heterogeneity was considered to be high between studies of head circumference.

4.27.5 Dose-response relationship maternal fish intake and birth length and head circumference

The evidence for a dose-response relationship was limited to primary studies that reported test for linear trend across categories of fish intake, or continuous effect estimates. Most studies of birth length reported no association (high-low analysis) without a trend. For head circumference, the two largest studies (MoBa and DNBC) reported linear trends in the opposite direction for total fish or seafood intake.

4.27.6 Weight of evidence for maternal fish intake and birth length and head circumference

Published evidence of maternal fish intake and birth length and head circumference

The evidence base for an association of maternal intake of total seafood or fish with birth length is six primary studies, including the national birth cohorts from Norway (MoBa, seafood) and Denmark (DNBC, fish). None of the six studies, except one with a retrospective cohort design, found an association with birth length. However, MoBa found a small increase in birth length for higher intake of lean fish (borderline statistically significant), and DNBC found a small decrease in birth length for higher intake of fatty fish. For head circumference, the Norwegian and Danish birth cohorts found significant associations in opposite directions for total fish or seafood that appeared to be driven by lean fish in MoBa (higher HC) and fatty fish in DNBC (lower HC).

Heterogeneity

Most primary studies of birth length found no association, and heterogeneity was considered low to moderate. For head circumference, heterogeneity was considered to be higher as two large studies reported significant associations in opposite directions.

Mechanisms/biological plausibility

There are several plausible mechanisms related to an effect of LC n-3 FA from fatty fish on gestational length and fetal growth, but not specific to birth length or head circumference. Mechanisms for an effect of lean fish on gestational length and fetal growth are less established.

Upgrading factors

No substantial upgrading factors were found.

There was little evidence of a dose-response relationship for height, consistent with predominantly null findings. For statistically significant results on head circumference for seafood or total fish, and for lean and fatty fish, significant trends were also reported.

4.27.6.1 *Conclusion weight of evidence maternal fish intake and birth length and head circumference*

The current evidence from six prospective cohort studies do not suggest an association with birth length, and the evidence is graded "limited, suggestive" of no association. For head circumference, the evidence is inconsistent, and therefore graded "limited, no conclusion".

4.28 Introduction to fish intake and asthma and allergies in children and adolescents

This chapter is an introduction to the weight of evidence analysis chapters for asthma and allergic diseases related to maternal fish intake or child fish intake (Chapters 4.29-4.33).

In this introductory chapter we have included both the systematic reviews and an overview of the primary studies because many studies contain multiple outcomes. Additionally, in this introductory chapter we show an overlap table between VKM's included primary studies, and the those included in the one meta-analysis found for asthma and allergic diseases and a pooled analysis.

Overview of asthma and allergy outcomes

Asthma and allergies are common chronic conditions in both children and adults, but disease onset is typically during childhood or adolescence. Therefore, there has been a special interest in early life exposures. The current chapter summarizes epidemiological studies of fish intake in relation to development of asthma and allergic diseases in infants, pre-school and school age children up to and including age 16 years. Disease development has been studied in relation to maternal fish intake (mostly during pregnancy, but also pre-pregnancy and during lactation) and child fish intake at different ages ranging from first introduction to age 8 years.

Asthma is a chronic inflammatory disease of the airways characterized by episodic, reversible airflow obstruction and respiratory symptoms (wheeze, shortness of breath/dyspnea) and is often triggered by allergens (referred to as atopic asthma) but can also be non-allergic (non-atopic). Wheeze is a symptom of asthma but may also be caused by transient respiratory tract infections that are common in children, such as virus-induced bronchiolitis. Thus, many children experiencing wheezing episodes during infancy and early childhood will not develop asthma, and wheeze has been summarized separately from asthma, as customarily done.

Allergic diseases are a broad disease group that include atopic eczema, atopic asthma, allergic rhinitis and rhinoconjunctivitis (nose- and/or eye-related allergy, including hay fever), and food allergies. Atopic eczema (or atopic dermatitis) is an allergy-related skin reaction, whereas allergic rhinitis and rhinoconjunctivitis ("itchy eyes") are caused by airborne allergens, such as from pollen, pets and domestic animals, dust mites, and molds. Food allergies are triggered by allergens in foods and may give many different symptoms. Fish and shellfish are among the 14 major food allergens specified in Norwegian and EU nutrition labelling legislation, but allergy to fish is relatively rare.

There is a certain time-based order of onset of allergic diseases, where atopic eczema and food allergies tend to appear in infants and young children, before atopic asthma and allergic rhinitis. Development of allergic disease begins with sensitization to an allergen and may occur before symptoms of disease. Although sensitization is a necessary step in the development of allergic diseases, it is not sufficient.

There is also a hereditary component that on a genetic basis predisposes subjects to asthma and/or allergies. The strong hereditary factor is a challenge in epidemiological studies, because the family history of allergy may cause families to modify their diet. The introduction of fish may be delayed or avoided in infants with a family history of allergic disease, or with early symptoms of allergic disease. To avoid heredity- and disease-related modification of exposure (so called reverse causation), it is important that epidemiological studies of fish intake and allergic diseases in children, control for the family history of allergy.

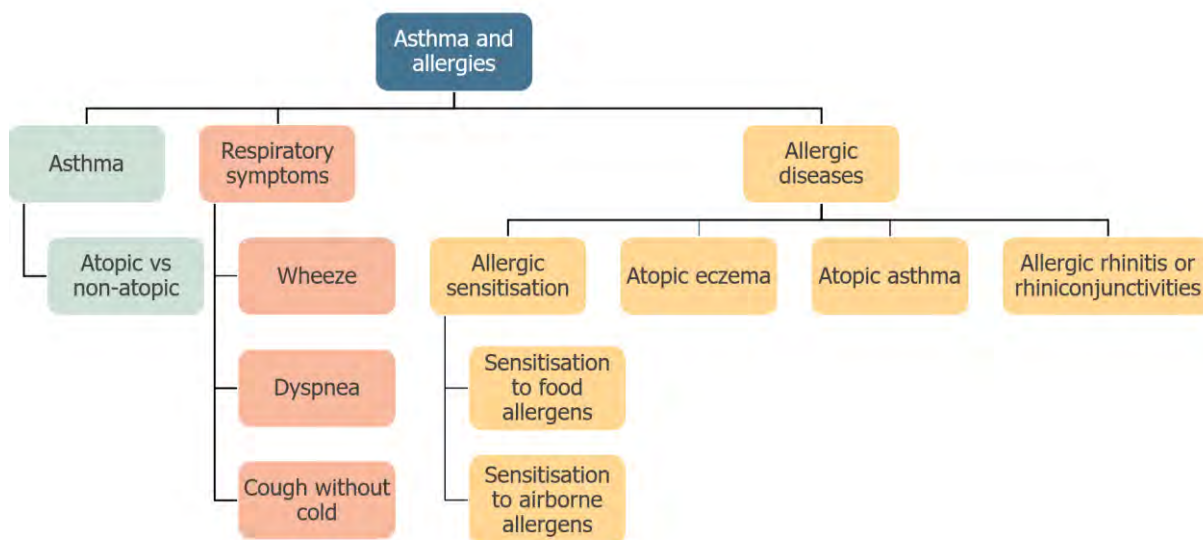


Figure 4.28-1 An overview over evaluated asthma and allergy outcomes.

Mechanisms/biological plausability

The causes of the development of allergic sensitization and atopy-related diseases are not well established, other than the requirement for a genetic predisposition and allergen exposure. Additionally, lifestyle and environmental factors in general are probably important. However, few environmental factors have been well documented to promote or counteract the development of allergy and allergy-related diseases like asthma and atopic dermatitis.

The immune system undergoes rapid development during fetal life and in the early postnatal period and continues to develop and mature during the first years of life. The intrauterine period, i.e. mother's diet during pregnancy, and the child's diet during the very first years of life are therefore of particular interest in relation to food intake as a factor in allergy development and prevention. It is not well understood why the immune response to antigens/allergens results in tolerance for most individuals, but in sensitization in others, and why only some of the sensitized individuals develop clinical symptoms. Regular exposure to antigens in early life could be important, and the age of introduction of fish and other foods has been hypothesized to play a role in the development of allergic diseases.

Fish contains, in addition to allergens, a number of substances that could have the potential to influence the immune system, both defense against infections, allergy, and inflammation. Fish intake could therefore affect susceptibility to infectious diseases, susceptibility to allergy

and allergy-related diseases, and susceptibility to chronic inflammatory diseases such as asthma. The influence of fish allergens is likely to be limited to the development of allergy to fish. The time period of most interest in relation to fish allergy is possibly the prenatal period, that is whether maternal intake of fish promotes the development of tolerance to fish in the unborn child, or primes for tolerance or fish allergy in postnatal life.

Regarding substances in fish that may influence allergy development, some are natural components of fish, while others are environmental contaminants. Both may act by direct interaction with the immune system, or indirectly by modifying the intestinal flora, or both. Among natural components of fish with immunoregulatory properties are vitamin D, LC n-3 FA, melatonin, tryptophan, taurine and polyamines. Among contaminants that have immunoregulatory properties can be mentioned dioxins and dioxin-like substances and other Ah-receptor ligands, and mercury.

4.28.1 VKM's search for published systematic reviews and meta-analyses on fish intake and asthma and allergic diseases

4.28.1.1 *Description of the identified publications*

VKM's search for systematic reviews and meta-analyses (see Chapter 3.1.2.2) identified six publications on the association between fish intake and asthma and allergic diseases that were assumed to fulfill the inclusion criteria and were read as full papers. Three papers were excluded, see Table 4.28.1.1-1 for reason for exclusions.

Table 4.28.1.1-1 Reasons for exclusion of identified papers from the search of systematic reviews and meta-analyses of fish intake and asthma or allergic diseases 2016-2021.

Included papers	Excluded paper and reasons for exclusions
Malmir et al., 2021: systematic review and meta-analysis of fish intake in pregnancy and allergic diseases	de Silva et al., 2020: no fish intake, only fish oil supplementation, or introduction of 6 multiple food allergens (including whitefish) to the infant diet
Papamichael et al., 2018: systematic review and meta-analysis of fish intake (infancy) and asthma	Garcia-Larsen et al., 2018: no fish intake, only fish oil supplementation
Zhang et al., 2017: systematic review and meta-analysis of fish intake (pregnancy or infancy) and allergic diseases	Best et al, 2016: studies of fish intake summarized qualitatively

The systematic reviews are described below; first, main descriptions of the methods used and then main results from the meta-analyses.

Meta-analyses and systematic reviews

Malmir et al. (2021) is a systematic review and meta-analysis of fish intake during pregnancy and development of allergic diseases in the offspring before age 10 years. Allergic disease included asthma, wheeze, eczema, dermatitis, allergic rhinitis, allergy to inhalants, and food

allergy. The databases Medline/PubMed, ISI web of Science, EMBASE, SCOPUS and Google Scholar were searched for publications prior to February 2020. A total of 24 observational studies were included with a cross-sectional, case-control or cohort design. (Only results excluding cross-sectional studies were emphasized by VKM). The risk of bias in individual studies was assessed by the Newcastle-Ottawa Scale (NOS) using a cut-off value of ≤ 6 (maximum 9) for high risk of bias. Study quality was not described overall, but in heterogeneity analyses, 2 of 9 estimates of asthma, 4 of 11 estimates of wheeze, and 7 of 12 estimates of eczema were evaluated as coming from high quality studies (NOS score > 6). Supplementary Tables 1-8 with study information were unavailable. Authors were contacted but did not respond. Some data on the studies is therefore missing.

Papamichael et al. (2018) is a systematic review and meta-analysis of fish intake during infancy in relation to current asthma or wheeze in children younger than 18 years. The databases Cochrane Central Register of Controlled Trials, MEDLINE, PubMed, CINAHL (EBSCO), SCOPUS and EMBASE were searched for studies (all study designs) until July 2017. Supplementary studies were sought from conference proceedings, clinical trials registries and by hand searching the reference lists of relevant articles. A total of 23 studies (9 cohort, 2 case-control, 12 cross-sectional) were included in the qualitative review. Quantitative meta-analyses were performed for all fish versus no fish intake. The risk of bias in individual studies was assessed by the quality assessment tool Data S2 (maximum score of 24 presented as percentage of total score) with a cut-off value $\geq 70\%$ (median value) for high quality. The score was above 90% for 8 of 9 cohort studies.

Zhang et al. (2017) is a systematic review and meta-analysis of fish intake during pregnancy or infancy and development of five allergic outcomes in children between birth and age 18 years. Allergic outcomes included asthma, wheeze, eczema, allergic rhinitis, and sensitization/food allergy. The data bases PubMed, EMBASE, and Cochrane Central Register of Controlled Trials were searched for randomized controlled trials (RCTs) and prospective cohort studies until February 7, 2016. A total of 22 prospective studies, one RCT and 21 cohort studies, were included in the review. The risk of bias in individual studies was assessed by the Cochrane risk-of-bias tool for the only RCT (found to have high risk of bias) and the Newcastle-Ottawa Scale for the cohort studies using a cut-off value of ≤ 6 (maximum 9) for high risk of bias (found in five included studies).

4.28.1.2 *Results from the meta-analyses*

Below is a summary table of results on maternal or child fish intake in relation to asthma and allergic outcomes in children (Table 4.28.1.2-1) based on the identified meta-analyses by Malmir et al. (2021), Papamichael et al. (2018) and Zhang et al. (2017).

The meta-analysis by Malmir et al. (2021) included some cross-sectional studies, which were not eligible for inclusion by VKM. However, estimates from cohort studies were selected from sensitivity analyses stratified by study design. Zhang et al. (2017) included studies with results unadjusted for potential confounding factors, and adjusted estimates were selected from sensitivity analyses stratified by adjustment.

Table 4.28.1.2-1 Summary of results from meta-analyses of maternal or child fish intake and child asthma and allergies outcomes.

Author, year	Fish intake, study design	Total no studies	No of cases	Comparison	Summary RR/OR (95 % CI)	Heterogeneity I^2 , P -value heterogeneity	Overall result
Eczema							
Malmir, 2021	Maternal intake, cohort studies	10 (excl. one cross sectional)	NA	High-low	RR=0.93 (0.84, 1.03)	$I^2=41\%$, $P=0.084$, cohorts only	No sig. assoc.
		NA	NA	Per 30 g/wk	RR 0.96 (0.92, 0.99) $P_{\text{non-linearity}}=0.042$	NA	4% decreased risk of eczema per 30 grams fish intake during pregnancy
Zhang, 2017	Maternal intake, cohort studies	8 (ex 2 studies with unadjusted results)	15945 children (all 10 studies)	High-low	RR=0.84 (0.69, 1.01)	$I^2=56\%$	Borderline protective association with moderate heterogeneity
	Infant intake, cohort studies	3 (ex 1 study, unadjusted results)	13823 children (all 4 studies)	High-low	RR=0.71 (0.61, 0.82)	$I^2=0\%$	Protective assoc.
Wheeze							
Malmir, 2021	Maternal intake, cohorts and one cross-sectional study	10 (11 estimates)	NA	High-low	RR=0.97 (0.96, 0.99)	$I^2=32.4\%$, $P=0.139$, fixed effects	Protective assoc.
	Maternal intake, cohort studies	9, ex cross-sectional study	NA	High-low	RR=0.97 (error in CI, reported as 0.99 to 0.99)	$I^2=0\%$, $P=0.45$	Protective assoc.
				Per 30 g/wk	RR=1.01 (0.97, 1.05), $P_{\text{non-linearity}}=0.01$	NA	No sig. assoc., increased risk for intake >30 – 150 g/wk
Papamichael, 2018	Infant intake, cohort studies	2	NA	All vs no intake	RR=0.62 (0.48, 0.80)	$I^2=0\%$, $P=0.809$, random effects	Protective assoc.
Zhang, 2017	Maternal intake, cohort studies	8	42096 children	High-low	RR=0.94 (0.83, 1.07)	$I^2=26\%$	No sig. assoc.
	Infant intake, cohort studies	2	8597 children	High-low	RR=0.94 (0.77, 1.14)	$I^2=0\%$	No sig. assoc.
Asthma							

Author, year	Fish intake, study design	Total no studies	No of cases	Comparison	Summary RR/OR (95 % CI)	Heterogeneity I^2 , P -value heterogeneity	Overall result
Malmir, 2021	Maternal intake, cohort and case-control studies	8 (9 estimates)	NA,	High-low	RR=0.99 (0.89, 1.11)	$I^2=76.3\%$, $P<0.001$	No sig. assoc. with significant heterogeneity
	Maternal intake, cohort studies	6	NA	High-low	RR=0.92 (0.82, 1.04)	$I^2=22.7\%$, $P=0.26$	No sig. assoc.
Papamichael, 2018	Infant intake, cohort studies	3	NA	All vs no intake	OR=0.75 (0.60, 0.95)	$I^2=11.5\%$; $P=0.32$	Protective assoc.
Zhang, 2017	Maternal intake, cohort studies	3 (excl 1 study, unadjusted results)	37 295 children, 4 studies	High-low	RR=0.93 (0.68, 1.28)	$I^2=66\%$	No sig. assoc.
	Infant intake, cohort studies	2 (excl 1 study, unadjusted results)	8 902 children, 3 studies	High-low	RR=0.87 (0.67, 1.12)	$I^2=0.0\%$	No sig. assoc.
Allergic rhinitis							
Malmir, 2021	Maternal intake – cohort and case-control studies	3	NA	High-low	RR=0.91 (0.75, 1.09), $P=0.409$	$I^2=0.0\%$, $P_{\text{heterogeneity}}=0.32$	No sig. assoc.
Zhang, 2017	Maternal intake – cohort studies	3	32 589 children	High-low	RR=0.95 (0.62, 1.45)	$I^2=44\%$	No sig. assoc.
	Infant intake	2 (excl 1 study, unadjusted results)	9 987 children, all 3 studies	High-low	RR=0.61 (0.37, 0.98)	$I^2=72\%$	Protective assoc.
Sensitization							
Malmir, 2021	Maternal intake	2 (3 estimates)	NA	High-low	RR=0.86 (0.66, 1.13)	$I^2=35.6\%$, $P_{\text{heterogeneity}}=0.21$	No sig. assoc.
Zhang, 2017	Maternal intake	1 (2 estimates)	NA	High-low	No summary RR		

4.28.2 VKM's systematic review of primary studies on fish intake and asthma and allergies

4.28.2.1 *Included studies from search*

We evaluated a total of 22 publications graded A or B, 21 single studies (Jedrychowski et al., 2008; Jedrychowski et al., 2011; Kieft-de Jong et al., 2012; Kull et al., 2006; Leermakers et al., 2013; Li et al., 2013; Lumia et al., 2012; Lumia et al., 2011; Lumia et al., 2015; Magnusson et al., 2013; Magnusson et al., 2015; Maslova et al., 2013; Miyake et al., 2009; Nafstad et al., 2003; Oien et al., 2019; Pele et al., 2013; Romieu et al., 2007; Sausenthaler et al., 2007; Willers et al., 2007; Willers et al., 2008; Willers et al., 2011; Talaei et al., 2021) and one large, pooled analysis (Stratakis et al., 2017) with one or more outcomes on asthma and/or allergic disease (Figure 4.28-1). Studies assessed outcomes in children from the first year of life up to age 16 years. Studies or study results on the age of introduction of fish in infants (usually any versus no intake, without frequency or amounts) are not part of this summary. One study of adult-onset asthma (Li et al., 2013) was considered insufficient for a summary and excluded from further analysis.

Several studies contributed with multiple publications on different exposure windows (pregnancy, lactation, childhood), on different outcomes, or the same outcome at different ages during follow-up of the children. Overall, there were 15 unique studies among the 22 publications (not counting the pooled analysis). Stratakis et al. (2017) (pooled analysis) used data from 18 birth cohorts (17 European and 1 US): ABCD (the Netherlands); DNBC (Denmark); FLEHS I (Belgium); GASPII (Italy); Generation R study (the Netherlands); Generation XXI study (Portugal); HUMIS (Norway); INMA (Spain); KOALA (the Netherlands); Lifeways Cross Generation (Ireland); Lucki (the Netherlands); NINFEA (Italy); PELAGIE (France); PIAMA (the Netherlands); RHEA (Greece); SWS (UK); Bologna Birth Cohort (Italy); Project Viva (Massachusetts, USA), and other publications were checked for overlap to not include the same studies multiple times. VKM used the results for the highest versus lowest category of fish intake when available for comparisons of results between primary studies and with previous high-low meta-analyses.

A description of the 22 evaluated studies (study name, design, time period, size of the study population, and dietary assessment method) can be found in (Table 4.28.2.1-1).

Table 4.28.2.1-1 Overview of 22 studies that were evaluated for inclusion in weight of evidence analysis of asthma and allergic outcomes.

Author, year, country	Study name	Study design	Inclusion year(s), end, follow-up time	Study size, age	Dietary assessment method	Dietary assessment period
Jedrychowski, 2008, Poland	Krakov birth cohort	Birth cohort	2001-2004 (births), follow-up to age 2 yrs	465 mother-child pairs (50.1% male), Maternal age 18-35 yrs, mean 27.6 yrs	Repeated FFQ by interview, 2nd and 3rd trimester of pregnancy, validated	Not specified, probably during pregnancy
Jedrychowski, 2011, Poland			2001-2004 (births), follow-up to age 12 months	469 mother-child pairs (50.5% male), Maternal age 18-35 yrs, mean 27.6 yrs	Repeated FFQ by interview, 2nd and 3rd trimester of pregnancy, validated	Not specified, probably during pregnancy
Kiefte-de Jong, 2012, the Netherlands	Generation R study	Birth cohort	2002-2006 (births), follow-up to age 4 yrs (48 mths)	7210 children (50.6% male), Children up to age 4 yrs	Parental FFQ, semi-quant, at 14 mths, validated	Intake in first year of life, and at 14 months (past month)
Kull, 2006, Sweden	Children, Asthma, Milieu, Stockholm, Epidemiology (BAMSE)	Birth cohort	1994–1996 (births), follow-up to age 4 yrs	3670 children (50.6% male), 2614 with blood samples (IgE ¹) analysis), Children at age 4 yrs, intake at age 1 yr	Parental questionnaire	Child's age (in months) when fish was first introduced and current consumption
Leermakers, 2013, the Netherlands	Generation R study	Birth cohort	2002-2006 (births), follow-up to age 4 yrs (48 mths)	2796 mother-child pairs (50.1% male), Maternal mean age 31.8 yrs	FFQ, semi-quant, modified version, validated (original, not modified version)	Previous 3 months, 1st trimester intake
Li, 2013, USA	CARDIA study	Prospective cohort, multicenter	1985–1986 to 2005, follow-up 20 yrs	4162 (47% male), Adults 18-30 (mean age 24. 9) yrs	FFQ by interview, repeated (1985, 1992, 2005), validated	Habitual intake, at baseline and follow-up
Lumia, 2011, Finland	Type 1 Diabetes Prediction and Prevention (DIPP) Nutrition Study	Birth cohort	1997-2004 (births), follow-up to age 5 yrs	2679 mother-child pairs (52.2% male), children with a high or moderate genetic risk of type 1 diabetes, Children up to age 5 yrs	FFQ, semi-quant, self-completed, validated	Maternal diet during the 8th month of pregnancy, suppl for whole pregnancy

Author, year, country	Study name	Study design	Inclusion year(s), end, follow-up time	Study size, age	Dietary assessment method	Dietary assessment period
Lumia, 2012, Finland	Type 1 Diabetes Prediction and Prevention (DIPP) Nutrition Study	Birth cohort	1998-2004 (births), follow-up to age 5 yrs	1798 mother-child pairs, children with a high or moderate genetic risk of type 1 diabetes, Children up to age 5 yrs	FFQ, semi-quant, self-completed, validated	Maternal diet during the third month of lactation, suppl during whole lactation
Lumia, 2015, Finland	Type 1 Diabetes Prediction and Prevention (DIPP) Nutrition Study	Case-control, nested	1996-2004 (births), follow-up to age 5 yrs	182 children with asthma and 728 matched controls, children (61.1% male) with high or moderate genetic risk of type 1 diabetes, Children up to age 5 yrs	3-day food records, completed by parents for ages 3-6-12 months, then annually to age 6 yrs. Timing of introduction of new foods up to 2 yrs by structured dietary questionnaires. Quantity of breast milk based on estimated energy requirements and growth by 1 year of age	Current diet, 3 days
Magnusson, 2013, Sweden	Children, Asthma, Milieu, Stockholm, Epidemiology (BAMSE)	Birth cohort	1994–1996 (births), follow-up at age 8 and 12 yrs (range 11-14 yrs)	3285 children (50.6% male), 2404 children without early symptoms of allergic disease, Children up to age 12 yrs (range 11-14), intake at age 1 year and 8 yrs	Parental questionnaire (1 year), FFQ at age 8 yrs completed by parents or together with child	Average intake, previous year
Magnusson, 2015, Sweden	Children, Asthma, Milieu, Stockholm, Epidemiology (BAMSE)	Birth cohort	1994–1996 (births), follow-up at age 8 and 16 yrs	1970 children (49.2% male), Children age 16 yrs, intake at age 8 yrs	FFQ at age 8 yrs completed by parent (57%) or together with the child (40%)	Average intake, previous year

Author, year, country	Study name	Study design	Inclusion year(s), end, follow-up time	Study size, age	Dietary assessment method	Dietary assessment period
Maslova, 2013, Denmark	Danish National Birth Cohort (DNBC)	Birth cohort	1996 and 2002 (pregnancies), follow-up to age 7 yrs	28,936 mother-child pairs (51% male), Children up to age 7 yrs	Repeated telephone interview, at 1 and 30 weeks of gestation. Semi-quant FFQ at 25 weeks gestation, validated	Uncertain for interview, past 4 weeks for FFQ
Miyake, 2009, Japan	Osaka Maternal and Child Health Study (OMCHS)	Birth cohort	2001-2003 (pregnancies), follow-up to age 16-24 months	763 mother-child pairs (52.8% male), Maternal mean (SD) age 30 (4) yrs, children 16-24 mo	Self-adm dietary history questionnaire, validated	Dietary habits preceding month, at baseline (any stage of pregnancy)
Oien, 2019, Norway	Prevention of Allergy among Children in Trondheim (PACT) study	Cohort, based on community-based lifestyle intervention with control cohort	2000 (controls) and 2002 (intervention cohort) to 2006 (pregnant women), inclusion of one-, two- and six-year-olds until 2008, 2009, and 2014, respectively. Follow-up to age 6 yrs	2955 (mother-child pairs) to 1952 children, dependig on analyses, Maternal mean age 30.3 yrs, children up to age 6 yrs	FFQ questions, semi-quant, validated	Pregnancy period assessed in pregnancy (median 13 weeks gestation) or retrospectively, age at introduction, at age 1 and 2 yrs
Pele, 2013, France	PELAGIE	Birth cohort	2002-2006 (pregnancy), follow-up to age 2 yrs	1550 mother-child pairs (51.5% male), Children up to age 2 yrs	FFQ, designed to capture dioxins and furanes	Usual intake, prior to pregnancy
Romieu, 2007, Spain	Antenatal care cohort, Menorca	Birth cohort	1997-1998 (pregnancy), follow-up to age 6 yrs	458 mother-child pairs (52% male), blood sample at 4 yrs in 75% of children, Children up to age 6 yrs	FFQ by interview, translated and modified version of EPIC-Norfolk, validated (original, not modified version)	Pregnancy period, retrospectively assessed 3 month after delivery

Author, year, country	Study name	Study design	Inclusion year(s), end, follow-up time	Study size, age	Dietary assessment method	Dietary assessment period
Sausenthaler, 2007, Germany	LISA (Influences of Lifestyle-related Factors on the Immune System and the Development of Allergies in Childhood) study	Birth cohort	1997-1999 (newborns), follow-up to age 2 yrs	2641 mother-child pairs (52% male), Children up to age 2 yrs	FFQ, semi quant.	Last 4 wks of pregnancy, retrospectively assessed 3 month after delivery
Stratakis, 2017, Europe/USA	Pooled analysis of 18 European and US birth cohorts	Birth cohorts - 18 pooled	1996 to 2011 (deliveries), maximum follow-up to age 8 years	60 774 mother-child pairs (51% male), Maternal median (IQR) age 30.4 (28.0-33.2) yrs	Cohort-specific FFQs or questionnaires specifically designed to assess fish intake during pregnancy, validated (in most cohorts)	During pregnancy, no further details
Talaei, 2021, the UK	Avon Longitudinal Study of Parents and Children (ALSPAC)	Birth cohort	1991-1992 (births), follow-up to age 14 yrs	4543 children (49.2% male), Maternal mean age 29.5 yrs, children up to age 14 yrs, intake at 7 yrs	Maternal completed FFQ on child consumption, validated (original, not modified version)	Usual intake, age 7 yrs
Willers, 2007, Scotland	Aberdeen Maternity Hospital cohort	Birth cohort	1997-1999 (pregnancy), follow-up to age 5 yrs	1212 mother-child pairs (50.3% male), Maternal mean age 29.9 yrs, children up to age 5 yrs	FFQ semi-quant (V 5.4 of Scottish Collaborative Group FFQ), validated	Previous 2-3 months at 32 weeks gestation
Willers, 2008, the Netherlands	Prevention and Incidence of Asthma and Mite Allergy (PIAMA)	Birth cohort, with intervention part (mite-allergen avoidance) and the natural history part	1996/97 (births), follow-up to age 8 yrs	2832 mother-child pairs (51.3% male), Maternal mean (SD) age 30.6 yrs, children up to age 8 yrs	Pregnancy questionnaire (at 30-36 gestational weeks in majority)	Past month

Author, year, country	Study name	Study design	Inclusion year(s), end, follow-up time	Study size, age	Dietary assessment method	Dietary assessment period
Willers, 2011, the Netherlands	Prevention and Incidence of Asthma and Mite Allergy (PIAMA)	Birth cohort, with intervention part (mite-allergen avoidance) and the natural history part	1996/97 (births), follow-up to age 8 yrs	2145 children (50.6% male), Up to age 8 yrs	Annual questionnaires for ages 2-8 yrs with food frequency questions	Past month

¹IgE: immunoglobulin E.

The pooled analysis by Stratakis et al. (2017) (18 birth cohorts) was based on European birth cohorts participating in an analysis on fish intake in pregnancy and birth outcomes (Leventakou et al., 2014). Among 29 invited cohorts (identified from the European inventory of birth cohorts or from individual websites or published articles assessed until June 2011), seven cohorts did not reply, and three cohorts declined participation for reasons not related to the objective of the article. Information about fish consumption during pregnancy, gestational age and weight at birth were the minimum requirements for inclusion. From the 19 potentially eligible birth cohorts for the current analysis, 16 cohorts provided relevant data for the analysis of asthma and allergies in the offspring: ABCD (The Netherlands); DNBC (Denmark); FLEHS I (Belgium); GASPII (Italy); Generation R study (The Netherlands); Generation XXI study (Portugal); HUMIS (Norway); INMA (Spain); KOALA (The Netherlands); Lifeways Cross Generation (Ireland); Lucki (The Netherlands); NINFEA (Italy); PELAGIE (France); PIAMA (The Netherlands); RHEA (Greece); and SWS (UK). In addition, the Project Viva cohort from Massachusetts (USA) and the Bologna Birth Cohort (Italy) were included in the current analysis. Overall, the study population in the 18 cohorts (17 European and one US) included 60 774 mother-child pairs with information on fish intake during pregnancy, selected confounding variables and at least one of the health outcomes studied: wheeze (infants, preschool age, school age), persistent wheeze (preschool age, school age), asthma (preschool age, school age), and allergic rhinitis (school age). Eczema was not included. Most outcomes were analyzed in relation to maternal intake in two ways; as a continuous variable (time per week), and for fish intake as a categorical variable with 3 levels (≥ 3 times/week, > 1 but < 3 times/wk, and ≤ 1 time/week). When available, VKM has emphasized results from the categorical analysis and the highest versus lowest intake level for comparison with other studies and previous meta-analyses.

The main difference between the included meta-analyses (Malmir et al., 2021; Zhang et al., 2017, Papamichael et al., 2018) and the pooled analysis by Stratakis et al. (2017), is that the meta-analyses are based on systematic literature reviews, whereas Stratakis et al. (2017) is a pooled analysis of primary data from a European research collaboration. VKM treated Stratakis 2017 as a multicenter study included among other primary studies, whereas the meta-analyses were performed independently of Stratakis et al. (2017). The results from the European cohorts in Stratakis et al. (2017) were only included in the meta-analyses if found as separate publications. Thus, smaller cohort studies that have not published independently, were missed in these meta-analyses.

4.28.2.2 VKM's search compared to previous meta-analyses

Table 4.28.2.2-1 presents overlap between VKM's included meta-analyses.

This table covers overlap for all the included asthma and allergic outcomes in Chapters 4.29-4.33.

Table 4.28.2.2-1 Overview of studies included by VKM compared with three identified meta-analyses on asthma and allergic diseases.

Publication	Exposure timing	Outcomes	VKM	Malmir, 2021	Papamichael, 2018	Zhang, 2017
Alm, 2009	Pregnancy	Eczema				X
Alm, 2011	First year of life	Allergic rhinitis				X
Alyarez Zallo, 2018	Pregnancy	Wheeze and eczema		X		
Chatzi, 2008						X
Chatzi, 2013	Pregnancy	Wheeze and eczema		X		X
Dotterud, 2013	Pre, postnatal				X	
Erkkola, 2012	Pregnancy	Asthma, wheeze		X		X
Goksör, 2011	Age introduction	Wheeze			X	X
Jedrychowski, 2008	Pregnancy	Respiratory symptoms (cough, difficult breathing, chest wheeze)	X	X		
Jedrychowski, 2011	Pregnancy	Eczema	X	X		X
Kiefte-de Jong, 2012	First yr of life	Asthma-like symptoms, wheeze (main tables), shortness of breath (Appendix)	X		X	X
Kull, 2006	First yr of life	Allergic diseases (asthma, allergic rhinitis, eczema) at age 4	X – overlap Magnusson 2013		X	X
Leermakers, 2013	Pregnancy	Wheezing and eczema	X	X		X
Li, 2013	Adulthood	Adult onset asthma	X			
Lumia, 2011	Pregnancy	Child asthma	X	X		X
Lumia, 2012	Lactation	Child asthma	X			
Lumia, 2015	First yr of life and childhood	Child asthma, total, atopic, non-atopic	X			
Magnusson, 2013	First yr of life and childhood	Allergic diseases (asthma, allergic rhinitis, eczema), IgE ¹	X		X	X
Magnusson, 2015	Childhood	Rhinitis, allergic and non-allergic	X			
Maslova, 2013	Pregnancy	Asthma	X	X		X
Miyake, 2009	Pregnancy	Wheeze, eczema	X	X		X
Miyake, 2013	Pregnancy	Wheeze and eczema		X		X
Nafstad, 2003	First year of life	Asthma, allergic rhinitis	X	X	X	X
Noakes, 2012	Pregnancy	Immune responses and clinical outcomes				X
Nwaru, 2010	Pregnancy	Allergic sensitization				X
Nwaru, 2013	First year of life	Asthma and allergic diseases			X	
Oien, 2010	Pregnancy	Asthma, eczema		X		X
Oien, 2019	First yr of life	Asthma, wheeze, eczema, ever allergic rhinoconjunctivitis	X			
Ozawa, 2014	Pregnancy	Eczema		X		X

Publication	Exposure timing	Outcomes	VKM	Malmir, 2021	Papamichael, 2018	Zhang, 2017
Pele, 2013	Pregnancy	Wheeze, eczema, food allergy	X	X		
Romieu, 2007	Pregnancy	7 outcomes (Eczema 1 yrs, IgE ¹ 4 yrs, SPT ² 6 yrs, wheeze 6 yrs)	X	(X) not used		X
Saito, 2010	Pregnancy	Atopic eczema				X
Salam, 2005	Pregnancy	Asthma		X		
Sausenthaler, 2007	Pregnancy	Eczema, allergic sensitization	X	X		X
Stratakis, 2017	Pregnancy	Asthma (preschool, school age), wheeze (infant, preschool, school age), persistent wheeze (preschool, school age) allergic rhinitis (school age)	X			
Talaei, 2021	Childhood	Asthma	X			
Viljoen, 2018	Pregnancy	Asthma		X		
Willers, 2007	Pregnancy	Eczema (doctor confirmed, current med, ever); hay fever (doctor confirmed, current med, ever)	X	X		X
Willers, 2008	Pregnancy	Asthma symptoms (wheeze, dyspnea, steroid use, composite asthma symptoms)	X	X		X
Willers, 2011	Childhood	Asthma symptoms (wheeze, dyspnea, steroid use, composite asthma symptoms), sensitization (inhaled allergenes, food), BHR	X			
Xu, 2015	Pregnancy	Asthma		X		

¹IgE: immunoglobulin E. ²SPT: skin prick test.

4.29 Fish intake and eczema in children

4.29.1 VKM's search for primary studies of fish intake and eczema

4.29.1.1 Included studies from search

A total of 10 studies had eczema in children as outcome (Jedrychowski et al., 2011; Kull et al., 2006; Leermakers et al., 2013; Magnusson et al., 2013; Miyake et al., 2009; Oien et al., 2019; Pele et al., 2013; Romieu et al., 2007; Sausenthaler et al., 2007; Willers et al., 2007). Eczema was assessed at different ages ranging from 3 months to 12 years in relation to maternal or child fish intake. One study was excluded due to overlap, as described below, leaving 9 studies for further analysis, one with results on pre-pregnancy intake, seven on pregnancy intake, one on intake during lactation, and three on intake in children.

4.29.1.2 *Overlapping publications*

There were multiple publications on eczema from the Swedish BAMSE study (Kull et al., 2006; Magnusson et al., 2013). Both assessed child fish intake at age 1 year, Kull et al. (2006) in relation to child eczema at age 4 years and Magnusson et al. (2013) up to age 12 years (with follow-up at ages 1-2-4-8-12 years). Magnusson et al. (2013) also assessed child fish intake at age 8 years in relation to eczema at 12 years. Kull et al. (2006) was excluded, as Magnusson et al. (2013) also covered age 4 years.

4.29.1.3 *Studies by design and geographic region*

The body of evidence on child eczema had a skewed geographic distribution with nine studies from Europe and one from Asia (Japan). All studies were based on cohorts, except Oien 2019 (Norwegian PACT study) which was based on a community-based lifestyle intervention with control cohort. The intervention involved structured advice to increase fish and cod liver oil intake, reduce tobacco exposure and reduce indoor dampness during pregnancy and the first two years of life.

4.29.1.4 *Studies by sub-groups and potential effect modification*

Magnusson et al. (2013) presented results separately for eczema with and without allergic sensitization at age 8 years, which could represent different phenotypes of eczema. Other studies also included multiple sub-groups of outcome definitions (Willers et al., 2007), such as ever eczema with or without doctor-confirmation, and current treatment (past 12 months) for eczema at age 5 years.

4.29.1.5 *Studies by fish exposure (type and timing)*

Child eczema was assessed in relation to maternal fish intake in pre-pregnancy in one study (Pele et al., 2013), during pregnancy in seven studies (Jedrychowski et al., 2011; Leermakers et al., 2013; Miyake et al., 2009; Oien et al., 2019; Romieu et al., 2007; Sausenthaler et al., 2007; Willers et al., 2007), during lactation in one study (Oien et al., 2019), and in children in two studies (Magnusson et al., 2013; Oien et al., 2019). Studies of intake prior to pregnancy or during lactation were too limited for a summary.

All studies included total fish exposure (sum of all fish, unspecified fish, or fish including shellfish and/or processed fish such as fish fingers and fish sandwich spread). Two studies additionally presented sub-classification of fish as lean and fatty fish intake during pregnancy (Leermakers et al., 2013; Oien et al., 2019) or in children (Oien et al., 2019).

4.29.1.6 *Studies assessing potential non-linearity*

None of the included primary studies on eczema presented a non-linear dose-response curve or dose-response information that could not be conveyed without a figure. One study (Romieu et al., 2007) assessed potential non-linearity in regression effects using Generalized

Additive Models (GAM) but found that the relationship was linear on the log-scale which was used for result presentation.

4.29.2 Results from the included primary studies on maternal fish intake and eczema

4.29.2.1 *Studies of maternal total fish intake and eczema*

We included eight publications with 15 estimates of the association between maternal total fish intake (14 during pregnancy, and one prior to pregnancy) and child eczema in the weight of evidence analysis. The exposure levels and results for maternal intake are shown in Table 4.29.2.1-1. All studies had a prospective observational design, so the design has been left out of the table, whereas details on the outcome have been included due to multiple definitions of the outcome in some studies.

Table 4.29.2.1-1 Results from prospective observational studies included in the weight of evidence analysis of maternal total fish intake and child eczema.

Author, year, country	Outcome measure	Fish exposure	Intake unit	High-low intake	Total cases	RR high-low (95 % CI)	Overall result
Total fish intake prior to pregnancy							
Pele, 2013, France	Eczema, age 2 yrs	Fish, pre-pregnancy intake	Times/wk or month, 3 cat	≥2 times/wk vs <1 time/mo	475	OR=0.92 (0.58, 1.46)	No sig. assoc.
Total fish intake during pregnancy							
Jedrychowski, 2011, Poland	Eczema, age 3-12 mo - any timepoint	Fish, pregnancy intake	g/wk, tertiles	>205 vs ≤90 g/wk, one fish meal set to 150 g	183 (prev 39%)	OR=0.57 (0.35, 0.93)	Sig. protective effect of higher maternal fish intake on child's eczema
	Eczema, age 3-12 mo (symptom frequency)		g/wk, tertiles	>205 vs ≤90 g/wk, one fish meal set to 150 g	183 (prev 39%)	IRR (Poisson)=0.72 (0.52, 0.99)	Sig. protective effect of higher maternal fish intake on frequency of child's eczema symptoms
Leermakers, 2013, the Netherlands	Eczema (doctor attended), age 6-12 mo	Total fish incl shellfish - pregnancy intake	g/wk, 5 cat	Cat 5 vs 1, >210 vs 0 g/wk	643	OR=1.02 (0.66, 1.57)	No sig. assoc., <i>P</i> -trend 0.87
	Eczema (doctor attended), age 2 yrs		g/wk, 5 cat	Cat 5 vs 1, >210 vs 0 g/wk	373	OR=0.95 (0.51, 1.75)	No sig. assoc., <i>P</i> -trend 0.96
	Eczema (doctor attended), age 3 yrs		g/wk, 5 cat	Cat 5 vs 1, >210 vs 0 g/wk	254	OR=0.91 (0.47, 1.78)	No sig. assoc., <i>P</i> -trend 0.84
	Eczema (doctor attended), age 4 yrs		g/wk, 5 cat	Cat 5 vs 1, >210 vs 0 g/wk	240	OR=0.88 (0.48, 1.63)	No sig. assoc., <i>P</i> -trend 0.51
	Eczema (doctor attended), age 1-4 yrs overall		g/wk, 5 cat	Cat 5 vs 1, >210 vs 0 g/wk	NA, see ages 1-4 yrs	OR=0.96 (0.73, 1.28)	No sig. assoc., <i>P</i> -trend 0.73
Miyake, 2009, Japan	Eczema 16-24 mo	Fish - pregnancy intake	g/d, quartiles (energy adjusted)	Quartile 4 vs 2, 73.2 vs 23.4 (quartile medians)	142	OR=0.73 (0.30, 1.75)	No sig. assoc., <i>P</i> -trend 0.68

Author, year, country	Outcome measure	Fish exposure	Intake unit	High-low intake	Total cases	RR high-low (95% CI)	Overall result
Oien, 2019, Norway	Eczema, age 6 yrs	Fish - pregnancy intake	Times/wk, binary	≥1 vs <1 time/wk	NA, estimated 278 (child intake analysis)	OR=0.95 (0.67, 1.35)	No sig. assoc.
Romieu, 2007, Spain	Eczema (doctor confirmed), age 1 year	Fish - pregnancy intake	Times/wk, Continuous, log transformed score		132	OR=0.73 (0.55, 0.98)	Protective assoc., <i>P</i> =0.04
Sausenthaler, 2007, Germany	Eczema, age 2 yrs	Fish - pregnancy intake (last 4 wks)	Times/mo or wk, tertiles	Tertile 3 vs 1-2 combined based on 5 frequencies (≥4 times/wk vs <2 times/mo or never)	446 (17.7%)	OR=0.75 (0.57, 0.98)	Protective assoc.
Willers, 2007, Scotland	Eczema, ever – age 5 yrs	Fish - pregnancy intake	Times/mo or wk, 3 cat	≥1/wk vs never	406 (32.4%)	OR=0.68 (0.43, 1.10)	Borderline protective trend without sig. estimates, <i>P</i> -trend 0.05
	Eczema, ever (doctor confirmed) – age 5 yrs		Times/mo or wk, 3 cat	≥1/wk vs never	380 (30.4%)	OR=0.57 (0.35, 0.92)	Protective association, <i>P</i> -trend 0.008
	Eczema, current treatment – age 5 yrs		Times/mo or wk, 3 cat	≥1/wk vs never	191 (15.3%)	OR=0.58 (0.32, 1.06)	Protective trend without sig estimates, <i>P</i> -trend 0.028

Of the seven studies that investigated maternal intake of total fish during pregnancy and child eczema, estimates were on the protective side or close to unity. One study of maternal intake prior to pregnancy (Pele et al., 2013) supported a protective association, but was not statistically significant, and one study of maternal intake during lactation was at unity (Oien et al., 2019, result not shown).

4.29.2.2 *Studies of maternal lean and fatty fish intake and eczema*

Two publications reported on fatty and lean fish intake during pregnancy and risk of child eczema. These publications reported no statistically significant associations for total fish, and associations with fatty fish and lean fish were also statistically non-significant and close to unity, see Table 4.29.2.2-1.

Table 4.29.2.2-1 Results from prospective observational studies included in the weight of evidence analysis of maternal fatty and lean fish intake and child eczema.

Author, year, country	Outcome measure	Child age	Intake unit	High-low intake	Total cases	OR high-low (95 % CI)	Overall result
Fatty fish intake during pregnancy							
Leermakers, 2013, the Netherlands	Eczema (doctor attended)	1-4 yrs overall	g/wk, 4 cat	Cat 4 vs 1, >70 vs 0 g/wk	NA, 240 at age 4 yrs	OR=1.06 (0.88, 1.27)	Sig. increased risk in cat 2 and 3 (intake 1-34 and 25- 69 g/d) but not cat 4, no sig. trend (<i>P</i> -trend 0.68)
Oien, 2019, Norway	Eczema	6 yrs	Times/wk, binary	≥1 vs <1 time/wk	NA, estimated 278 (child intake analysis)	OR=0.97 (0.69, 1.36)	No sig. assoc.
Lean fish intake during pregnancy							
Leermakers, 2013, the Netherlands	Eczema (doctor attended)	1-4 yrs overall	g/wk, 4 cat	Cat 4 vs 1, >70 vs 0 g/wk	NA, 240 at age 4 yrs	OR=0.99 (0.79, 1.24)	No sig. assoc., <i>P</i> -trend 0.67
Oien, 2019, Norway	Eczema	6 yrs	Times/wk, binary	≥1 vs <1 time/wk	NA, estimated 279 (child intake analysis)	OR=0.98 (0.70, 1.39)	No sig. assoc.

4.29.2.3 Studies of total child fish intake and eczema

We included two studies of total fish intake in children with 13 estimates of eczema, in the weight of evidence analysis. The exposure levels and results for child intake are shown in Table 4.29.2.3-1. Both studies found reduced risk of eczema for intake in the first year of life, but not at later ages (age 2 years in Oien et al., 2019, or age 8 years in Magnusson et al., 2013).

The high number of estimates in Magnusson et al. (2013) was due to presentation of both prevalence and incidence, estimates before and after restriction to children with early symptoms of allergic disease (to assess the potential influence of disease-related modification of exposure), for eczema at age 8 years with and without allergic sensitization (specific IgE-positivity to food or airborne allergen), and for two different intake categorizations (both binary). Child intake at age 1 year showed a protective association with both the prevalence and incidence of eczema up to age 12 years and for both reference categories (>1 time/week vs Never, and for ≥ 2–3 vs <1 time/month). The association with eczema at

age 8 years (stratified by sensitization) was stronger for eczema with sensitization. Sensitization was defined based on at least one allergen-specific IgE-result (≥ 35 kU/L) for a food or airborne allergen. The food allergens tested for were hen egg, cow's milk, cod fish, wheat, soybean, and peanut, and the airborne allergens *Dermatophagoides pteronyssinus* (dust mite), cat, dog, horse, timothy, birch, mugwort, and *Cladosporium herbarium* (mold).

However, all associations with eczema at age 8 years or 12 years were attenuated when restricted to analyses of children without early symptoms of allergic disease, suggesting an influence of disease-related modification of exposure. Only prevalent eczema at age 12 years remained statistically significant after restriction for intake $\geq 2-3$ vs <1 time/month.

Table 4.29.2.3-1 Results from prospective observational studies included in the weight of evidence analysis of fish intake in children and risk of eczema.

Author, year, country	Outcome measure	Fish exposure timing	Intake unit	High-low intake	Total cases	Adjusted OR high-low (95% CI)	Overall result
Magnusson, 2013, Sweden	Eczema, prev up to age 12 yrs (1-2-4-8-12 yrs)	Age 1 yr	Times/wk or mo, 5 cat	>1 time/wk vs never	392 (12%)	OR=0.43 (0.35, 0.54)	Protective assoc., all intake levels above never, P -trend ≤ 0.001
	Eczema, prev up to age 12 yrs (restricted)	Age 1 yr	Times/wk or mo, 5 cat	>1 time/wk vs never	NA, sample 2040 of 3285	OR=0.74 (0.52, 1.03)	Borderline protective assoc., P -trend 0.008
	Eczema, prev age 12 yrs	Age 1 yr	Times/wk or mo, binary	$\geq 2-3$ vs <1 time/mo	392 (12%)	OR=0.61 (0.52, 0.70)	Protective assoc. for regular ($\geq 2-3$ times/mo) vs irregular (≤ 1 time/mo) intake
	Eczema, prev up to age 12 yrs (restricted)	Age 1 yr	Times/wk or mo, binary	$\geq 2-3$ vs <1 time/mo	NA, sample 2040 of 3285	OR=0.78 (0.63, 0.97)	Protective assoc. for regular ($\geq 2-3$ times/mo) vs irregular (≤ 1 time/mo) intake
	Eczema, incidence age 12 yrs	Age 1 yr	Times/wk or mo, binary	$\geq 2-3$ vs <1 time/mo	94 (5%), since age 8 yrs	OR=0.63 (0.55, 0.73)	Protective assoc. for regular ($\geq 2-3$ times/mo) vs irregular (≤ 1 time/mo) intake
	Eczema, incidence age 12 yrs (restricted)	Age 1 yr	Times/wk or mo, binary	$\geq 2-3$ vs <1 time/mo	NA, sample 2040 of 3285	OR=0.87 (0.70, 1.08)	No sig. assoc.
	Eczema without sensitization, 8 yrs	Age 1 yr	Times/wk or mo, binary	$\geq 2-3$ vs <1 time/mo	169	OR=0.70 (0.47, 1.03)	Borderline protective assoc.
	Eczema with sensitization, 8 yrs	Age 1 yr	Times/wk or mo, binary	$\geq 2-3$ vs <1 time/mo	144	OR=0.51 (0.34, 0.75)	Protective assoc.

Author, year, country	Outcome measure	Fish exposure timing	Intake unit	High-low intake	Total cases	Adjusted OR high-low (95 % CI)	Overall result
	Eczema without sensitization, 8 yrs, restricted	Age 1 yr	Times/wk or mo, binary	≥2–3 vs <1 time/mo	NA	OR=0.74 (0.47, 1.24)	No sig. assoc.
	Eczema with sensitization, 8 yrs, restricted	Age 1 yr	Times/wk or mo, binary	≥2–3 vs <1 time/mo	NA	OR=0.84 (0.41, 1.71)	No sig. assoc.
	Eczema, incidence age 12 yrs	Age 8 yrs	Times/wk or g/d, not specified	Tertile 3 vs 1	NA/2456 children	OR= 0.82 (0.47, 1.46)	No sig. assoc., <i>P</i> -trend 0.50
Oien, 2019, Norway	Eczema, age 6 yrs	Age 1 yr	Times/wk, binary	≥1 vs <1 time/wk	278	OR=0.69 (0.53, 0.91)	Protective assoc.
	Eczema, age 6 yrs	Age 2 yrs	Times/wk binary	≥1 vs <1 time/wk	278	OR=1.28 (0.88, 1.88)	No sig. assoc.

4.29.2.4 *Summary relative risks (RR) based on VKM's inclusion of primary studies*

VKM calculated a summary RR for child eczema in relation to the highest versus lowest intake of total fish during pregnancy based on six prospective observational studies. One study was excluded from the summary RR as fish intake was reported on a continuous scale (Romieu et al., 2007). Leermakers et al. (2013) presented risk of eczema at ages 1 to 4 years, separately and combined. Estimates were relatively similar, and the combined estimate for age 1-4 years was selected. Willers et al. (2007) presented results on different definitions on eczema at age 5 years (ever, ever doctor confirmed, and current medication use) and the estimate for ever doctor confirmed eczema was found to be most comparable with other studies and included.

VKM's summary RR for maternal intake during pregnancy indicate a statistically significant protective effect on the risk of eczema (RR=0.78, 95% CI: 0.65, 0.94), without significant heterogeneity ($p_{\text{heterogeneity}}=0.26$, six studies). In contrast, previous meta-analyses by Malmir et al., 2021 (RR=0.93, 95% CI: 0.84, 1.03, $I^2=41\%$; $P_{\text{heterogeneity}}=0.08$, 10 cohort studies) and Zhang et al. (2017) (RR=0.84, 95% CI 0.69, 1.01; $I^2=56\%$; $P_{\text{heterogeneity}}=0.07$, 8 cohort studies), found associations that were only borderline statistically significant and more heterogeneous (Table 4.28.1.2-1). Additional analyses were performed to explain the difference in results. Both meta-analyses (Malmir et al., 2021; Zhang et al., 2017) included more studies than VKM, some did not fulfill VKM's eligibility criteria regarding study quality or study design, but other studies were not detected in VKM's search (as described in following section). The inclusion of these studies, of which two from Japan, would have attenuated VKM's results and increased heterogeneity.

VKM did not calculate summary RRs for maternal intake of fatty and lean fish (two studies), child intake of total fish (two studies) or fatty and lean fish (one study), in relation to eczema. In comparison, Zhang et al. (2017) reported a protective association for age of introduction or infant intake and later eczema (RR=0.71; 95% CI 0.61, 0.82; $p<0.001$; $I^2=0\%$, 3 studies) as described in more detail below.

4.29.2.5 *VKM's search compared to previous meta-analyses of child eczema*

An overview of overlapping studies in the included meta-analyses of eczema (Malmir et al., 2021; Zhang et al., 2017) is included in 4.29.2.2-1.

VKM identified one recent publication from the Norwegian PACT study (Oien et al., 2019) whereas previous meta-analyses included an older publication with shorter follow-up for eczema (Oien et al., 2010) that did not meet VKM's quality criteria. Among the 10 cohort studies in Malmir 2021 and 8 cohort studies in Zhang et al. (2017), three were not identified by VKM (Chatzi et al., 2013; Miyake et al., 2013; Ozawa et al., 2014) as the main focus was not fish, but Mediterranean diet or other dietary aspects. The inclusion of these studies would have attenuated VKM's high-low summary RR to some extent.

Zhang et al. (2017) presented a summary RR for infant intake of fish and risk of eczema based on 3 studies (excluding a crude OR from Alm et al., 2009). All included studies were identified, but one (Nafstad et al., 2003) presented any fish intake and did not fulfill VKM's eligibility criteria of frequency or amount of fish intake, and one study (Oien et al., 2010) did not meet VKM's quality criteria. These studies were therefore excluded by VKM.

4.29.3 Heterogeneity maternal fish intake and eczema

In the high-low meta-analysis of total maternal fish intake during pregnancy and risk of child eczema, there was no significant heterogeneity between studies included by VKM (7 studies), whereas heterogeneity in previous meta-analyses (Malmir et al., 2021; Zhang et al., 2017) was moderate ($I^2 = 41\%$ to 56%). Most estimates were on the protective side or close to null and estimates on the adverse side were not statistically significant. Heterogeneity analysis performed on the high-low estimates in Malmir et al. (2021) suggested some potential methodological issues, as there was no significant association with low heterogeneity among studies with the largest sample size (>1000 , 8 of 12 estimates). The protective association was stronger among studies with a low-quality score (5 of 12 estimates), or self-reported diagnosis (6 of 12 estimates).

4.29.4 Dose-response relationship maternal fish intake and eczema

Malmir et al. (2021) performed a meta dose-response analysis (intake range 0-200 grams per week) and found a protective association with significant departure from linearity ($P_{\text{nonlinearity}} = 0.042$). Risk began to decrease from 50 g/week. However, the confidence limits of the curve were too wide to conclude that the relationship was statistically significant.

4.29.5 Weight of evidence for maternal fish intake and eczema

In this section the evidence of the association between maternal fish intake during pregnancy and eczema is weighed according to the WCRF criteria presented in Chapter 3.1.6 (Box 2).

Published evidence of maternal fish intake during pregnancy and child eczema

Two previous meta-analyses of maternal intake (high-low) of fish during pregnancy and risk of child eczema, have found overall associations on the protective side, but only borderline statistically significant, based on ten (Malmir et al., 2021) or eight (Zhang et al., 2017) prospective studies. VKM evaluated seven prospective studies of pregnancy intake and found a statistically significant protective association in a high-low meta-analysis of six of these studies, but three potentially eligible studies (two from Japan, and one from Spain/Greece) were not identified in the search. Re-analysis suggest that the inclusion of these studies would have attenuated the association. No conclusions can be drawn regarding a differential effect of fatty and lean fish (two studies included by VKM, no previous meta-analyses). Prospective studies of fish intake in children and risk of eczema remain fewer than for pregnancy intake. One previous meta-analysis of child intake (Zhang et al., 2017) included

three studies (four including one study reporting an unadjusted estimate) and VKM found two eligible studies. Associations are protective for intake in the first year of life, but not later. Results suggest that disease-related modification of exposure occur.

Heterogeneity

The heterogeneity between studies of maternal intake of fish and child eczema is low to moderate with most associations either on the protective side or null, with no statistically significant adverse associations. However, the heterogeneity analysis by Malmir et al. (2021) suggest potential methodological limitations.

Mechanisms/biological plausibility

LC n-3 FA have established anti-inflammatory and immunoregulatory properties that may protect against the development of eczema.

Upgrading factors

Evidence of dose-response was not found to be an upgrading factor for eczema.

One non-linear dose-response meta-analysis of maternal fish intake during pregnancy and child eczema suggests a protective association but is not statistically significant for any part of the curve.

4.29.5.1 *Conclusion weight of evidence fish intake and eczema*

The evidence of an association between maternal fish intake in pregnancy and development of eczema in the offspring is based on nine cohort studies included by VKM (six in high-low summary RR), and two independent meta-analyses of eight or ten studies. Previous meta-analyses have found associations on the protective side (high-low or meta dose-response analysis), but they did not reach statistical significance despite a relatively large number of studies. VKM found a significant association but based on fewer studies than the most recent meta-analysis. Some potentially eligible studies were not identified by VKM. Heterogeneity analysis in the most recent meta-analysis (Malmir et al., 2021) suggests some potential methodological limitations. There was no significant association among studies with the largest sample size or highest study quality. A clear dose-response relationship was not found to be an upgrading factor. Therefore, the evidence that maternal fish consumption during pregnancy reduces risk of eczema is graded "limited, suggestive". Evidence on fish intake in the lactation period and risk of eczema (one study) is too limited for a conclusion.

The evidence that fish intake in infants reduces risk of eczema is graded "limited, suggestive" based on one previous meta-analysis of three studies and two studies included by VKM showing protective associations for intake around age 1 year, but not older ages. Associations with eczema at age 8 years or 12 years were attenuated when restricted to analyses of children without early symptoms of allergic disease (one study), suggesting an influence of disease-related modification of exposure.

No conclusions could be drawn for the effects of fatty fish or lean fish due to limited evidence.

4.30 Fish intake and wheeze in children

4.30.1 VKM's search for primary studies of fish intake and wheeze

4.30.1.1 *Included studies from search*

A total of 11 studies, including the pooled analysis by Stratakis et al. (2017), had wheeze in children as outcome (Jedrychowski et al., 2008; Kiefte-de Jong et al., 2012; Leermakers et al., 2013; Maslova et al., 2013; Miyake et al., 2009; Oien et al., 2019; Pele et al., 2013; Romieu et al., 2007; Stratakis et al., 2017; Willers et al., 2008; Willers et al., 2011). Wheeze was assessed at different ages ranging from infants to school age children, and in relation to maternal and child fish intake. Four studies were excluded due to overlap as described below, leaving seven for further analysis of wheeze, five with results on pregnancy intake and three on child intake, of which one study included both time periods.

4.30.1.2 *Overlapping publications*

Stratakis et al. (2017) pooled data on pregnancy fish intake from 18 birth cohorts (17 European and 1 US): ABCD (the Netherlands); DNBC (Denmark); FLEHS I (Belgium); GASPII (Italy); Generation R study (the Netherlands); Generation XXI study (Portugal); HUMIS (Norway); INMA (Spain); KOALA (the Netherlands); Lifeways Cross Generation (Ireland); Lucki (the Netherlands); NINFEA (Italy); PELAGIE (France); PIAMA (the Netherlands); RHEA (Greece); SWS (UK); Bologna Birth Cohort (Italy); Project Viva (Massachusetts, USA). Separate publications on pregnancy fish intake and wheeze prior to 2017 were found from four of these cohorts which were excluded from the summary to not count the same studies twice; the Generation R (Leermakers et al., 2013); DNBC (Maslova et al., 2013); PELAGIE (Pele et al., 2013); and PIAMA (Willers et al., 2008). Publications on child intake were not overlapping with Stratakis et al. (2017) and were kept from Generation R (Kiefte-de Jong et al., 2012) and PIAMA (Willers et al., 2011).

4.30.1.3 *Studies by design and geographic region*

As for eczema, the body of evidence was predominantly from studies conducted in Europe except for one US cohort (part of Stratakis et al., 2017), and one Japanese study (Miyake et al., 2009). Two Norwegian studies contributed to the analyses, the HUMIS study (part of Stratakis et al., 2017) and the PACT study (Oien et al., 2019).

All studies had a prospective observational design (birth cohort, or cohort based on intervention study). As described under eczema, the PACT study (Oien et al., 2019) is a community-based lifestyle intervention (including advice to increase fish and cod liver oil intake) with a control cohort. The PIAMA study is a birth cohort with an intervention part

(mite-allergen avoidance) and a natural history part (Willers et al., 2011). Both studies were analyzed as cohorts by combining all data and adjusting for the different study arms.

4.30.1.4 *Studies by sub-groups and potential effect modification*

Stratakis et al. (2017) assessed wheeze at different ages: infancy (first 2 years), preschool age (3-4 years), and school age (5-8 years), and made the distinction between wheeze and persistent wheeze. Persistent wheeze was defined as presence of wheeze both in infancy and the respective period examined (preschool age or school age). Romieu et al. (2007) divided wheeze at age 6 years into persistent wheeze (wheezing at 6 years and in any preceding years) and atopic wheeze (any positive skin prick test in addition to wheeze), and additionally stratified by breastfeeding.

4.30.1.5 *Studies by fish exposure (type and timing)*

All five studies of maternal fish intake (Jedrychowski et al., 2008; Miyake et al., 2009; Oien et al., 2019; Romieu et al., 2007; Stratakis et al., 2017) included total fish exposure (sum of all fish, unspecified fish, or fish including shellfish). Two of five studies of total fish additionally included fatty and lean fish intake (Stratakis et al., 2017; Oien et al., 2019). Of three studies on child intake (Kiefte-de Jong et al., 2012; Oien et al., 2019; Willers et al., 2011) all included total fish, two also included fatty fish (Kiefte-de Jong et al., 2012; Oien et al., 2019) and one included lean fish (Oien et al., 2019). Other classifications of fish were not used. Maternal intake during lactation (only study Oien et al., 2019) and age of introduction (any intake in two studies; Kiefte-de Jong et al., 2012; Oien et al., 2019) was not summarized.

4.30.1.6 *Studies assessing potential non-linearity*

None of the included primary studies on wheeze presented a non-linear dose-response curve or dose-response information that could not be conveyed without a figure. As for eczema, Romieu et al. (2007) reported to have investigated the shape of the dose-response relationship using generalized additive models (GAM) and found that the relationship was linear on the log-scale which was used for result presentation.

4.30.2 Results from the included primary studies on fish intake and child wheeze

4.30.2.1 *Studies of maternal total fish intake and wheeze*

We included five publications with ten estimates of the association between total fish intake during pregnancy and child wheeze in the weight of evidence analysis. The exposure levels and results for maternal intake are included in Table 4.30.2.1-1. All studies had a prospective observational design, so the design was left out of the table, whereas details on the outcome was included due to multiple definitions of the outcome in several studies. Three studies

assessed wheeze in the first two years of life, and three studies at age six years or school age (5 to 8 years). Associations were either null or protective. The pooled analysis by Stratakis et al. (2017) was the largest and did not report any statistically significant associations for any age group. Associations were similar for wheeze and persistent wheeze. Two smaller studies reported protective associations, for the number of days of wheezing among children up to age 2 years (Jedrychowski et al., 2008), and for atopic wheeze at age 6 years (Romieu et al., 2007, 19 cases only).

Table 4.30.2.1-1 Results from studies included in the weight of evidence analysis of maternal total fish intake during pregnancy and child wheeze.

Author, year, country	Outcome	Child age	Intake unit	High-low intake	Total cases	OR/HR/RR high-low (95 % CI)	Overall result
Jedrychowski, 2008, Poland	Wheezing irrespective of respiratory infection	3-24 mo	g/wk, dichotomized at median	>150 vs ≤150 g/wk, one meal set to 150 g	125 (prev 27%)	IRR=0.97 (0.95, 0.99), Poisson	Sig. protective effect of higher maternal fish intake on child wheezing (no. of days)
Stratakis, 2017, Europe/USA	Wheeze infancy	First 2 yrs	Times/wk, 3 cat	>3 vs ≤1time/wk	17518 (29.2%)	RR (pooled, 14 cohorts)=0.96 (0.89, 1.03)	No sig. assoc., non sig. heterogeneity ($I^2=16.8\%$, $P=0.27$)
	Wheeze	Preschool age (3-4 yrs)	Times/wk, 3 cat	>3 vs ≤1time/wk	1949 (15.4%)	RR (pooled, 8 cohorts)=0.98 (0.84, 1.14)	No sig. assoc., non sig. heterogeneity ($I^2=0\%$, $P=0.44$)
	Wheeze	School age (5-8 yrs)	Times/wk, 3 cat	>3 vs ≤1time/wk	3050 (13.1%)	RR (pooled, 9 cohorts)=1.05 (0.95, 1.17)	No sig. assoc., non sig. heterogeneity ($I^2=0\%$, $P=0.69$)
	Persistent wheeze	Preschool age (3-4 yrs)	Times/wk, 3 cat	>3 vs ≤1time/wk	1228 (14.1%)	RR (pooled, 8 cohorts)=0.97 (0.79, 1.20)	No sig. assoc., non sig. heterogeneity ($I^2=4\%$, $P=0.40$)
	Persistent wheeze	School age (5-8 yrs)	Times/wk, 3 cat	>3 vs ≤1time/wk	1525 (10.4%)	RR (pooled, 6 cohorts)=1.08 (0.94, 1.24)	No sig. assoc., non sig. heterogeneity ($I^2=0\%$, $P=0.96$)
Miyake, 2009, Japan	Wheeze	16-24 mo	g/d, quartiles (energy adjusted)	Quartile 4 vs 1, 73.2 vs 23.4 (quartile medians)	169	OR=0.67 (0.30, 1.48)	No sig. assoc., P -trend 0.28
Oien, 2019, Norway	Current wheeze	6 yrs	Times/wk, binary	≥1 vs <1 time/wk	NA, sample 2024	OR=1.15 (0.76, 1.74)	No sig. assoc.
Romieu, 2007, Spain	Persistent wheeze	6 yrs	Times/wk, continuous, log transformed score		27	OR=0.87 (0.51, 1.49)	No sig. assoc., $P=0.62$
	Atopic wheeze	6 yrs	Times/wk, continuous, log transformed score		19	OR=0.55 (0.31, 0.96)	Protective assoc., $P=0.034$

4.30.2.2 Studies of maternal lean and fatty fish intake and wheeze

Two publications, including one pooled analysis, reported on fatty and lean fish intake during pregnancy and risk of child wheeze, see Table 4.30.2.2-1. These publications reported no statistically significant associations for total fish, and no associations emerged from the analysis of fatty fish and lean fish. In Stratakis et al. (2017), estimates were close to unity (no association) for all three age intervals (only reported on continuous scale). Estimates in Oien et al. (2019) were also non-significant for both fatty- and lean fish.

Table 4.30.2.2-1 Results from studies included in the weight of evidence analysis of maternal fatty and lean fish intake during pregnancy and child wheeze.

Author, year, country	Outcome, measure	Child age	Intake unit	High-low intake	Total cases	OR/RR high-low (95 % CI)	Overall result
Fatty fish intake during pregnancy							
Stratakis, 2017, Europe/USA	Wheeze infancy	First 2 yrs	Times/wk, continuous, per 1-time/week		17518 (29.2%)	RR (pooled, 11 cohorts) =1.00 (0.99, 1.01)	No sig. assoc., non sig. heterogeneity ($I^2=0\%$, $P=0.79$)
	Persistent wheeze	Preschool age (3-4 yrs)	Times/wk, continuous, per 1-time/week		1228 (14.1%)	RR (pooled, 8 cohorts) =0.99 (0.94, 1.05)	No sig. assoc., non sig. heterogeneity ($I^2=0\%$, $P=0.97$)
	Persistent wheeze	School age (5-8 yrs)	Times/wk, continuous, per 1-time/week		1525 (10.4%)	RR (pooled, 7 cohorts) =0.99 (0.95, 1.03)	No sig. assoc., non sig. heterogeneity ($I^2=0\%$, $P=0.98$)
Oien, 2019, Norway	Current wheeze	6 yrs	Times/wk, binary	≥ 1 vs <1 time/wk	NA, sample 2031	OR 1.32 (0.90, 1.93)	No sig. assoc.
Lean fish intake during pregnancy							
Stratakis, 2017, Europe/USA	Wheeze infancy	First 2 yrs	Times/wk, continuous, per 1-time/week		17518 (29.2%)	RR (pooled, 11 cohorts) =1.00 (0.99, 1.01)	No sig. assoc., non sig. heterogeneity ($I^2=0\%$, $P=0.76$)
	Persistent wheeze	Preschool age (3-4 yrs)	Times/wk, continuous, per 1-time/week		1228 (14.1%)	RR (pooled, 8 cohorts) =1.04 (0.98, 1.10)	No sig. assoc., non sig. heterogeneity ($I^2=13.1\%$, $P=0.33$)
	Persistent wheeze	School age (5-8 yrs)	Times/wk, continuous, per 1-time/week		1525 (10.4%)	RR (pooled, 7 cohorts) =1.03 (0.99, 1.05)	No sig. assoc., non sig. heterogeneity ($I^2=0\%$, $P=0.83$)
Oien, 2019, Norway	Current wheeze	6 yrs	Times/wk, binary	≥ 1 vs <1 time/wk	NA, sample 2105	OR=1.05 (0.71, 1.55)	No sig. assoc.

4.30.2.3 Studies of child total fish intake and wheeze

We included three publications with five estimates of the association between total fish intake in children and wheeze in the weight of evidence analysis. The exposure levels and results for child intake are included in Table 4.30.2.3-1. Estimates were not statistically significant, except for a protective association with intake at age 1 year on wheeze at 6 years (Oien et al., 2019). The protective association for intake around 1 year of age was not confirmed in Kiefte-de Jong et al. (2012) where child intake at 14 months was studied in relation to wheeze at 3 years and 4 years. Willers et al. (2011) analyzed the longitudinal fish intake during ages 2 to 8 years overall in relation to wheeze at age 8 years and found a borderline adverse association.

Table 4.30.2.3-1 Results from studies included in the weight of evidence analysis of child total fish intake and child wheeze.

Author, year, country	Outcome measure, timing	Fish exposure timing	Intake unit	High-low intake	Total cases	Adjusted OR high-low (95 % CI)	Overall result
Kiefte-de Jong, 2012, the Netherlands	Wheezing, age 36 mo	14 mo	Servings/wk, 3 cat	At least ½ serving/wk vs no intake, 120 g raw fish counted as one serving	Prev. 34.4%, sample 2480	OR=0.99 (0.80, 1.24)	No sig. assoc.
	Wheezing, age 48 mo	14 mo	Servings/wk, 3 cat	At least ½ serving/wk vs no intake, 120 g raw fish counted as one serving	Prev. 33.8%, sample 2439	OR=0.94 (0.76, 1.18)	No sig. assoc.
Oien, 2019, Norway	Current wheeze, age 6 yrs	Age 1 yr	Times/wk, binary	≥1 vs <1 time/wk	224	OR=0.62 (0.45, 0.83)	Protective assoc.
	Current wheeze, age 6 yrs	Age 2 yrs	Times/wk, binary	≥1 vs <1 time/wk	224	OR=1.10 (0.72, 1.67)	No sig. assoc.

Author, year, country	Outcome measure, timing	Fish exposure timing	Intake unit	High-low intake	Total cases	Adjusted OR high-low (95 % CI)	Overall result
Willers 2011, the Netherlands	Wheeze, age 8 yrs	Age 2-8 yrs	Days/week, continuous, per 1 day increase per wk, median 0.5, 0.5, 0.7 consumption days for age 2-3 yrs, 7-8 yrs, and average long-term intake (2-8) yrs respectively		213 (6.5%) age 8 yrs	OR=1.32 (0.97, 1.80)	Borderline adverse association with higher intake

4.30.2.4 *Summary relative risks (RR) based on VKM's inclusion of primary studies*

VKM calculated summary RRs for risk of wheeze in relation to the highest versus lowest maternal intake of total fish during pregnancy.

In addition to the pooled analysis of high-low maternal intake by Stratakis et al. (2017) (14 cohorts for wheeze in first 2 years of life; 8 cohorts for preschool age; and 9 cohorts for school age) VKM identified two prospective studies on wheeze in the first 2 years of life (Jedrychowski et al., 2008; Miyake et al., 2009), and two on wheeze at 6 years of age (Oien et al., 2019; Romieu et al., 2007) with non-overlapping results. Romieu et al. (2007) only reported maternal fish intake on a continuous scale and could not be included in the summary RR.

The pooled RR (high-low) in Stratakis et al. (2017) for child wheeze in the first 2 years ($RR=0.96$, 95% CI: 0.89, 1.03, heterogeneity $I^2=16.8\%$, $P=0.27$, 11 cohorts) was similar in magnitude and statistically significant after VKM added two additional primary studies of wheeze at age 2 years ($RR=0.97$, 95% CI: 0.95, 0.99, $P_{\text{heterogeneity}}=0.63$). Stratakis et al. (2017) did not report the cohort-specific estimates in the high-low analysis. Therefore, the single, pooled estimate was used, which contributed little to the relative weight despite the large sample (17 518 cases, 8% weight) compared with the protective estimate in Jedrychowski et al. (2008) (125 cases, 92% relative weight due to smaller standard error). Thus, the statistical significance could potentially be an artefact of using the pooled estimate. The contribution by Miyake et al. (2009) was negligible ($<0.1\%$ relative weight) due to the wide confidence interval (CI) of the estimate. Romieu et al. (2007) (not added) also had a wide CI and few cases.

The pooled RR (high-low) in Stratakis et al. (2017) for wheeze at school age (5 to 8 years) ($RR=1.05$, 95% CI: 0.95, 1.17, heterogeneity $I^2=0\%$, 9 cohorts) was combined with current wheeze at age 6 years in one recent study (Oien et al., 2019), which had little effect. VKM's summary RR was not statistically significant ($RR=1.09$, 95% CI: 0.95, 1.22) and without significant heterogeneity ($P_{\text{heterogeneity}}=0.70$).

In comparison, two previous high-low meta-analyses (Malmir et al., 2021; Zhang et al., 2017) have summarized maternal fish intake and risk of wheeze in the offspring (Table 4.28.1.2-1). Malmir et al. (2021) found a protective association small in magnitude, but statistically significant ($RR=0.97$, 95% CI: 0.96, 0.99, 9 cohort studies). Malmir et al. (2021) was to a large extent an update of Zhang et al. (2017) ($RR=0.94$, 95% CI 0.83, 1.07).

VKM did not calculate a summary RR for child intake due to only two studies with similar reporting.

Two previous high-low meta-analyses of infant or child intake (Papamichael et al., 2018; Zhang et al., 2017) included the same two publications but may have emphasized different estimates which could explain different results (Table 4.28.1.2-1). Papamichael et al. (2018) focused on infant intake (all fish versus no fish) and found a statistically significant protective

association (RR=0.62, 95% CI 0.48, 0.80), but not Zhang et al. (2017) (RR=0.94, 95% CI: 0.83, 1.07).

4.30.2.5 VKM's search compared to the previous meta-analysis and pooled analysis on wheeze

An overview of overlapping studies in the included meta-analyses (Malmir et al., 2021; Papamichael et al., 2018; Zhang et al., 2017) is found in Table 4.28.2.2-1. All meta-analyses assessed maternal fish intake, and all except Malmir et al. (2021) also assessed child intake, in relation to child wheeze.

VKM identified one recent primary study (Oien et al., 2019) not included in previous meta-analyses, and neither Malmir et al. (2021) nor Zhang et al. (2017) included results from Stratakis et al. (2017). Despite being an older publication, the pooling project by Stratakis et al. (2017) captured more studies (14 on wheeze in infancy) than the systematic literature reviews. Of the 10 studies included by Malmir et al. (2021), one (Alvarez Zallo et al., 2018, cross-sectional design) did not meet VKM's eligibility criteria, and three were not identified (Chatzi et al., 2013; Erkkola et al., 2012; Miyake et al., 2013). Malmir et al. (2021) covered all studies in Zhang et al. (2017) and included additional studies on pregnancy intake, but Malmir et al. (2021) did not assess child intake.

Regarding child fish intake, VKM identified three studies of intake at ages 1 to 8 years. Previous meta-analyses (Papamichael et al., 2018; Zhang et al., 2017) focused on age of introduction (not assessed by VKM) or infant intake only and were not directly comparable. Both meta-analyses identified the same primary studies (Goksor et al., 2011; Kiefte-de Jong et al., 2012), but summary estimates differed. Papamichael et al. (2018) used estimates for age of introduction (all fish versus no fish intake), whereas Zhang et al. (2017) may have emphasized other estimates in the same publications (not specified).

4.30.3 Heterogeneity maternal fish intake and wheeze

In the high-low meta-analysis of total maternal fish intake during pregnancy and risk of child wheeze (at age 2 years or 6 years) performed by VKM, there was no significant heterogeneity between studies. Heterogeneity was low to moderate ($I^2=26\%$ to 32%) between studies of maternal intake in the meta-analyses by Malmir et al. (2021) and Zhang et al. (2017). Most estimates were on the protective side or close to null and estimates on the adverse side were not statistically significant. However, heterogeneity analysis performed on the high-low estimates in Malmir 2021 suggested some potential methodological issues (much similar to eczema). Associations were close to unity or statistically non-significant among studies with the largest sample size (9 of 11 estimates) or highest quality score (7 of 11 estimates).

There was no heterogeneity ($I^2=0\%$) in previous meta-analyses of intake in children (Papamichael et al., 2018; Zhang et al., 2017) but only based on two studies.

4.30.4 Dose-response relationship maternal fish intake and wheeze

Malmir et al. (2021) performed both a linear and non-linear meta dose-response analysis of maternal fish intake and risk of child wheeze and found significant departure from linearity (P non-linearity 0.01). The non-linear dose-response curve (spline model) suggested increased risk of wheeze for intake higher than 30 grams per week. However, the confidence limits of the curve were too wide to conclude that the relationship was statistically significant. High-low analyses did not reflect increased risk.

Stratakis et al. (2017) (pooled analysis) meta-analyzed two categorical intake levels; ≥ 3 vs ≤ 1 time/week (high-low) and > 1 but < 3 times/week vs ≤ 1 time/week (midrange-low). Estimates were close to unity and non-significant for both levels in all age groups (infancy, preschool age, school age) and did therefore not suggest a gradient in the association of maternal fish intake during pregnancy with risk of wheeze.

4.30.5 Weight of evidence for maternal fish intake and wheeze

Published evidence of maternal fish intake during pregnancy and child wheeze

The association of maternal total fish intake (high-low) during pregnancy with risk of wheeze in the offspring has been examined in a large number of birth cohorts from Europe, with less evidence from other populations or study designs. One pooled analysis (Stratakis et al., 2017) reported an association in the protective direction that was small in magnitude and borderline statistically significant for wheeze in infants (based on 14 studies), but not in preschool children (eight studies) or school age children (nine studies). One recent meta-analysis (Malmir et al., 2021, nine cohort studies) that to a large extent cover a previous meta-analysis (Zhang et al., 2017) also reported a protective association small in magnitude, but statistically significant. VKM's summary estimates (based on Stratakis et al., 2017 and two additional primary studies) were protective and statistically significant for wheeze in the first two years of life, but not later ages.

Studies of child fish intake and wheeze remain limited. VKM identified three studies, and two previous meta-analyzed included two studies. Associations are null or on the protective side for fish intake in the first year of life, but on the adverse side for older ages.

Heterogeneity

The heterogeneity between studies of maternal intake of fish and child wheeze is low to moderate with most associations either on the protective side or null, with no statistically significant adverse associations. However, the heterogeneity analysis by Malmir et al. (2021) suggest potential methodological limitations.

Mechanisms

Wheeze is a symptom of asthma but is not unique to asthma. LC n-3 FAs have established anti-inflammatory and immunoregulatory properties that may protect against the

development of asthma. Vitamin D may prevent transient forms of wheezing due to respiratory tract infections in preschool children, but probably has no effect on wheezing due to allergy-related asthma.

Upgrading factors

Evidence of dose-response was not found to be an upgrading factor for wheeze.

One non-linear dose-response meta-analysis of maternal fish intake during pregnancy and child wheeze suggests an association on the adverse side for intakes higher than 30 grams per week, but the association is not statistically significant for any part of the curve.

4.30.5.1 *Conclusion weight of evidence fish intake and wheeze*

The evidence that maternal fish intake during pregnancy reduces the risk of child wheeze is graded "limited, suggestive" for wheeze in the first two years of life and "limited, no conclusions" for wheeze at older ages. Results are only borderline statistically significant despite relatively many studies. Results may be limited by methodological issues (no association among studies with the largest sample size or highest quality score), and the evidence of a dose- response relation was not found to be an upgrading factor. No conclusions can be drawn regarding a differential effect of fatty and lean fish intake during pregnancy (two studies included by VKM, no previous meta-analyses). Evidence on fish intake in the lactation period and risk of wheeze (one study) is too limited for a conclusion.

Studies on child intake remain limited with inconsistent results for intake and outcome at different ages and no conclusion can be drawn.

4.31 Fish intake and asthma in children

4.31.1 **VKM's search for primary studies of** fish intake and asthma

4.31.1.1 *Included studies from search*

A total of 11 publications, 10 single (Kull et al., 2006; Lumia et al., 2011; Lumia et al., 2012; Lumia et al., 2015; Magnusson et al., 2013; Maslova et al., 2013; Oien et al., 2019; Talaei et al., 2021; Willers et al., 2008; Willers et al., 2011) and one pooled analysis (Stratakis et al., 2017) had asthma in children as outcome. Asthma was assessed at different ages ranging from 1 to 14 years, in relation to maternal or child fish intake. Three studies were excluded due to overlap as described below, leaving eight for further analysis: three with results on intake during pregnancy and two during lactation, and five on child intake.

4.31.1.2 *Overlapping publications*

Stratakis et al. (2017) pooled data on pregnancy fish intake and child asthma from 16 birth cohorts (15 European and one US). Separate publications on pregnancy fish intake and

asthma prior to 2017 were found from two of these cohorts which were excluded from the summary to not count the same studies twice; the DNBC (Maslova et al., 2013) and PIAMA (Willers et al., 2008). Publications on child intake were not overlapping with Stratakis et al. (2017) and were kept from PIAMA (Willers et al., 2011).

There were multiple publications on asthma from the Swedish BAMSE study (Kull et al., 2006; Magnusson et al., 2013). Both assessed child fish intake at age 1 year; Kull et al. (2006) in relation to child asthma at age 4 years and Magnusson et al. (2013) at 8 years (with and without allergic sensitization) and up to age 12 years (follow-up at ages 1-2-4-8-12 years). Magnusson et al. (2013) also assessed child fish intake at age 8 years in relation to eczema at 12 years. Kull et al. (2006) was excluded, as Magnusson et al. (2013) also covered age 4 years.

There were also multiple publications from the Finnish DIPP study (Lumia et al., 2011; Lumia et al., 2012; Lumia et al., 2015), but on fish intake at different time points (pregnancy, lactation, in children) and all were kept.

4.31.1.3 *Studies by design and geographic region*

The body of evidence on asthma consisted of European studies, except for one US cohort (part of Stratakis et al., 2017). Two Norwegian studies contributed to the analyses, the HUMIS study (part of Stratakis et al., 2017) and the PACT study (Oien et al., 2019). All studies had a prospective observational design (birth cohort, nested case-control, or cohort based on intervention study). As described previously, two publications were based on interventions studies, but analyzed as cohorts (PACT study, Oien et al., 2019 and PIAMA, Willers et al., 2011).

4.31.1.4 *Studies by sub-groups and potential effect modification*

Stratakis et al. (2017) assessed asthma at different ages; preschool age (3-4 years), and school age (5-8 years). Two studies (Lumia et al., 2015; Magnusson et al., 2013) stratified asthma by sensitization (referred to as atopic and non-atopic asthma), and one study (Talaie et al., 2021) stratified results by a fatty acid desaturase (*FADS*) polymorphism (selected SNP rs1535 in the *FADS2* gene) associated with blood levels of LC n-3 FA.

All children in the Finnish DIPP study (Lumia et al., 2011; Lumia et al., 2012; Lumia et al., 2015) have moderate or high genetic risk of type 1 diabetes (human leucocyte antigen (HLA)-conferred susceptibility, *HLA-DQB1*), but a genetic interaction for type 1 diabetes with allergic diseases or asthma has not been established, and therefore the DIPP study population was included.

4.31.1.5 *Studies by fish exposure (type and timing)*

All studies (three on maternal fish intake in pregnancy, two on intake during lactation, and five on child intake) included total fish exposure (sum of all fish, unspecified fish, or fish including shellfish). Fewer studies (two of three on pregnancy intake, one of two on lactation

intake and one of five on child intake) additionally included fatty and lean fish intake. No other classifications of fish intake were presented.

Among studies in children, three analyzed intakes in the first year of life at age 1 year or longitudinally up to age 5 years (Lumia et al., 2015; Magnusson et al., 2013; Oien et al., 2019), two analyzed intake at 2 years or used longitudinal intake from ages 2-8 years (Oien et al., 2019; Willers et al., 2011), and two used intake at age 7 or 8 years (Talaie et al., 2021; Magnusson et al., 2013).

4.31.1.6 *Studies assessing potential non-linearity*

None of the included primary studies on asthma presented a non-linear dose-response curve or dose-response information that could not be conveyed without a figure. Talaie et al. (2021) explored potential non-linearity in the association of the fatty acids EPA and DHA from fish with risk of incident asthma, but not fish intake itself.

4.31.2 Results from the included primary studies on fish intake and asthma in children

4.31.2.1 *Studies of maternal total fish intake during pregnancy and lactation and asthma*

We included three publications, two primary studies and one pooled analysis, with four estimates of the association between total fish intake during pregnancy and child asthma in the weight of evidence analysis. The exposure levels and results for maternal intake are included in Table 4.31.2.1-1. All studies had a prospective observational design, so the design was left out of the table, whereas details on the outcome was included due to multiple definitions of the outcome in several studies. Associations were either null or on the adverse side. The pooled analysis by Stratakis et al. (2017) was the largest and reported an association close to unity for asthma at preschool age (seven cohorts), and a borderline adverse association for asthma at school age (nine cohorts).

Table 4.31.2.1-1 Results from studies included in the weight of evidence analysis of maternal total fish intake during pregnancy or lactation, and child asthma.

Author, year, country	Outcome measure	Child age	Intake unit	High-low intake	Total cases	RR high-low (95 % CI)	Overall result
Total fish intake during pregnancy							
Stratakis, 2017, Europe/USA	Asthma	Preschool age (3-4 yrs)	Times/wk, 3 cat	>3 vs ≤1 time/wk	921 (9.2%)	RR (pooled, 7 cohorts) =1.00 (0.68, 1.45)	No sig. assoc., non sig. heterogeneity ($I^2=37.1\%$, $P=0.15$)
	Asthma	School age (5-8 yrs)	Times/wk, 3 cat	>3 vs ≤1 time/wk	2479 (10.5%)	RR (pooled, 9 cohorts) =1.09 (0.97, 1.23)	No sig. assoc., non sig. heterogeneity ($I^2=0\%$, $P=0.00$)
Lumia, 2011, Finland	Asthma	Up to age 5 yrs	g/d, quartiles	Quartile 4 vs 1	158	HR=0.95 (0.51, 1.77), reported as 1.06 (0.68, 1.65) for quartile 4 and 1.12 (0.74, 1.70) for quartile 1 vs mid-half (ref quartiles 2-3)	No sig. assoc., P -trend 0.86
Oien, 2019, Norway	Current asthma	6 yrs	Times/wk, binary	≥1 vs <1 time/wk	NA, estimated 94 (child intake analysis), sample 2046	OR=1.83 (1.01, 3.32)	Adverse assoc.
Total fish intake during lactation							
Lumia, 2012, Finland	Asthma	Up to age 5 yrs	g/d, quartiles	Quartile 4 vs 1, >29.2 vs <8.8 g/d	96	HR=close to unity, for quartile 4 vs mid-half (ref) and quartile 1 vs mid-half (visual inspection of figure, estimates not given)	No sig. assoc.
Oien, 2019, Norway	Current asthma	6 yrs	Times/wk, binary	≥1 vs <1 time/wk	NA, estimated 94 (child intake analysis), sample 2040	OR=0.69 (0.39, 1.22)	No sig. assoc.

4.31.2.2 Studies of maternal lean and fatty fish intake during pregnancy and asthma

Two studies of total fish, including the pooled analysis, reported on fatty and lean fish intake during pregnancy and risk of child asthma. Associations were on the adverse side (statistically significant or borderline statistically significant) for total fish and risk of asthma in children aged 5-8 years or 6 years (previous table). When stratified by fatty and lean fish, estimates were on the adverse side (not statistically significant) for lean fish in the pooled analysis (Stratakis et al., 2017), and for both lean and fatty fish in the Norwegian PACT study (Oien et al., 2019).

Table 4.31.2.2-1 Results from studies included in the weight of evidence analysis of maternal fatty and lean fish intake during pregnancy and child asthma.

Author, year, country	Outcome measure	Child age	Intake unit	Total cases	RR high-low or continuous (95 % CI)	Overall result
Fatty fish intake during pregnancy						
Stratakis, 2017, Europe/USA	Asthma	Preschool age (3-4 yrs)	Times/wk, continuous, per 1-time/week	921 (9.2%)	RR (pooled, 8 cohorts) =0.96 (0.88, 1.06)	No sig. assoc., non sig. heterogeneity ($I^2=12.4\%$, $P=0.33$)
	Asthma	School age (5-8 yrs)	Times/wk, continuous, per 1-time/week	2479 (10.5%)	RR (pooled, 7 cohorts) =1.00 (0.97, 1.03)	No sig. assoc., non sig. heterogeneity ($I^2=0\%$, $P=0.63$)
Oien, 2019, Norway	Current asthma	6 yrs	Times/wk, binary	NA, estimated 94 (child intake analysis)	OR=1.66 (0.96, 2.89)	Borderline adverse assoc.
Lean fish intake during pregnancy						
Stratakis, 2017, Europe/USA	Asthma	Preschool age (3-4 yrs)	Times/wk, continuous, per 1-time/week	921 (9.2%)	RR (pooled, 8 cohorts) =1.06 (0.99, 1.14)	No sig. assoc., non sig. heterogeneity ($I^2=0\%$, $P=0.81$)
	Asthma	School age (5-8 yrs)	Times/wk, continuous, per 1-time/week	2479 (10.5%)	RR (pooled, 7 cohorts) =1.04 (0.96, 1.12)	No sig. assoc., non sig. heterogeneity ($I^2=34.3\%$, $P=0.17$)
Oien, 2019, Norway	Current asthma	6 yrs	Times/wk, binary	NA, estimated 94 (child intake analysis)	OR=1.61 (0.91, 2.85)	No sig. assoc.

4.31.2.3 *Studies of child total fish intake and asthma*

We included five publications with 21 estimates of the association between total fish intake in children and asthma in the weight of evidence analysis. The exposure levels and results for child intake are included in Table 4.31.2.3-1.

Two studies assessed atopic vs non-atopic asthma (Lumia et al., 2015; Magnusson et al., 2013). Atopic asthma was defined as at least one allergen-specific IgE-result (≥ 35 kU/L) for a food or airborne allergen in addition to criteria for asthma. In Lumia et al. (2015) the food allergens specified were egg, cow's milk, fish, and wheat and the airborne allergens were house dust mite, cat, timothy grass, or birch. Magnusson et al. (2013) included some additional allergens, the specified food allergens were hen egg, cow's milk, cod fish, wheat, soy bean, and peanut, and the airborne allergens *Dermatophagoides pteronyssinus* (dust mite), cat, dog, horse, timothy, birch, mugwort, and *Cladosporium herbarium* (mold).

As described for eczema, the high number of estimates in Magnusson et al. (2013) was due to presentation of two different reference categories for fish intake (>1 time/week vs Never, and $\geq 2-3$ vs <1 time/month), both prevalence and incidence of asthma, estimates before and after restriction to children without early symptoms (to control for possible disease-related modification of exposure), as well as atopic vs non-atopic asthma.

Like eczema in Magnusson et al. (2013), child intake at age 1 year showed a protective association with both the prevalence and incidence of asthma up to age 12 years and for both reference categories (>1 time/week vs Never, and for $\geq 2-3$ vs <1 time/month). The association with asthma at age 8 years (stratified by sensitization) years was stronger for atopic than non-atopic asthma. However, all associations with asthma at age 8 years or 12 years were attenuated when restricted to analyses of children without early symptoms of allergic disease, suggesting an influence of disease-related modification of exposure. Only prevalent eczema at age 12 years remained statistically significant after restriction for intake $\geq 2-3$ vs <1 time/month.

Table 4.31.2.3-1 Results from studies included in the weight of evidence analysis of child total fish intake and child asthma.

Author, year, country	Outcome measure, timing	Fish exposure timing	Intake unit	High-low intake	Total cases	OR high-low (95% CI)	Overall result
Lumia, 2015, Finland	Asthma, up to age 5 yrs	Fish, incl fish products, infancy (3, 6,12 mo) and childhood (2-3-4-5) yrs	g/d, continuous, log transformed		154	OR=0.87 (0.77, 0.98)	Protective assoc.
	Asthma, up to age 5 yrs, adj for age introduction	Fish, incl fish products, infancy (3, 6,12 mo) and childhood (2-3-4-5) yrs	g/d, continuous, log transformed		154	OR=0.93 (0.82, 1.05)	No sig. assoc.
Magnusson, 2013, Sweden	Asthma, prev up to age 12 (1-2-4-8-12 yrs)	Fish, age 1 yr	Times/wk or mo, 5 cat	>1 time/wk vs never	218 (7%)	OR=0.54 (0.40, 0.74)	Protective assoc., all intake levels above never, <i>P</i> -trend ≤0.001
	Asthma, prev up to age 12 yrs (restricted)	Fish, age 1 yr	Times/wk or mo, 5 cat	>1 time/wk vs never	NA, sample 2040 of 3285	OR=0.81 (0.48, 1.37)	No sig. assoc., <i>P</i> -trend 0.30
	Asthma, prev up to age 12 (1-2-4-8-12 yrs)	Fish, age 1 yr	Times/wk or mo, binary	≥2–3 vs <1 time/mo	218 (7%)	OR=0.71 (0.57, 0.87)	Protective assoc. for regular (≥2–3 times/mo) vs irregular (≤1 time/mo) intake
	Asthma, prev up to age 12 yrs (restricted)	Fish, age 1 yr	Times/wk or mo, binary	≥2–3 vs <1 time/mo	NA, sample 2040 of 3285	OR=0.89 (0.63, 1.27)	No sig. assoc.
	Asthma, incidence age 12 yrs	Fish, age 1 yr	Times/wk or mo, binary	≥2–3 vs <1 time/mo	83 (3%), since age 8 yrs	OR=0.80 (0.65, 0.98)	Protective association for regular (≥2–3 times/mo) vs irregular (≤1 time/mo) intake
	Asthma, incidence age 12 yrs (restricted)	Fish, age 1 yr	Times/wk or mo, binary	≥2–3 vs <1 time/mo	NA, sample 2040 of 3285	OR=0.85 (0.61, 1.19)	No sig. assoc.

Author, year, country	Outcome measure, timing	Fish exposure timing	Intake unit	High-low intake	Total cases	OR high-low (95% CI)	Overall result
	Asthma, incidence age 12 yrs	Fish, age 8 yrs	Times/wk or g/d, not specified		NA/2456 children	OR=1.12 (0.62, 2.05)	No sig. assoc., <i>P</i> -trend 0.69
Oien, 2019, Norway	Current asthma, 6 yrs	Fish, age 1 yr	Times/wk, binary	≥1 vs <1 time/wk	94	OR=0.55 (0.35, 0.87)	Protective association
	Current asthma, 6 yrs	Fish, age 2 yrs	Times/wk, binary	≥1 vs <1 time/wk	94	OR=1.07 (0.58, 1.96)	No sig. assoc.
Talaie, 2021, the UK	Asthma incidence, 11 or 14 yrs (since 8 yrs)	Fish, incl shellfish, age 7 yrs,	g/d, quartiles	Quartile 4 vs 1, median 46.5 vs 6.07 g/d	393	OR=0.83 (0.62, 1.13)	No sig. assoc., <i>P</i> -trend 0.22
	Asthma incidence, 11 or 14 yrs (since 8 yrs)	Fish, incl shellfish, age 7 yrs, FADS2 genotype rs1535: AA	g/d, quartiles	Quartile 4 vs 1, median 46.5 vs 6.07 g/d	145	OR=1.06 (0.61, 1.85)	No sig. assoc., <i>P</i> -trend 0.81
Willers, 2011, the Netherlands	Asthma symptoms, age 8 yrs	Fish, child intake age 2-8 yrs	Days per week, continuous, per 1 consumption day increase per wk, Median for early age (2-3 yrs), later age (7-8 yrs) and average long-term intake (2-8) yrs were 0.5, 0.5 and 0.7 days respectively		425 (13.0%) age 8 yrs	OR=1.23 (0.97, 1.57)	No sig. assoc.
	Asthma incidence, 11 or 14 yrs (since 8 yrs)	Fish, incl shellfish, child intake age 7 yrs, FADS2 genotype rs1535: GA/GG	g/d, quartiles	Quartile 4 vs 1, median 46.5 vs 6.07 g/d	171	OR=0.59 (0.37, 0.93)	Protective assoc. for intake in quartiles 3 and 4, <i>P</i> -trend 0.03 (limited to genotype)

Table 4.31.2.3-2 Results from studies included in the weight of evidence analysis of child total fish intake and atopic versus non atopic asthma.

Author, year, country	Outcome measure, timing	Fish exposure, timing	Intake unit	High-low intake	Total cases	OR high-low (95 % CI)	Overall result
Atopic vs non atopic asthma							
Lumia, 2015, Finland	Asthma atopic, up to age 5 yrs	Fish, incl fish products, infancy (3, 6,12 mo) and childhood (2-3-4-5) yrs	g/d, continous, log transformed		86	OR=0.84 (0.70, 1.01)	Borderline protective assoc.
	Asthma non-atopic, up to age 5 yrs	Fish, incl fish products, infancy (3, 6,12 mo) and childhood (2-3-4-5) yrs	g/d, continous, log transformed		62	OR=0.99 (0.83, 1.17)	No assoc.
	Asthma atopic, up to age 5 yrs, adj for age introduction	Fish, incl fish products, infancy (3, 6,12 mo) and childhood (2-3-4-5) yrs	g/d, continous, log transformed		86	OR=0.89 (0.74, 1.07)	No sig. assoc.
	Asthma non-atopic, up to age 5 yrs, adj for age introduction	Fish, incl fish products, infancy (3, 6,12 mo) and childhood (2-3-4-5) yrs	g/d, continous, log transformed		62	OR=1.03 (0.85, 1.25)	No sig. assoc.
Magnusson, 2013, Sweden	Asthma non-atopic, 8 yrs	Fish, age 1 yr	Times/wk or mo, binary	≥2–3 vs <1 time/mo	54	OR=0.82 (0.41, 1.62)	No sig. assoc.
	Asthma atopic, 8 yrs	Fish, age 1 yr	Times/wk or mo, binary	≥2–3 vs <1 time/mo	113	OR=0.51 (0.33, 0.78)	Protective assoc.
	Asthma non-atopic, 8 yrs (restricted)	Fish, age 1 yr	Times/wk or mo, binary	≥2–3 vs <1 time/mo	NA	OR=0.57 (0.24, 1.37)	No sig. assoc.
	Asthma atopic, 8 yrs (restricted)	Fish, age 1 yr	Times/wk or mo, binary	≥2–3 vs <1 time/mo	NA	OR=0.73 (0.35, 1.50)	No sig. assoc.

4.31.2.4 *Summary relative risks (RR) based on VKM's inclusion of primary studies*

The pooled RR in Stratakis et al. (2017) for pregnancy intake (high-low, nine cohorts) and child asthma at school age defined as 5-8 years (RR=1.09, 95% CI: 0.97, 1.23, $I^2=0\%$, $P_{\text{heterogeneity}}=0.61$) remained statistically non-significant but on the adverse side after VKM added two additional primary studies (Lumia et al., 2011; Oien et al., 2019) of asthma at age 5 or 6 years (RR=1.16, 95% CI: 0.88, 1.54, $P_{\text{heterogeneity}}=0.22$). Stratakis et al. (2017) did not report the cohort-specific estimates in the high-low analysis. Therefore, the single, pooled estimate was used (67% relative weight). In comparison, the previous meta-analysis by Malmir et al. (2021) also reported a statistically non-significant association close to unity for all studies (RR=0.99, 95% CI: 0.89, 1.11, $I^2=76.3\%$, $P_{\text{heterogeneity}}<0.001$, eight studies). The association was on the protective side with lower heterogeneity when limited to cohort studies (RR=0.92, 95% CI: 0.82, 1.04, $I^2=22.7\%$, $P=0.26$, six studies).

Of three studies that included fish intake in the first year of life, two provided high-low estimates that could be summarized in relation to prevalent asthma at age 6 or 12 years (Magnusson et al., 2013; Oien et al., 2019). Magnusson et al. (2013) presented several intake classifications and outcome definitions, and the results selected for pooled analysis were prevalent asthma at age 12 restricted to children without early symptoms of disease. When the estimate for regular versus irregular intake ($\geq 2-3$ vs <1 time/month) was used, the summary RR was on the protective side (RR=0.72, 95% CI: 0.45, 1.14, $P_{\text{heterogeneity}}=0.10$, two studies), but was stronger and statistically significant when intake >1 time/wk vs Never was used (RR=0.65, 95% CI: 0.45, 0.95, $P_{\text{heterogeneity}}=0.24$, two studies).

Compared with previous meta-analyses, Papamichael et al. (2018) found a similar significant protective association for any fish intake versus no intake during infancy (RR=0.75, 95% CI: 0.60, 0.95, $I^2=11.5\%$; $P=0.32$, three studies), but not Zhang et al. (2017) (RR=0.87, 95% CI 0.67, 1.12, $I^2=0\%$, two studies)

VKM's summary RR for studies of fish intake from age 2 or later (Magnusson et al., 2013; Oien et al., 2019; Talaei et al., 2021; Willers et al., 2011) was closer to unity and not statistically significant (RR=0.91, 95% CI: 0.68, 1.21, $P_{\text{heterogeneity}}=0.33$, four studies). In Talaei et al. (2021), the overall estimate was used (without stratification by genotype).

Summary RRs could not be estimated for pregnancy intake of fatty and lean fish, intake during the lactation period, or atopic versus non-atopic asthma due to heterogenous reporting (continuous or categorical effects, or as figure without estimates).

4.31.2.5 *VKM's search compared to previous meta-analyses and one pooled analysis of asthma*

An overview of overlapping studies in the included meta-analyses is included in Table 4.28.2.2-1. All meta-analyses included asthma as outcome (Malmir et al., 2021; Papamichael et al., 2018; Zhang et al., 2017).

VKM identified one recent publication on asthma and other outcomes from the Norwegian PACT study (Oien et al., 2019) whereas previous meta-analyses included an older publication with shorter follow-up (Oien et al., 2010) that did not meet VKM's quality criteria. None of the meta-analyses included results from the pooled analysis by Stratakis et al. (2017) on maternal intake and asthma (nine cohorts with data on asthma at school age). Malmir et al. (2021) (six cohorts and two case-control studies) covered all studies in Zhang et al. (2017) on maternal intake and asthma. VKM identified all studies in Malmir et al. (2021), but four were excluded after quality assessment (Salam et al., 2005; Oien et al., 2010; Viljoen et al., 2018) or due to non-English full-text (Xu et al., 2015) and VKM identified a different publication from the Finnish DIPP study (Lumia et al., 2011) than Malmir et al. (2021) (Erkkola et al., 2012).

Both previous meta-analyses of child intake (Papamichael et al., 2018; Zhang et al., 2017) included three studies (Nafstad et al., 2003; Oien et al., 2010, and either Kull et al., 2006 or Magnusson et al., 2013 from the Swedish BAMSE study). VKM identified all studies, but two were excluded due to the fish exposure (age of introduction) or after quality assessment (Oien et al., 2010).

4.31.3 Heterogeneity fish intake and asthma

Previous meta-analyses of maternal intake and child asthma (Malmir et al., 2021; Zhang et al., 2017) have reported moderate to high ($I^2=66\%$ to 76.3%) heterogeneity for overall null associations. In heterogeneity analyses (high-low estimates), significantly increased risk of asthma was limited to case-control studies and studies with the smallest sample sizes (≤ 1000). Heterogeneity was lower among cohort studies only ($I^2=22.7$).

Stratakis et al. (2017) used meta-analysis to pool estimates which were at unity or on the adverse side (but not statistically significant) with low to moderate heterogeneity ($I^2=0\%$ to 37%). When VKM added two estimates of asthma at age five or six years to the pooled estimate for asthma at school age (5-8 years) in Stratakis et al. (2017), heterogeneity remained non-significant ($P_{\text{heterogeneity}} 0.22$).

Previous meta-analyses of infant intake reported a protective association (Papamichael et al., 2018, $I^2=11.5\%$) or null association (Zhang et al., 2017, $I^2=0\%$) with low heterogeneity, but based on few studies (three studies in Papamichael et al., 2018 and two in Zhang et al., 2017). Heterogeneity was non-significant within each of VKM's summary RR by age of intake, also based on few studies. But results suggest that age of intake could be a source of heterogeneity in studies of children.

4.31.4 Dose-response relationship maternal fish intake and asthma

Malmir et al. (2021) performed both a linear and non-linear meta dose-response analysis of maternal fish intake and risk of child asthma and did not find significant departure from linearity ($P_{\text{non-linearity}} 0.437$). The non-linear model suggested a slightly increased risk for maternal intake in the range 30 to 150 grams per week, but the confidence limits of the

curve were too wide to conclude that the relationship was statistically significant. As previously described under heterogeneity, significantly increased risk of asthma was limited to case-control studies and studies with the smallest sample sizes.

As for wheeze, Stratakis et al. (2017) (pooled analysis) meta-analyzed two categorical intake levels of total fish: ≥ 3 vs ≤ 1 time/week (high-low) and > 1 but < 3 times/week vs ≤ 1 time/week (midrange-low). Estimates were non-significant for both intake levels in both age groups (preschool age, school age) and did not suggest a clear gradient in the association of maternal fish intake during pregnancy with risk of asthma. Estimates for fish intake (total-, fatty or lean fish) on a continuous scale (times/week) were close to unity and did not suggest a gradient in any age group.

4.31.5 Weight of evidence for maternal fish intake and asthma

Published evidence of maternal fish intake during pregnancy and child asthma

The association of maternal total fish intake (high-low) during pregnancy with risk of asthma in the offspring has been examined in several birth cohorts from Europe, with less evidence from other populations or study designs. One pooled analysis (Stratakis et al., 2017) reported an association at unity in preschool children (seven studies), and on the adverse side for school age children (based on nine studies) but not statistically significant. VKM's summary estimate for pregnancy intake and asthma in school age children (based on Stratakis et al., 2017 and additional primary studies) was also on the adverse side, but not statistically significant. No conclusions can be drawn regarding a differential effect of fatty and lean fish intake during pregnancy (two studies included by VKM, including one pooled, no previous meta-analyses). Evidence for fish intake in the lactation period (two studies) and risk of asthma was too limited for a conclusion.

Studies of infant fish intake and asthma remain limited. Two previous meta-analyzed included three studies. Associations are null or on the protective side for fish intake in the first year of life, but on the adverse side for older ages.

Heterogeneity

Heterogeneity was moderate to high in the most recent meta-analysis of maternal intake during pregnancy (Malmir et al., 2021, nine cohort studies). Study design was identified as a source of heterogeneity with adverse association limited to case-control studies and studies with the smallest sample sizes (≤ 1000). In cohort studies, the association was on the protective side but not statistically significant.

Mechanisms/biological plausibility

LC n-3 FA have established anti-inflammatory and immunoregulatory properties that may protect against the development of asthma.

Upgrading factors

Evidence of dose-response was not found to be an upgrading factor for asthma.

One dose-response meta-analysis of maternal fish intake during pregnancy and child asthma suggests an association on the adverse side for intakes higher than 30 grams per week, but the association is not statistically significant for any part of the curve.

Conclusion weight of evidence maternal fish intake and birth length and head circumference

4.31.5.1 *Conclusion weight of evidence fish intake and asthma*

The evidence that maternal fish intake during pregnancy can affect the risk of asthma in the offspring is graded “limited, no conclusion” based on no statistically significant findings overall in one previous pooled analysis, two meta-analyses, or analysis conducted by VKM, and no clear dose-response relationship. In primary studies, there are reports of associations on the adverse side, but few are statistically significant. One recent meta-analysis suggests that study design is a source of heterogeneity in studies of intake during pregnancy. No conclusions can be drawn regarding a differential effect of fatty and lean fish intake during pregnancy (two studies included by VKM, no previous meta-analyses). Evidence on fish intake in the lactation period and risk of child asthma (two studies) is too limited for a conclusion.

Studies on child intake remain limited with inconsistent results for intake and outcome at different ages, and no conclusion can be drawn. Associations differed by atopic status in two studies, and by genotype (*FADS2*) in one recent study.

4.32 Fish intake and allergic rhinitis in children

4.32.1 **VKM’s search for primary studies of** fish intake and allergic rhinitis

4.32.1.1 *Included studies from search*

A total of seven studies, including one pooled analysis, had rhinitis or rhinoconjunctivitis in children or adolescents as outcome (Kull et al., 2006; Magnusson et al., 2013; Magnusson et al., 2015; Maslova et al., 2013; Oien et al., 2019; Stratakis et al., 2017; Willers et al., 2007). The outcome was assessed at ages 4 to 16 years in relation to maternal or child fish intake. Two studies were excluded due to overlap, as described below, leaving five for further analysis, three with results on maternal intake and four with results on child intake, of which one included both.

4.32.1.2 *Overlapping publications*

Stratakis et al. (2017) pooled data on pregnancy fish intake and child allergic rhinitis from ten birth cohorts (nine European and one US). The DNBC cohort (Maslova et al., 2013) was

found to publish separately on allergic rhinitis prior to 2017, and the study was excluded to not count the same study twice. Publications on child intake were not overlapping with Stratakis et al. (2017).

There were three publications on rhinitis from the Swedish BAMSE study (Kull et al., 2006; Magnusson et al., 2013; Magnusson et al., 2015). Both Kull et al. (2006) and Magnusson et al. (2013) assessed child fish intake at age 1 year; Kull et al. (2006) in relation to allergic rhinitis at age 4 years and Magnusson et al. (2013) up to age 12. Magnusson et al. (2013) also assessed child fish intake at age 8 years in relation to rhinitis (with and without sensitization) from 8 to 12 years, and Magnusson et al. (2015) assessed fish intake at 8 years in relation to rhinitis with and without sensitization from 8 to 16 years. Kull et al. (2006) was excluded, as Magnusson et al. (2013) covered age 4 years. For intake at age 8 years, the result in Magnusson et al. (2015) with the longest follow-up was used.

4.32.1.3 *Studies by design and geographic region*

The body of evidence on allergic rhinitis or rhinoconjunctivitis consisted of European studies, except for one US cohort (part of Stratakis et al., 2017). Two Norwegian studies contributed to the analyses, the HUMIS study (part of Stratakis et al., 2017) and the PACT study (Oien et al., 2019). All studies had a prospective observational design (birth cohort, or cohort based on intervention study).

4.32.1.4 *Studies by sub-groups and potential effect modification*

Magnusson et al. (2013) and Magnusson et al. (2015) both stratified rhinitis by sensitization, and Willers et al. (2007) included multiple sub-groups of the outcome; ever hay fever with or without doctor-confirmation, and current medication (past 12 months) for hay fever, all at age 5 years.

4.32.1.5 *Studies by fish exposure (type and timing)*

Most studies (two of three on maternal fish intake in pregnancy and all four on child intake) included total fish exposure (sum of all fish, unspecified fish, or fish including shellfish), but one study in children (Magnusson et al., 2015) did not present adjusted results for total fish (as the unadjusted results were close to null). All three studies on pregnancy intake included fatty fish, and two also included lean fish intake. Two of four studies on child intake included fatty- and lean fish intake, and one study additionally presented results on intake of fish fingers (Magnusson et al., 2015). Maternal intake during lactation in one study (Oien et al., 2019) was not summarized.

4.32.1.6 *Studies assessing potential non-linearity*

None of the included studies on allergic rhinitis presented a non-linear dose-response curve or dose-response information that could not be conveyed without a figure.

4.32.2 Results from the included primary studies on fish intake and allergic rhinitis

4.32.2.1 *Studies of maternal fish intake (total, fatty, lean) and allergic rhinitis*

We included three publications, two single studies and the pooled analysis by Stratakis et al. (2017), in the weight of evidence analysis. The exposure levels and results for maternal intake during pregnancy for total fish, fatty fish and lean fish are included in Table 4.32.2.1-1. Associations were either null or on the adverse side, except for one estimate with very wide confidence limits for fatty fish and hay fever. In Oien et al. (2019), a borderline adverse association for total fish was strengthened and became statistically significant for fatty fish, while the association was close to null for lean fish. The pooled analysis by Stratakis et al. 2017 (largest) reported associations close to unity for all types of fish.

Table 4.32.2.1-1 Results from studies included in the weight of evidence analysis of maternal fish intake (total-, fatty-, and lean fish) during pregnancy and child rhinitis.

Author, year, country	Outcome measure	Child age	Intake unit	High-low intake	Total cases	RR high-low (95 % CI)	Overall result
Total fish intake during pregnancy							
Stratakis, 2017, Europe/USA	Allergic rhinitis	School age (5-8 yrs)	Times/wk	>3 vs ≤1 time/wk	1914 (5.4%)	RR (pooled, 7 cohorts) =0.99 (0.77, 1.26)	No sig. assoc., non sig. heterogeneity ($I^2=25.7\%$, $P=0.23$)
Oien, 2019, Norway	Allergic rhinoconjunctivitis	6 yrs	Times/wk, binary	≥1 vs <1 time/wk	NA, estimated 239 (child intake analysis)	OR=1.36 (0.91, 2.02)	No sig. assoc.
Fatty fish intake during pregnancy							
Stratakis, 2017, Europe/USA	Allergic rhinitis	School age (5-8 yrs)	Times/wk, Continuous, per 1-time/week		1914 (5.4%)	RR (pooled, 6 cohorts) =1.02 (0.98, 1.05)	No sig. assoc., non sig. heterogeneity ($I^2=0\%$, $P=0.83$)
Oien, 2019, Norway	Allergic rhinoconjunctivitis	6 yrs	Times/wk, binary	≥1 vs <1 time/wk	NA, estimated 239 (child intake analysis)	OR=1.62 (1.13, 2.33)	Sig. adverse assoc.
Willers, 2007, Scotland	Hay fever, ever doctor confirmed	5 yrs	Times/mo or wk, 3 cat	≥1 time/wk vs never	68 (5.4%)	OR=0.28 (0.06, 1.19)	Protective trend without sig. estimates, P -trend 0.04
Lean fish intake during pregnancy							
Stratakis, 2017, Europe/USA	Allergic rhinitis	School age (5-8 yrs)	Times/wk, Continuous, per 1-time/week		1914 (5.4%)	RR (pooled, 6 cohorts) =0.99 (0.86, 1.14)	No sig. assoc., non sig. heterogeneity ($I^2=47.7\%$, $P=0.09$)
Oien, 2019, Norway	Allergic rhinoconjunctivitis	6 yrs	Times/wk, binary	≥ 1 vs < 1 time/wk,		OR=0.97 (0.67, 1.40)	No sig. assoc.

4.32.2.2 Studies of child total fish intake and allergic rhinitis

We included two studies (three publications) with 15 estimates of the association between total fish intake in children and allergic rhinitis in the weight of evidence analysis. Both Magnusson et al. (2013) and Magnusson et al. (2015) presented results from the Swedish BAMSE study, but for different exposure and outcome time points. The exposure levels and results for child intake are included in Table 4.32.2.2-1.

Intake in the first year of life was associated with reduced risk of rhinitis in one of two studies, whereas intake at later ages (2 to 8 years) was not in any of the two studies.

As described for previous outcomes (eczema, and asthma) the high number of estimates in Magnusson et al. (2013) was due to presentation of two different reference categories for fish intake (>1 time/week vs Never, and $\geq 2-3$ vs <1 time/month), both prevalence and incidence, and estimates before and after restriction to children without early symptoms (to control for possible disease-related modification of exposure). Magnusson et al. (2013) and also Magnusson et al. (2015) presented result on rhinitis with and without allergic sensitization.

Table 4.32.2.2-1 Results from studies included in the weight of evidence analysis of child total fish intake and child rhinitis.

Author, year, country	Outcome measure, timing	Fish exposure timing	Intake unit	High-low intake	Total cases	RR high-low (95 % CI)	Overall result
Rhinitis overall							
Magnusson, 2013, Sweden	Rhinitis, prev up to age 12 yrs (1-2-4-8-12 yrs)	Age 1 yr	Times/wk or mo, 5 cat	>1 time/wk vs never	681 (21%)	OR 0.53 (0.43, 0.66)	Protective assoc., all intake levels above never, P -trend ≤ 0.001
	Rhinitis, prev up to age 12 yrs, restricted	Age 1 yr	Times/wk or mo, 5 cat	>1 time/wk vs never	NA	OR 0.63 (0.46, 0.87)	Protective assoc., P -trend 0.001
	Rhinitis, prevalence age 12 yrs	Age 1 yr	Times/wk or mo, binary	$\geq 2-3$ vs <1 time/mo	681 (21%)	OR 0.68 (0.59, 0.79)	Protective assoc. for regular ($\geq 2-3$ times/mo) vs irregular (≤ 1 time/mo) intake
	Rhinitis, incidence age 12 yrs	Age 1 yr	Times/wk or mo, binary	$\geq 2-3$ vs <1 time/mo	295 (13%) since age 8 yrs	OR 0.74 (0.63, 0.86)	Protective assoc. for regular ($\geq 2-3$ times/mo) vs irregular (≤ 1 time/mo) intake
	Rhinitis, incidence age 12 yrs	Age 8 yrs	Times/wk	Tertile 3 vs 1	295 (13%) since age 8 yrs	OR 1.20 (0.85, 1.70)	No sig. assoc., P -trend 0.30

Author, year, country	Outcome measure, timing	Fish exposure timing	Intake unit	High-low intake	Total cases	RR high-low (95 % CI)	Overall result
Oien, 2019, Norway	Allergic rhinoconjunctivitis, 6 yrs	Age 1 yr	Times/wk, binary	≥1 vs <1 time/wk	239	OR 0.86 (0.64, 1.16)	No sig. assoc.
	Allergic rhinoconjunctivitis, 6 yrs	Age 2 yrs	Times/wk, binary	≥1 vs <1 time/wk	239	OR 0.94 (0.63, 1.41)	No sig. assoc.
Atopic vs non-atopic rhinitis							
Magnusson, 2013, Sweden	Rhinitis with sensitization, 8 yrs	Age 1 yr	Times/wk or mo, binary	≥2–3 vs <1 time/mo	246	OR 0.70 (0.50, 0.97)	No sig. assoc.
	Rhinitis without sensitization, 8 yrs	Age 1 yr	Times/wk or mo, binary	≥2–3 vs <1 time/mo	105	OR 0.50 (0.32, 0.79)	No sig. assoc.
	Rhinitis with sensitization, 8 yrs, restricted	Age 1 yr	Times/wk or mo, binary	≥2–3 vs <1 time/mo	NA	OR 0.98 (0.58, 1.67)	No sig. assoc.
	Rhinitis without sensitization, 8 yrs, restricted	Age 1 yr	Times/wk or mo, binary	≥2–3 vs <1 time/mo	NA	OR 0.36 (0.21, 0.63)	Protective assoc.
Magnusson, 2015, Sweden	Rhinitis with sensitization, 8-16 yrs	Age 8 yrs	Times/wk, binary	≥2 vs <2 times/wk	337	OR 1.04 (0.80, 1.35)	No sig. assoc.
	Rhinitis without sensitization, 8-16 yrs	Age 8 yrs	Times/wk, binary	≥2 vs <2 times/wk	236	OR 0.98 (0.72, 1.32)	No sig. assoc.
	Rhinitis with sensitization, 8-16 yrs	Fish fingers, age 8 yrs	Times/wk, binary	≥1 vs <1 time/wk	337	OR 1.26 (0.95, 1.66)	No sig. assoc.
	Rhinitis without sensitization, 8-16 yrs	Fish fingers, age 8 yrs	Times/wk, binary	≥1 vs <1 time/wk	236	OR 1.04 (0.75, 1.44)	No sig. assoc.

4.32.2.3 *Summary relative risks (RR) based on VKM's inclusion of primary studies*

No summary RR estimates were calculated by VKM as no more than two studies could be combined for any analysis of pregnancy intake (total fish, fatty- or lean fish) or child intake. Among studies of pregnancy intake, one was already a pooled analysis (Stratakis et al., 2017).

4.32.2.4 *VKM's search compared to previous meta-analyses and one pooled analysis of allergic rhinitis*

An overview of overlapping studies in the included meta-analyses is included in Table 4.28.2.2-1. Two previous meta-analyses included rhinitis as outcome (Malmir et al., 2021; Zhang et al., 2017).

VKM identified one recent publication on allergic rhinoconjunctivitis from the Norwegian PACT study (Oien et al., 2019) not included in previous meta-analyses of rhinitis. None of the meta-analyses included results from Stratakis et al. (2017) (pooled analysis) on maternal intake and allergic rhinitis at school age.

Malmir et al. (2021) combined three studies (Calvani et al., 2006; Maslova et al., 2013; Sausenthaler et al., 2007) and Zhang et al. (2017) also combined three studies (Erkkola et al., 2012; Maslova et al., 2013; Willers et al., 2007) of maternal fish intake in relation to allergic rhinitis or allergic sensitization to inhalant allergens. VKM identified all studies except Erkkola et al. (2012). One study had a cross sectional design (Calvani et al., 2006) and did not fulfill VKM's eligibility criteria. Maslova et al. (2013) was covered by the Stratakis et al. (2017) (pooled analysis), and the outcome in Sausenthaler et al. (2007) was allergic sensitization which VKM considered to be a separate outcome, as not all sensitized individuals develop clinical allergy.

Zhang et al. (2017) also combined three studies of infant fish intake in relation to allergic rhinitis (Alm et al., 2011; Magnusson et al., 2013; Nafstad et al., 2003). Only Magnusson et al. (2013) met VKM's eligibility criteria.

4.32.3 Heterogeneity fish intake and allergic rhinitis

Stratakis et al. (2017) (pooled analysis) reported a summary estimate for high-low intake of total fish and risk of rhinitis that was close to unity with low to moderate heterogeneity (I^2 25.7%, 7 studies).

Two previous meta-analyses of maternal intake (Malmir et al., 2021; Zhang et al., 2017) and one of infant intake (Zhang et al., 2017) included 2-3 studies each which may be too few to reliably estimate between study heterogeneity (I^2 was 0% and 44% for maternal intake and 74% for infant intake). The consistency in direction and magnitude of associations could not be evaluated from the individual study-specific estimates as they were not provided.

4.32.4 Dose-response relationship fish intake and allergic rhinitis

As for wheeze and asthma, Stratakis et al. (2017) (pooled analysis) meta-analyzed allergic rhinitis in relation to two categorical intake levels of total fish; ≥ 3 vs ≤ 1 time/week (high-low, 7 studies) and > 1 but < 3 times/week vs ≤ 1 time/week (midrange-low, ten studies). There was no evidence of a gradient as estimates were close to unity (RR 0.99) for both intake levels. Estimates for fish intake (total-, fatty or lean fish) on a continuous scale (times/week) were also close to unity in Stratakis et al. (2017). Previous meta-analyses based on literature review (Malmir et al., 2021; Zhang et al., 2017) included few studies on rhinitis and did not perform a meta dose-response analysis.

4.32.5 Weight of evidence for maternal fish intake and allergic rhinitis

Published evidence maternal fish intake and allergic rhinitis

The evidence for an association of fish intake in pregnancy or in infants with development of rhinitis is based on one pooled analysis of birth cohorts (six European and one US) and two meta-analyses previously described for other outcomes, but the meta-analyses include few (two to three) primary studies on rhinitis. Associations are not statistically significant for pregnancy intake. Associations are protective for infant intake based on very limited data. Intake in the first year of life was associated with reduced risk of rhinitis in one of two studies, whereas intake at later ages (2 to 8 years) was not in any of the two studies included by VKM.

Heterogeneity

Heterogeneity was low to moderate in the largest study (Stratakis et al. 2017, pooled analysis) showing no association, and moderate to high two meta-analyses based on few studies.

Mechanisms/biological plausibility

Plausible mechanisms have been proposed for a protective effect of fish intake on rhinitis. The timing of fish exposure could also be of importance.

Upgrading factors

Evidence of dose-response was not found to be an upgrading factor for allergic rhinitis.

4.32.5.1 *Conclusion weight of evidence maternal fish intake and allergic rhinitis*

The evidence that maternal fish intake during pregnancy affect the risk of rhinitis in the offspring is graded "limited, no conclusion" based on no statistically significant findings overall in one previous pooled analysis, and two meta-analyses, and no clear dose-response relationship.

The evidence that child fish intake in the first year protects against rhinitis is graded “limited, no conclusion” based on one meta-analysis of two studies, and two primary studies on total fish. Associations differed by age of intake, and atopic status in one study.

4.33 Fish intake and allergic sensitization in children

4.33.1 VKM’s search for primary studies of fish intake and allergic sensitization

4.33.1.1 *Included studies from search*

A total of four studies had allergic sensitization in children as outcome in relation to fish intake during pregnancy (Kull et al., 2006; Willers et al., 2011) or child intake (Romieu et al., 2007; Sausenthaler et al., 2007). Sensitization was diagnosed as elevated serum immunoglobulin (Ig) E to any food or inhalant allergen, or as a positive skin prick test (SPT) at ages 2 to 8 years. All studies used panels of pediatric food allergens and/or inhalant allergens. Results on individual allergens were not presented, except for house dust mite (HDM) in one study (Romieu et al., 2007). One study included cod fish among other food allergens (Romieu et al., 2007).

4.33.1.2 *Overlapping publications*

There were no overlapping publications.

4.33.1.3 *Studies by design and geographic region*

All included studies were European birth cohorts. The PIAMA study (Willers et al., 2011) is a cohort study with an intervention part (mite-allergen avoidance) and a natural history part.

4.33.1.4 *Studies by sub-groups and potential effect modification*

All studies made the distinction between sensitization to food allergens or airborne allergens. Kull et al. (2006) stratified result and tested for statistical interaction by parental heredity, Romieu et al. (2007) by breast feeding.

4.33.1.5 *Studies by fish exposure (type and timing)*

All studies included total fish exposure (sum of all fish, unspecified fish, or fish including shellfish). No sub-classifications were used in relation to sensitization. Intake in children was assessed in the first year of life or at ages 2-8 years.

4.33.1.6 *Studies assessing potential non-linearity*

Romieu et al. (2007) investigated the shape of the dose response relationship between maternal fish intake during pregnancy (frequency range 0.02 to 10 times per week) on the log scale, and the predicted probability of house dust mite (HDM) sensitivity at age 6 years using Generalized Additive Models (GAM). GAMs are flexible statistical methods that may be used to identify and characterize non-linear regression effects. The association was reported to be linear and was also presented in a figure. Confidence limits were not included for the regression line.

4.33.2 Results from the included primary studies on fish intake and allergic sensitization

4.33.2.1 *Studies of maternal total fish intake and allergic sensitization*

Two studies of pregnancy intake included seven estimates of allergic sensitization. Romieu et al. (2007) used both IgE testing and skin prick testing at two ages (4 or 6 years).

Romieu et al. (2007) tested specific IgE levels against house dust mite (Der p 1), cat (Fel d 1) and mixed grass pollens (determined using Uni cap). Skin tests included house dust mite (Der p 1), cat (Fel d 1), grass pollen, olive tree (olea), mixed gramineae, and parietaria.

Sausenthaler et al. (2007) tested specific IgE levels against pediatric food allergens (fx5 panel for egg, cow milk, wheat, peanut, soybean, and codfish) and inhalants: house dust allergens (hx2 panel for *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, i.e. German cockroach, and house dust respectively), cat dander (e1), mixed molds (mx1 panel for *Penicillium notatum*, *Cladosporium herbarum*, *Aspergillus fumigatus*, and *Alternaria alternata*), or seasonal allergens (rx1 panel for timothy grass, mugwort, English plantain, ribwort, wall pellitory, and birch pollen).

Both studies used a concentration of ≥ 0.35 kU/L (kilounits per liter) as cut-off for a positive IgE test.

Associations were close to null for sensitization (positive IgE tests) to food and inhalant allergens at ages 2, 4, or 6 years in both studies, whereas a borderline protective association for sensitization to house dust mite was observed using skin prick testing in one study.

Table 4.33.2.1-1 Results from studies included in the weight of evidence analysis of maternal total fish intake during pregnancy, and sensitization.

Author, year, country	Outcome measure, timing	Fish exposure, timing	Intake unit	High-low intake	Total cases	RR high-low (95 % CI)	Overall result
Romieu, 2007, Spain	Allergic sensitization, IgE ¹ to inhalant at 4 yrs	Fish, pregnancy intake	Times/wk, continuous, log transformed score		42	OR 0.93 (0.59, 1.47)	No sig. assoc., <i>P</i> 0.77
	Allergic sensitization, IgE ¹ to HDM ² at 4 yrs	Fish, pregnancy intake	Times/wk, continuous, log transformed score		40	OR 1.00 (0.62, 1.62)	No sig. assoc., <i>P</i> 0.98
	Allergic sensitization, specific SPT ³ to inhalant at 6 yrs	Fish, pregnancy intake	Times/wk, continuous, log transformed score		57	OR 0.74 (0.50, 1.09)	No sig. assoc., <i>P</i> 0.12
	Allergic sensitization, SPT ³ to HDM ² at 6 yrs	Fish, pregnancy intake	Times/wk, continuous, log transformed score		51	OR 0.68 (0.46, 1.01)	Borderline protective assoc., <i>P</i> 0.058
Sausenthaler, 2007, Germany	Allergic sensitization, IgE ¹ to food or inhalant allergen at 2 yrs	Fish, pregnancy intake (last 4 wks)	Times/mth or wk: tertiles	Tertile 3 vs 1-2 combined based on 5 frequencies (≥ 4 times/wk vs <2 times/mo or never)	264 (12.3%)	OR 1.02 (0.73, 1.43)	No sig. assoc.
	Allergic sensitization, IgE ¹ to foods (fx5 panel) at 2 yrs	Fish, pregnancy intake (last 4 wks)	Times/mo or wk, tertiles	Tertile 3 vs 1-2 combined based on 5 frequencies (≥ 4 times/wk vs <2 times/mo or never)	200 (9.3%)	OR 1.01 (0.69, 1.48)	No sig. assoc.
	Allergic sensitization, IgE ¹ to panel of inhalant allergen at 2 yrs	Fish, pregnancy intake (last 4 wks)	Times/mo or wk, tertiles	Tertile 3 vs 1-2 combined based on 5 frequencies (≥ 4 times/wk vs <2 times/mo or never)	103 (4.8%)	OR 0.94 (0.56, 1.57)	No sig. assoc.

¹IgE: immunoglobulin E, ²HDM: house dust mite, ³SPT: skin prick test.

4.33.2.2 Studies of child total fish intake and allergic sensitization

Two studies of child intake included five estimates of allergic sensitization at age 4 or 8 years.

In Willers et al. (2011), children were considered to be sensitized against inhalant allergens if one or more allergen-specific immunoglobulin E (IgE) levels to house dust mite (*Dermatophagoides pteronyssinus*), cat, dog, birch (*Betula verrucosa*), grass (*Dactylis glomerata*) or fungus (*Alternaria alternata*) were ≥ 0.35 international units (IU) per milliliter (mL). Sensitisation to food allergens was defined as a high level of allergen-specific IgE to milk or egg (also ≥ 0.35 IU per mL).

Table 4.33.2.2-1 Results from studies included in the weight of evidence analysis of child total fish intake and child sensitization.

Author, year, country	Outcome measure and timing	Fish exposure timing	Intake unit	High-low intake	Total cases	RR high-low (95 % CI)	Overall result
Kull, 2006, Sweden	Allergic sensitization, IgE ¹ - inhalants (Phadiatop), age 4 yrs	Age 12 mo	Times/mo or wk	≥ 1 time/wk vs never	390	OR 0.44 (0.30, 0.64)	Sig protective assoc., <i>P</i> -trend <0.001
	Allergic sensitization, IgE ¹ - foods (f×5 panel), age 4 yrs	Age 12 mo	Times/mo or wk	≥ 1 time/wk vs never	406	OR 0.47 (0.33, 0.69)	Sig. protective assoc., <i>P</i> -trend <0.001
	Allergic sensitization, IgE ¹ to food (fx5 panel) or inhalants (Phadiatop) at age 4 yrs	Age 12 mo	Times/mo or wk	≥ 1 time/wk vs never	612	OR 0.53 (0.38, 0.74)	Sig. protective assoc., <i>P</i> -trend <0.001
Willers, 2011, the Netherlands	Allergic sensitization, IgE ¹ inhalants	Age 2-8 yrs	Days per week, continuous, 1 consumption day per wk increase, median consumptions for early age (2-3 yrs), later age (7-8 yrs) and average long-term intake (2-8) yrs were 0.5, 0.5 and 0.7 respectively		550 (32.1%) age 8 yr	OR 0.98 (0.78, 1.24)	No sig. assoc.

Author, year, country	Outcome measure and timing	Fish exposure timing	Intake unit	High-low intake	Total cases	RR high-low (95 % CI)	Overall result
	Allergic sensitization, IgE ¹ food allergens (milk, egg), age 8 yrs	Age 2-8 yrs	Days per week, Days per week, continuous, 1 consumption day per wk increase, median consumptions for early age (2-3 yrs), later age (7-8 yrs) and average long-term intake (2-8) yrs were 0.5, 0.5 and 0.7 respectively		285 (16.6%) age 8 yr	OR 1.09 (0.82, 1.45)	No sig. assoc.

¹IgE: Immunoglobulin E.

4.33.2.3 *Summary relative risks (RR) based on VKM's inclusion of primary studies*

VKM did not calculate summary RRs for sensitization due to few studies with heterogenous reporting of results.

4.33.2.4 *VKM's search compared to previous meta-analyses of allergic sensitization*

4.33.3 Heterogeneity maternal fish intake and allergic sensitization

In one previous meta-analysis of pregnancy intake, heterogeneity was low, based on two studies only (I^2 35.6%, $P_{\text{heterogeneity}}$ 0.21).

In primary studies, estimates were generally null or on the protective side, with no statistically significant reports of increased risk.

4.33.4 Dose-response relationship maternal fish intake and allergic sensitization

One primary study reported a protective association between maternal fish intake during pregnancy and the predicted probability of house dust mite (HDM) sensitivity at age 6 years.

4.33.5 Weight of evidence for maternal fish intake and allergic sensitization

Published evidence maternal fish intake and allergic sensitization

The evidence for an association of fish intake with development of allergic sensitization is based on two primary studies on pregnancy intake, and two on child intake, as well as two previous meta-analyses including one or two studies of maternal intake.

Heterogeneity

Heterogeneity appears to be low, but cannot be reliably assessed due to few studies.

Mechanisms/biological plausibility

There is evidence for plausible mechanisms operating in humans.

Upgrading factors

Dose-response was not found to be an upgrading factor. No other upgrading factors were evaluated.

4.33.5.1 *Conclusion weight of evidence maternal fish intake and allergic sensitization*

The evidence that maternal fish intake during pregnancy affects the risk of allergic sensitization to food or inhalant allergens is graded “limited, no conclusion” based on no statistically significant findings in two studies included by VKM, or in two previous systematic reviews including one or two studies.

The evidence that child fish intake protects against sensitization is graded “limited, no conclusion” based on two primary studies where associations differed by age of intake.

4.33.6 Remaining outcomes for fish intake and asthma and allergies (not summarized)

Results on bronchial hyperresponsiveness (one study), cough without cold (one study), dyspnea (two studies), diagnosed food allergy (one study), and steroid use (two studies) from the included studies were not summarized.

4.34 Fish intake and multiple sclerosis

Multiple sclerosis (MS) is an immune-mediated inflammatory disorder of the central nervous system. The causes are largely unknown but believed to involve environmental exposure and genetic susceptibility. A central hypothesis regarding diet is linked to vitamin D, and therefore also to intake of fish.

4.34.1 VKM's search for previous systematic reviews and meta-analyses

VKM's search for systematic reviews and meta-analyses (see Chapter 3.1.2.2) identified one publication on the association between fish intake and MS (Rezaeizadeh et al., 2020). The publication was included after quality assessment (AMSTAR grade B).

The authors performed a systematic literature search in PubMed, Scopus and Web of Science from inception up to September 2019 for observational studies. The quality of the eligible papers was assessed by the Newcastle-Ottawa Scale (NOS). Six studies, all with a case-control design and high-quality rating (NOS score 8 or 9), were included in the analysis.

The result indicates that the consumption of fish decreases the risk of MS (Table 4.34.1-1). The moderate heterogeneity (I^2 54.7%) reflects differences in the magnitude of the associations, not direction, which is consistently protective or on the protective side.

Table 4.34.1-1 Summary of results from meta-analysis on fish intake and risk of multiple sclerosis (MS).

Author, year	Type of studies included	Total no studies	No of cases	Comparison	Summary RR (95 % CI)	Heterogeneity	Overall conclusion
Rezaeizadeh et al., 2020	Observational (all case-control) studies on fish intake and MS	6	2370 cases, 4800 controls	Highest vs lowest	0.77 (0.64, 0.92); p-value 0.004	I^2 54.7%, P 0.051, random effects	Increased intake of fish associated with sig. decreased risk of MS

In the search for primary studies, VKM identified four of six studies in Rezaeizadeh et al., 2020 (all with a case-control design) and included one (Baarnhielm et al., 2014). Three of four were excluded after quality assessment (Abdollahpour et al., 2018 and Ghadirian et al., 1998 were graded C) or because the fish intake variable (Halawani et al., 2018 presenting Never vs > 1 time/week) did not fulfil VKM's eligibility criteria. Two studies were not identified (Sedaghat et al. 2016; Kampman et al., 2007). But VKM identified a recent study (Hedström et al. 2020) that was not included in the review (6,914 MS cases and 6,590 controls). VKM did not calculate a summary RR based on the two identified studies (Baarnhielm et al., 2014, Hedström et al. 2020) as they were partially overlapping. Both used data from the Swedish Epidemiological Investigation of Multiple Sclerosis (EIMS) study. Hedström et al. 2020 additionally included data from the Swedish Genes and Environment in

Multiple Sclerosis (GEMS) study. The overall results for total fish were consistent between the two sub-cohorts in Hedström et al. 2020 (low-high estimate of OR 1.2, 95% CI 1.1, 1.4 in both studies) showing higher risk among those with the lowest intake. This applied to both fatty and lean fish when analysed separately. Fish intake, both fatty and lean, was found to be associated with MS independently of vitamin D status (mediation analysis). There was also a reported genetic interaction with the *DRB1*15:01* allele of the *HLA-DRB1* gene.

In conclusion, the current evidence suggests a protective association of fish intake with risk of MS that is graded “limited, suggestive” because the mechanisms are uncertain and because the evidence base consists of case-control studies only. Dietary intake was recalled after disease and some studies had a long recall period, increasing the potential for disease-related modification of dietary intake and/or recall bias.

4.35 Fish intake and cancer

Evidence for the association between intake of fish and cancer was summarised in the Third Expert Report of WCRF/AICR in 2018. The report concludes that there is evidence on the level "limited, suggestive" for that fish decreases the risk of liver and colorectal cancers. No evidence was found for an association between fish intake and any other cancer type (WCRF, 2018).

4.36 Summary fish intake and health outcomes

Table 4.36-1 shows a summary of the weight of evidence conclusions for fish intake and the included health outcomes based on VKM's summary of primary studies and previous systematic reviews, and WCRF criteria.

Table 4.36-1 Summary of weight of evidence conclusions for fish intake and all included health outcomes.

Health outcome	Total fish	Fatty fish	Lean fish
CVD outcomes (adults)			
CVD incidence	Limited, suggestive (protective)	Limited, no conclusion	Limited, no conclusion
CHD incidence	Probable (protective)	Limited, suggestive (protective)	Limited, suggestive (no assoc.)
CHD incidence, secondary prevention	Limited, suggestive (protective)	Limited, no conclusion	-
MI incidence	Limited, suggestive (protective)	Limited, suggestive (protective)	Limited, no conclusion
Stroke incidence	Probable (protective)	Limited, suggestive (protective)	Limited, suggestive (protective)
Stroke incidence, ischemic	Limited, suggestive (protective)		
Stroke incidence, haemorrhagic	Limited, suggestive (protective)		
Heart failure (HF)	Limited, suggestive (protective)		
Atrial fibrillation (AF)	Limited, suggestive (adverse)	Limited, no conclusion	Limited, suggestive (protective)
Venous thromboembolism (VTE)	Limited, no conclusion		
Mortality outcomes (adults)			
CVD mortality	Probable (protective)		
CHD mortality	Probable (protective)	Limited, no conclusion	Limited, no conclusion
MI mortality	Probable (protective)		
Stroke mortality	Probable (protective)	Limited, suggestive (protective)	Limited, suggestive (protective)
Stroke mortality, ischemic			
Stroke mortality, hemorrhagic			
T2D mortality	Limited, no conclusion		
All-cause mortality	Probable (protective)		
Alzheimer mortality	Limited, no conclusion		
Neurodevelopment (children)			

Health outcome	Total fish	Fatty fish	Lean fish
Child neurodevelopment (maternal exposure)	Limited, suggestive (beneficial)	Limited, no conclusion	Limited, no conclusion
Child neurodevelopment (exposure in child)	Limited, suggestive (beneficial)	Limited, suggestive (beneficial)	
Cognition and mental health (adults)			
Cognitive decline in adults, e.g. dementia and Alzheimer's disease	Probable (protective)	Limited, no conclusion	Limited, no conclusion
Mental health in adults (depression)	Limited, suggestive (protective)	Limited, no conclusion	Limited, no conclusion
Postpartum depression	Limited, suggestive (protective)	Limited, no conclusion	
Other chronic diseases in adults			
Type 2 diabetes	Limited, no conclusion	Limited, no conclusion	Limited, suggestive (no assoc.)
Rheumatoid arthritis	Limited, suggestive (protective)		
Bone health/hip fracture	Limited, suggestive (protective)		
Overweight in children and adults			
Overweight in adults	Limited, no conclusion		
Overweight in children –maternal exposure	Limited, no conclusion	Limited, suggestive (no assoc.)	Limited, no conclusion
Overweight in children –child exposure	Limited, no conclusion		
Birth outcomes			
Preterm birth (PTB)	Probable (protective)	Limited, no conclusion	Limited, no conclusion
Small for gestational age (SGA)	Limited, suggestive (protective)	Limited, no conclusion	Limited, no conclusion
Low birth weight (LBW)	Probable (protective)	Limited, no conclusion	Limited, no conclusion
High birth weight	Limited, suggestive (increase/adverse)	Limited, suggestive (increase/adverse)	Limited, suggestive (increase/adverse)
Birth weight (continuous)	Limited, suggestive (positive assoc.)	Limited, suggestive (positive assoc.)	Limited, suggestive (positive assoc.)
Birth length, and head circumference (continuous)	Limited, no conclusion	Limited, no conclusion	Limited, no conclusion
Asthma and allergies (children)			
Eczema in children – maternal exposure	Limited, suggestive for pregnancy and limited, no conclusion from lactation		
Eczema in children – child fish intake	Limited, suggestive		

Health outcome	Total fish	Fatty fish	Lean fish
Wheeze – maternal fish intake	Limited, suggestive first two years of life and Limited, no conclusion older age		
Asthma – maternal fish intake	Limited, no conclusion		
Allergic rhinitis – maternal fish intake	Limited, no conclusion		
Allergic rhinitis – child fish intake	Limited, no conclusion		
Allergic sensitization	Limited, no conclusion		
Other			
Multiple sclerosis (MS)	Limited, suggestive (protective)		
Colorectal cancer	Limited, suggestive		

4.37 References

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5 Evaluation of health outcomes related to nutrients where fish is an important contributor to the total dietary exposure

The main focus in this benefit and risk assessment is health outcomes related to fish as such, described in Chapter 3. However, as illustrated in Figure 5-1, the health outcomes associated with fish consumption may be mediated through nutrients and contaminants. In this section, we have evaluated nutrients where fish is an important source, and their association with health outcomes.

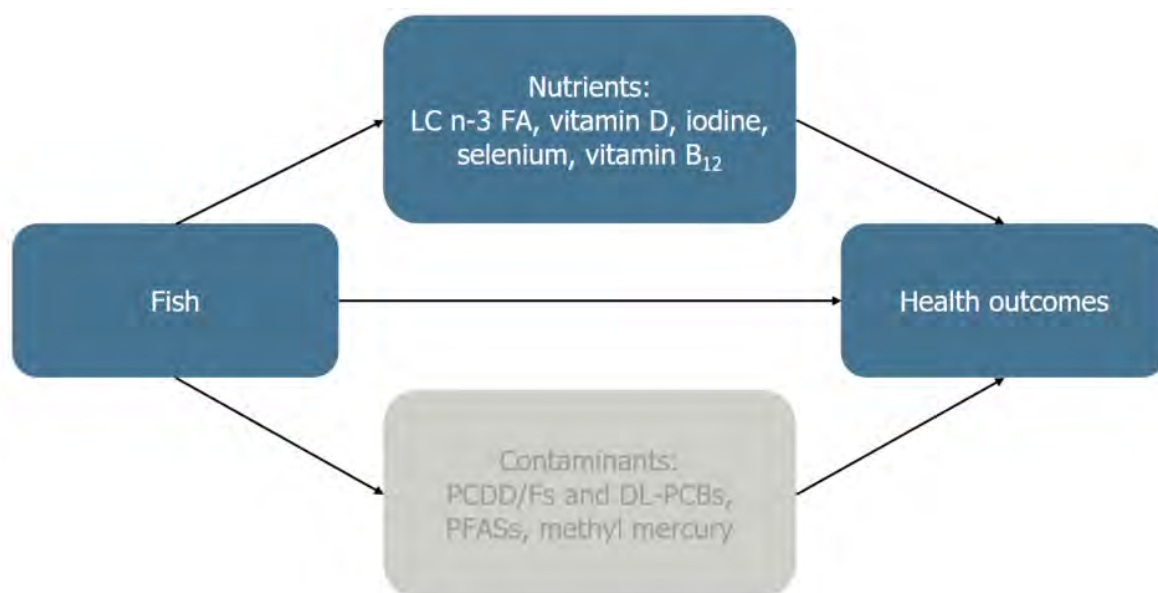


Figure 5-1 Illustration of how beneficial or adverse health effects from fish can be mediated through nutrients, contaminants or through unknown modes of action only ascribed to fish as such. This chapter covers nutrients in fish and associated health outcomes. Contaminants (illustrated in grey) are covered in Chapter 6.

5.1 Identification and characterisation of beneficial health effects associated with exposure to the included nutrients

In this section, we elaborate on identification of health outcomes associated with long chain n-3 fatty acids (LC n-3 FA), vitamin D, iodine, selenium, and vitamin B₁₂, which are the included nutrients in this benefit and risk assessment.

Overviews of selection of health outcomes, databases searched, and details about inclusion and exclusion criterias for the literature and the selection and data extraction process for the nutrients and related health outcomes are given in Chapter 3.2. In brief, the systematic searches were conducted back to 2015 or 2016 in the databases MEDLINE, Embase, Cochrane and Epistemonikos for systematic reviews and meta-analyses, the selection process of literature was conducted by two persons, independently. The quality of the systematic reviews/meta-analyses included after full text screening was evaluated using an adapted version of the AMSTAR tool for systematic reviews (see Chapter 3.1.3.2). The systematic reviews/meta-analyses judged as quality A or B were included as input in the weight of evidence for the specific health outcomes. The weighing of the evidence followed the guidelines described by the World Cancer Research Fund (WCRF) (see Chapter 3.1.6), but applied to evaluation of systematic reviews and meta-analyses. Also, for the weight of evidence for associations between health outcomes and nutrients, we considered evidence for the general population, including patient groups with type 2 diabetes (T2D), obesity, and musculoskeletal disorders. Studies on treatment of patients with the outcome of interest, including rheumatoid arthritis, multiple sclerosis, mental disorders, child neurodevelopmental disorders, and previous birth complications were excluded. However, studies on secondary prevention of cardiovascular diseases (CVD), prevention of cognitive impairment in the elderly and treatment of sub-infertility were included. The complete search strategies are given in Chapter 15, Appendix II.

The weight of evidence for health outcomes that have been evaluated for the nutrients in this benefit and risk assessment are further discussed in the Chapters 5.2 to 5.6.

For some associations, there were multiple relevant systematic reviews and meta-analyses, who included several of the same primary studies. For these situations we have presented so called “overlap tables”.

5.2 LC n-3 FA – introduction and mechanisms

In the revision of NNR (2012), Schwab et al. (2014) conducted an evidence-based systematic literature review of n-3 fatty acids and associated health effects. The objective was to assess the evidence of an effect of the amount and type of dietary fat on body weight, and risk of non-communicable diseases, that is T2D, CVD, and cancer in healthy subjects or subjects at risk for these diseases. None of the health outcomes relevant for this benefit and risk assessment of fish consumption were concluded to have “probable” or “convincing” evidence by Schwab et al. (2014). Schwab et al. (2014) concluded that there is “limited, suggestive” evidence for an inverse association with LC n-3 FA on cardiovascular disease (CVD) risk. We have therefore conducted an updated systematic literature review of all the associated health effects included in NNR (2012), and additionally for neurodevelopment in children, cognition, cognitive decline and mental health in adults, birth outcomes, rheumatoid arthritis, multiple sclerosis, and semen quality/ male infertility. The searches and the selection process are described in Chapter 3.2. The complete search strategies are given in Chapter 15, Appendix II.

Table 5.2-1 shows the number of included studies for each health outcome. No good quality systematic reviews or meta-analyses were identified for the prevention of rheumatoid arthritis or multiple sclerosis. Only papers graded A or B in the quality assessment are included in this benefit and risk assessment.

Table 5.2-1 Overview of number of included systematic reviews and meta-analyses per health outcomes.

Health outcome	Quality A or B
CVD/mortality	12
Neurodevelopment in children	4
Cognition and cognitive decline in adults	5
Mental health in adults	1
Birth weight and preterm birth	3
Type 2 diabetes	2
Rheumatoid arthritis and multiple sclerosis	0
Semen quality	3

Mechanisms

The long-chain n-3 (or omega-3) fatty acids (LC n-3 FA) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) incorporate into the phospholipid bilayer of cell membranes and thereby affect the membrane fluidity and the function of membrane ion-channels and receptors. In cardiac cells, this can explain why LC n-3 FA may prevent lethal arrhythmias and sudden cardiac death (London et al., 2007). DHA is an essential component of the phospholipid bilayer of brain cells, and incorporation of DHA into brain cell membranes may affect different neurotransmitter systems altering the regulation of dopaminergic and serotonergic neurotransmission. This may explain why LC n-3 FA may play a role in neuronal

function in the brain (cognitive development, cognitive decline, neuropsychiatric disorders, and depression) (Grosso et al., 2014; Patrick et al., 2015; Grant and Guest, 2016). The plasma membranes of spermatozoa contain high levels of LC n-3 FA, which increase the fluidity of the sperm membrane. However, this fluidity makes the sperm susceptible to reactive oxygen species (ROS) and lipid peroxidation, that may damage the sperm and lead to male subfertility (Lafuente et al., 2013; Marshburn et al., 2014 in Hosseini et al., 2019).

Moreover, LC n-3 FA convert to eicosanoids, which are signaling molecules affecting several cells involved in a wide range of processes such as the immune function, muscle contraction, dilatation of blood vessels, and aggregation of blood plates (Calder et al., 2017). These LC n-3 FA can also prevent the conversion of arachidonic acid (an n-6 fatty acid) into pro-inflammatory eicosanoids by serving as an alternative substrate for cyclooxygenases or lipoxygenases. In addition, a number of metabolites, called inflammation-resolving mediators (resolvins, protectins and maresins) are derived from LC-n-3 FA. These mediators can explain further the anti-inflammatory effects of LC n-3 FA. In relation to CVD risk, both eicosanoids and inflammation-resolving mediators derived from LC n-3 FA may act anti-inflammatory and inhibit atherosclerosis, the underlying cause of CVD. Eicosanoids derived from LC n-3 FA may also improve endothelial function by enhancing the arterial elasticity by increasing endothelium-derived vasodilators, and they have anti-arrhythmic effects by lowering heart rate (Calder et al., 2017). LC n-3 FA may also influence platelet-monocyte aggregation and thus may lower atherosclerotic plaques and arterial stiffness (Rimm et al., 2018). The anti-inflammatory effects via the eicosanoids are also important to maintain neuronal function in the brain (Grosso et al., 2014; Patrick et al., 2015; Grant and Guest, 2016) and may also have a protective effect on T2D (see e.g., Puglisi et al., 2011; Chen et al., 2021) and improve the course of established RA (Rennie et al., 2003; Gan et al., 2016; Gan et al., 2017a; Gan et al., 2017b). LC n-3 fatty acids might also influence the development of chronic inflammatory allergic diseases like asthma and atopic dermatitis because of the anti-inflammatory properties of eicosanoids. The mechanisms involved in human pregnancy maintenance and parturition are highly complex and involve mother, fetus, and placenta. The eicosanoids derived from LC n-3 fatty acids play an important role in these interactive paths and may explain why n-3 fatty acids are important for birth weight and preterm birth (Jones et al., 2014).

LC n-3 FA and their metabolites such as eicosanoids can furthermore act as ligands for key transcription factors to regulate gene expression of genes involved in lipid metabolism and inflammation, although differences are observed between different cells and tissues (Deckelbaum et al., 2006).

The effects of LC n-3 FA on adipocyte differentiation (Kim et al., 2006) and fat metabolism through altered gene expression (Deckelbaum et al., 2006), and secretion of healthy adipokines (e.g., adiponectin) may explain the effect of LC n-3 FA on T2D, weight gain (Hensler et al., 2011), and the reduction in serum triglyceride levels (Davidson et al., 2006).

5.2.1 LC n-3 FA and cardiovascular diseases

The Chapters 5.2.2-5.2.9 summarizes the results from epidemiological evidence of LC n-3 FA intake and risk of cardiovascular disease outcomes (CVD), including coronary heart disease (CHD), myocardial infarction (MI), stroke, atrial fibrillation (AF), and all-cause mortality. These results are all based on systematic reviews and meta-analyses described in this chapter below. The systematic reviews and meta-analyses primarily included randomized controlled trials (RCTs) on dietary supplement intake, but a few RCTs studied foods and advice to increase intake of LC n-3 FA. Two of the included systematic reviews and meta-analyses included prospective cohort studies. The systematic reviews and meta-analyses included RCTs with either primary or secondary prevention studies, or a mix of primary or secondary studies (an overview of overlapping RCTs among the meta-analyses is found in Table 5.2.1-2).

We performed a systematic search for systematic reviews and meta-analyses (see Chapter 3.2.3 for details) and identified 564 publications published between 2016 and 2021. Twenty-two papers were quality asses, and twelve of these were included to fill in knowledge about the association between LC n-3 FA and CVD/CHD outcome, and ten were excluded (see Table 5.2.1-1 for reasons for exclusions).

Table 5.2.1-1 Reasons for exclusion of identified papers from the search of systematic reviews and meta-analysis of LC n-3 FA and CVD outcome 2016-2021.

Included papers	Excluded paper and reasons for exclusions
Rizos et al., 2021 Abdelhamid et al., 2020 Casula et al., 2020 Lombardi et al., 2020 Campano et al., 2019 (therapy of stroke) Hu et al., 2019 Marston et al., 2019 Alexander et al., 2017 Balk et al., 2016 Kow et al., 2021 (atrial fibrillation) Jiang et al., 2018 (atrial fibrillation) Li et al., 2017 (atrial fibrillation)	The following were evaluated as quality C: Bernasconi et al., 2021: No comprehensive literature search was performed and data extraction and study selection were not done in duplicate. Jia et al., 2021: No consensus procedure if disagreement between the two persons performing data extraction and study selection was described, no assessment of quality of the included paper. Cabiddu et al., 2020: No assessment of quality of the included paper. Popoff et al., 2019: Graded C-included both marine and plant-based n-3 fatty acids with no separation of the analysis. Ueno et al., 2019: No assessment of quality of the included paper. Maki et al., 2017: No assessment of quality of the included paper. Waltz et al., 2016: No assessment of quality of the included paper, no consensus procedure if disagreement between the two persons performing data extraction and study selection was described. The following were excluded for other reasons: Abdelhamid et al., 2018: Abdelhamid et al. (2020) is an updated version. Aung et al., 2018: Hu et al. (2019) is an updated version. Chen et al. 2021: Serious flaws identified in the paper.

Table 5.2.1-2 Overview of overlap between RCTs included in the reviews. For Abdelhamid et al. (2020), only overlapping papers are listed. Additionally, 64 papers are included in Abdelhamid et al. (2020). One of the systematic reviews and meta-analyses included for atrial fibrillation (Jiang et al. 2018), included entirely different primary studies, and are therefore not included in this overlap table.

RCTs/ primary studies Author, year	Included in the meta-analyses									
	Kow, 2021	Rizos, 2021	Casula, 2020	Lombardi, 2020	Abdelhamid, 2020	Marston, 2019	Hu, 2019	Campano, 2019	Alexander, 2017	Balk, 2016
Bhatt, 2019	X	X	X	X	X	X	X	-	-	-
Bonds, 2014		X	X	X	X	X	X	-	-	-
Bosch, 2012		X	X	X	X	X	X	-	X	X
Bowman, 2018	X	-	-	X	X	X	X	-	-	-
Brouwer, 2006		-	X	X	X	-	-	-	X	X
Burr, 1989		-	-	-	X	-	-	-	X	X
Burr, 2003		-	-	-	X	-	-	-	X	X
Einvik, 2010		X	X	X	X	X	X	-	X	X
Eritsland, 1996		-	-	-	-	-	-	-	-	X
Galan, 2010		X	-	X	X	X	X	X	X	X
Garbagnati, 2009		X	-	-	X	-	-	X	-	-
GISSI-P, 1999		-	-	-	X	X	X	-	-	-
Ishikawa, 2010		-	-	-	-	-	-	-	X	-
Johansen, 1999		-	-	-	-	-	-	-	X	-
Kromhout, 2010		X	-	X	X	X	X	X	X	X
Kromhout, 2011		-	-	-	-	-	-	-	X	-
Laake, 2014	X	-	-	-	X	-	-	-	-	-
Leaf, 2005		-	-	-	-	-	-	-	X	X
Leng, 1998		X	-	-	-	-	-	-	-	-
Macchia, 2005		-	X	-	-	-	-	-	X	X
Macchia, 2013		X	X	-	X	-	-	X	X	-
Manson, 2019	X	X	-	X	X	X	X	-	-	-
Marchioli, 2001		-	X	X	-	-	-	-	X	X
Nicholls, 2020	X	-	-	-	-	-	-	-	-	-
Nilsen, 2001		X	X	-	-	-	-	-	X	X
Nosaka, 2017		-	X	-	-	-	-	X	-	-
OPAL, 2010		-	-	-	X	-	-	X	-	-
Raitt, 2005		-	-	-	X	-	-	-	X	X
Rauch, 2010		X	X	X	X	X	X	X	X	X
Roncaglioni, 2013	X	X	X	X	X	X	X	X	X	X
Sacks, 1995		X	-	-	X	-	-	-	-	X
Singh, 1997		X	X	-	-	-	-	-	X	-
Svensson, 2006		-	X	-	-	-	-	X	-	-
Tavazzi, 2008		X	X	X	X	X	X	X	-	X
Von Schacky, 1999		X	X	-	X	-	-	-	X	-
Yokoyama, 2007		-	X	X	X	X	X	X	X	X

Meta-analyses included for all CVD and mortality outcomes for LC n-3 FA

Rizos et al. (2021) is a systematic review and meta-analysis of RCTs with the aim to assess the association between dose-specific LC n-3 FA supplementation and CVD outcomes (all-cause mortality, cardiac death, sudden death, non-fatal MI and all types of strokes). They performed a systematic search of RCTs in Cochrane Central Register of Controlled Trials (CENTRAL), PubMed, and Embase up to September 2019. They included 17 double-blind RCTs (n=83 617) with duration ≥ 1 year assessing LC n-3 FA supplementation. The trials were both primary, secondary and a mix of primary/secondary prevention trials. All studies included either capsules with EPA+DHA or EPA (Leng et al., 1998 +REDUCE-IT), except one, which supplemented with EPA/DHA enriched margarine (Alpha-Omega). The methodological quality of the included trials and the risk of bias was assessed by using elements of the Cochrane collaboration tool for assessing risk of bias. Most studies were of high quality, all were double blind, but the methods used to ensure adequate allocation concealment were not always clearly reported.

Kow et al. (2021) is a meta-analysis (and Letter to the editor) of RCTs to summarize the existing evidence on the overall effect of LC n-3 FA on the development of atrial fibrillation (AF) (new-onset incident and recurrent atrial fibrillation following exposure to LC n-3 FA).

They performed a literature search in Cochrane Central Register of Controlled Trials (CENTRAL), PubMed and Embase (dates for the search was not given). The literature search was limited to records published in English with follow-up >1 year and a sample size of 500 participants or more. Six RCTs were included. The studies were conducted in men and woman with mean/median age 63-74 years. The risk of bias in the included studies was based on the Cochrane Collaboration risk of bias tool version 2 (RoB2), and was evaluated to be low in five studies, and "of some concern" in one study.

Abdelhamid et al. (2020) is a Cochrane systematic review and meta-analysis of RCTs with the aim to assess the effects of increased intake of fish- and plant-based n-3 fats for all-cause mortality, cardiovascular events, adiposity and lipids. They performed a systematic search of RCTs in Cochrane central library, Medline and Embase plus ClinicalTrials.gov and WHO international Clinical Trials Registry up to August 2019. They included 86 RCTs (n=162 796) with duration ≥ 1 year and compared supplementation or advice to increase LC n-3 intake or alpha-linolenic acid (ALA, a plant-based n-3 FA) intake, or both, versus usual or lower intake. The trials were both primary, secondary and a mix of primary/secondary prevention trials. They performed separate random effect meta-analysis for ALA and LC n-3 FA interventions. The methodological quality of the included trials and the risk of bias was assessed by using the Cochrane collaboration RoB2. All the papers included in the meta-analysis were overall high-quality articles (RoB was low), and they used GRADE to evaluate the overall evidence.

Lombardi et al. (2020) is a meta-analysis of RCTs to elucidate the benefit of different doses and LC n-3 FA supplementation in the setting of primary and secondary prevention. They performed a systematic search of RCTs in MEDLINE (PubMed), Embase (Ovid), Cochrane Central register of Controlled Trials (CENTRAL) and clinocaltrials.gov from 1946 to February 2019. They included 14 RCTs (n=125 763) with duration of ≥ 1 year, sample size of 500 participants or more, and compared supplementation with EPA alone (JELIS and REDUCE-IT)

or EPA+DHA with control. The methodological quality of the included trials and the risk of bias was assessed by using the Cochrane collaboration RoB2. The quality of all the papers included in the meta-analysis were overall high-quality articles (RoB was low), except of three studies (GISSI-P, JELIS, and R & P).

Casula et al. (2020) is an updated meta-analysis of RCTs on cardiovascular secondary prevention and patients at high and very high cardiovascular risk. The aim was to investigate the effect of LC n-3 FA supplementation on cardiovascular outcomes with focus on the role of dose, type of LC n-3 FA, and different cardiovascular risk at baseline. All studies used EPA+DHA or only EPA (JELIS, REDUCE-IT, Nosaka et al., 2017) supplementation versus placebo or no treatment. They performed a comprehensive search of RCTs in PubMed, Cochrane library and Embase up to March 2020. They included 16 RCTs (n=81 073) with duration of ≥ 1 year and supplementation of LC n-3 FA of ≥ 1 g/day. The quality of all the papers included in the meta-analysis were overall high-quality articles (Jadad score between 8-13 point, where 13 is rigorous).

Marston et al. (2019) is a systematic review and meta-regression analysis, which aim to examine the association between the magnitude of non-HDL-C, LDL-C, and triglyceride lowering, and the reduction in major vascular events across trials of fibrates, niacin, and LC n-3 FA, as well as statins as an established reference. They performed a systematic search of RCTs in Medline and Embase from 1968 until March 2019. They included 24 RCTs (n=197 270) with duration of ≥ 1 year and ≥ 400 subjects. In total 13 RCTs (n=125 544) studied the effect of LC n-3 FA. All studies used EPA+DHA or only EPA supplementation (JELIS, REDUCE-IT) versus placebo or no treatment. The quality of all the papers included in the meta-analysis were overall high-quality articles (RoB was low, with exception of JELIS, which had an open-label design).

Hu et al. (2019) is an updated meta-analysis of RCTs based on the published data of a previous meta-analysis (Aung et al., 2018), including ASCEND, VITAL, and REDUCE-IT. The aim was to update previous meta-analyses by adding three recent large randomized controlled clinical trials, increasing sample size by 64%. All 13 studies (n=127 477) used LC n-3 FA, EPA+DHA or EPA alone, (JELIS and REDUCE-IT) versus placebo or open label control (JELIS and GISSI-P), with a sample size of at least 500 participants and a follow up duration ≥ 1 year. The LC n-3 FA were given as capsules in all studies except of one, giving enriched margarine (Alpha-Omega). In Aung et al. (2018) they performed a systematic search of RCTs in PubMed and Medline data sets, supplemented by manual hand searching of reference lists from individual trials, review articles, or previous meta-analysis of LC n-3 FA and CVD. The quality of all the papers included in the meta-analysis were overall high-quality articles (RoB was low, with exception of two trials that did not use a placebo-treated control group) (Aung et al., 2018).

Campano et al. (2019) is a Cochrane systematic review and meta-analysis of RCTs with the aim to study the effects of administration of LC n-3 FA on functional outcomes and dependence in people with stroke. The secondary outcomes were vascular-related death, recurrent events, incidence of other type of stroke, adverse events, quality of life, and mood. They performed a systematic search in Cochrane Stroke Group trials register (August 2018),

the Cochrane Central Register of Controlled Trials (CENTRAL; January 2019), MEDLINEOvid (from 1948 to August 2018), EmbaseOvid (from 1980 to August 2018), CINAHL EBSCO (Cumulative Index to Nursing and Allied Health Literature; from 1982 to August 2018), Science Citation Index Expanded – Web of Science (SCI-EXPANDED), Conference Proceedings Citation Index-Science – Web of Science (CPCI-S), and BIOSIS Citation Index. They also searched ongoing trial registers, reference lists, relevant systematic reviews, and used the Science Citation Index Reference Search. They included RCTs that compared LC n-3 FA to placebo or open control (no placebo) in people with a history of stroke or transient ischemic attack (TIA), or both. They used the Cochrane bias assessment tool to assess risk of bias in the 29 studies. Overall, the quality of the papers was good (with low RoB, with exception of three trials that were open-label).

Alexander et al. (2017) is a systematic meta-analysis of RCTs aiming to estimate the effect of EPA+DHA from foods and supplements, and of prospective cohort studies to estimate the association between EPA+DHA intake and CHD risk. They performed a systematic search in Medline, PubMed, Embase, and the Cochrane Library, which covered studies published from January 1, 1946 until November 2, 2015. They included 18 RCTs (n=93 000) and 16 prospective cohort studies (n=732 000). In the RCTs, EPA+DHA was given as supplements, as fatty fish (DART) and n-3 enriched margarine trials (Alpha-Omega). The Cochrane Bias Assessment score was used to evaluate the quality of the RCTs. Overall, the quality of the papers was good (with low RoB, with exception of four trials that were open-label). Newcastle-Ottawa Score stars were used to evaluate the quality of the prospective cohort studies and ranged from 6-9.

Jiang et al. (2017) is a meta-analysis of RCTs. The aim was to determine the effectiveness of n-3 PUFA as a sole anti-arrhythmic agent or as an added therapy to existing pharmacological therapies in preventing recurrence of atrial fibrillation. The primary outcome was rate of atrial fibrillation recurrence measured by time to first recurrent episode. They performed a literature search in Cochrane Central Register of Controlled Trials (CENTRAL), PubMed, Embase, MEDLINE, Scopus and CINAHL from inception to December 2016. The literature search was limited to records published in English. Only four RCTs were included with follow-up ranging from 6-16 months. It is not clear whether some of the included studies also include ALA in the intervention. The studies were conducted in atrial fibrillation patients, mean age >60 years. The risk of bias in the included studies was based on the Cochrane Collaboration tool, and the overall risk of bias among studies were evaluated to be low.

Li et al. (2017) is a meta-analysis of prospective cohort studies. The aim was to examine the prospective associations between long-term dietary fish and n-3 PUFAs intakes and risk of atrial fibrillation. They performed a literature search in PubMed and Embase from inception to 18 May 2016. The literature search was limited to records published in English. Seven studies including men and women aged 45 to 100 years, and an average duration of follow-up ranging between 4 and 17.6 years was included in this meta-analysis. Newcastle-Ottawa Quality Assessment Scale was used to evaluate the quality, and all studies was evaluated to be of high quality.

Balk et al. (2016) is a technical report and contains a meta-analysis of RCTs. The aim was to evaluate the effect of n-3 FA on clinical and selected intermediate cardiovascular outcomes and the association of n-3 FA intake and biomarkers with cardiovascular outcomes. They performed a systematic search of RCTs in MEDLINE, Embase, the Cochrane Central Register of Controlled Trials (CENTRAL), Cochrane Database of Systematic Reviews, and CAB Abstracts from 2000 to June 8, 2015. They included 61 RCTs mostly with EPA, DHA, docosapentaenoic acid (DPA), and some with stearidonic acid (SDA), and ALA, with a duration of ≥ 1 year. They performed separate random effect meta-analysis for ALA, SDA, and LC n-3 FA interventions. The studies were conducted in healthy adults, those at risk for CVD, or those with CVD. They also included 37 prospective observational studies. The quality of all the papers included in the meta-analysis were overall high-quality articles (RoB was low).

5.2.2 LC n-3 FA and CVD mortality

5.2.2.1 *Results from the meta-analyses on LC n-3 FA and CVD mortality*

Below is a summary table for LC n-3 FA intake and CVD mortality (Table 5.2.2.1-1) based on the identified meta-analyses described above.

Table 5.2.2.1-1 Overview of results from meta-analyses included in the weight of evidence analysis of LC n-3 FA intake and CVD mortality.

Author, year	Study design, dose range, N	Total no studies	No of cases	Comparison	Summary RR/HR (95 % CI)	Heterogeneity	Overall results
Abdelhamid, 2020	RCT, EPA+DHA: 0.5-5 g/d n=117 837	29	2724 high intake vs 2934 low intake	Placebo or open-labelled control	RR 0.92 (0.86 to 0.99)	$I^2=21.9\%$	LC n-3 FA has a protective effect on CVD mortality
Hu, 2019 Hu, 2019	RCT, EPA+DHA: 0.4-4 g/d, n=108 778	12	2412 treatment vs 2605 CTR	Placebo or open-labelled control	RR 0.92 (0.88 to 0.97)	$I^2=5.7\%$	LC n-3 FA has a protective effect on CVD mortality. This risk reduction was strengthened after including REDUCE-IT ($P=0.003$)
	RCT (without REDUCE-IT), EPA: 4 g/d, n=100 599	11	2238 treatment vs 2392 CTR	Placebo or open-labelled control	RR 0.93 (0.88 to 0.99)	$I^2=0.2\%$	LC n-3 FA has a protective effect on CVD mortality ($P=0.013$)
Balk, 2016	RCT, EPA+DHA: 0.4-3.46 g/d n=49 070	7	1841 treatment vs 1958 CTR	Placebo or open labeled control	HR 0.92 (0.82-1.02)	$I^2=42.7\%$	LC n-3 FA has no significant effect on CVD mortality (including stroke)
	Prospective cohort studies (n=90 778)			0.066 to 1.58 g/d	ES* 0.88 (0.82 to 0.95)		Higher marine oil intake (including from dietary fish) is associated with lower risk of CVD mortality

*Effect size, overall (ES).

Overall, two out of three meta-analyses found a small protective effect of intake of LC n-3 FA and CVD mortality (Abdelhamid et al 2020 and Hu et al 2019). The meta-analysis by Balk et al. (2016) does not include the latest large RCTs performed (ASCEND, VITAL, and REDUCE-IT) and found no significant effect of LC n-3 FA and CVD mortality. The prospective cohort studies indicates that there is an inverse association between marine oil intake and CVD mortality.

Abdelhamid et al. (2020) included 29 RCTs, and the overall effect, based on RR and 95% CI, shows a small protective effect of LC n-3 FA on CVD mortality. Among the 29 trials, 19 show decreased risk, and four out of these shows significant decreased risk of CVD mortality (REDUCE-IT, ASCEND, DART and GISSI-P). One trial show significant increased risk (DART2). In this meta-analysis, all 12 large supplement studies that was included in Hu et al. (2019) was included. In addition, one study included supplemented food, and two studies used advice to increase intake of LC n-3 FA, and several smaller studies with few events were included. Since all studies have been weighted, and up-and downgraded based on risk of bias and other criteria by the authors of the meta-analysis, the GRADE assessment suggested moderate-certainty evidence that LC n-3 FA intake probably makes little or no difference to CVD deaths (downgraded once for imprecision).

Hu et al. (2019) included 12 RCTs with more than 500 participants in each trial and a follow up-duration of more than a year. The overall effect, based on RR and 95% CI, shows a small protective effect of LC n-3 FA on CVD mortality. Among the 12 trials (which was also included in Abdelhamid et al 2020), nine show decreased risk, and three out of these shows significant decreased risk of CVD mortality (REDUCE-IT, ASCEND and GISSI-P). No studies show significant increased risk. When the authors removed REDUCE-IT from the analysis (a study with 4 g EPA per day), the overall effect was that LC n-3 FA still had a protective effect on CVD mortality. In the sensitivity analysis that excluded DOIT, SU.FO-L.OM3, Alpha-Omega, and OMEGA (because of considerably lower dose, duration, or size), the inverse associations for CVD mortality was slightly strengthened (RR 0.92, 95% CI 0.87-0.97 and RR 0.93, 95% CI 0.88-0.98 without REDUCE-IT). In the sensitivity analysis that excluded two open-label trials, GISSI-P and JELIS, the point estimates for CVD mortality were reduced (RR 0.96, 95% CI 0.93-0.99 and RR 0.98, 95% CI 0.95-1.01, without REDUCE-IT).

Balk et al. (2016) included seven RCTs. The overall effect, based on HR and 95% CI shows a non-significant protective effect of LC n-3 FA on CVD mortality. Two of the conducted trials were performed with participants at risk of CVD, while the other five trials were performed with participants with CVD including diabetes, history of CVD, MI or heart failure. Among the seven trials, five show decreased risk, and two of these shows significant reduced effect. No studies show significant increased risk. In this meta-analysis, five trials overlap with Abdelhamid 2020 and Hu 2019. The latest large RCTs performed (ASCEND, VITAL, and REDUCE-IT) was not included. They also included prospective cohort studies with more than 90 000 individuals and based on overall effect size it seems like there is a protective effect of LC n-3 FA on CVD mortality. The authors of the meta-analysis state that there is low strength of evidence of associations between higher marine oil intake and decreased risk of CVD mortality.

5.2.2.2 Heterogeneity LC n-3 FA intake and CVD mortality

In Abdelhamid et al. (2020), heterogeneity was low ($I^2=21.9\%$, $P_{\text{heterogeneity}}=0.15$).

In Hu et al. (2019), heterogeneity was low (5.7%, $P_{\text{heterogeneity}}=0.388$), and when excluding REDUCE-IT, the heterogeneity was even lower (0.2%, $P_{\text{heterogeneity}}=0.439$).

In Balk et al. (2016), heterogeneity was moderate ($I^2=42.7\%$, $P_{\text{heterogeneity}}=0.134$).

5.2.2.3 Dose-response relationship LC n-3 FA intake CVD mortality

Hu et al. (2019) investigated a linear dose-response relationship between LC n-3 FA supplementation with and without including REDUCE-IT, and no linear dose-response was found.

Abdelhamid et al. (2020), found no significant overall effect of LC n-3 FA dose in pre-planned subgrouping or meta-regression.

Balk et al. (2016) reported no apparent differences in association between marine oil intake dose and outcome at lower or higher dose ranges.

5.2.2.4 Weight of evidence for LC n-3 FA intake and CVD mortality

In this section, the evidence of the association between LC n-3 FA and CVD mortality is weighed according to the WCRF criteria presented in the method chapter (Box 2 in Chapter 3.1.6, but applied to evaluation of systematic reviews and meta-analyses).

Published evidence of LC n-3 FA intake and CVD mortality

Two meta-analyses and systematic reviews of RCTs (Abdelhamid et al., 2020 and Hu et al., 2019) reported a small protective significant effect against CVD mortality, while one meta-analysis (Balk et al., 2016) did not find a significant effect of LC n-3 FA intake. All three meta-analysis had very similar RR and 95% CI, but the two who reported a significant protective effect included three new, large RCTs. Due to various inclusion criteria, the included studies differ between the three meta-analyses, even though there is some overlap between the studies. One meta-analysis of prospective cohort studies showed that high intake of LC n-3 FA is inversely associated with CVD mortality (Balk et al., 2016).

Heterogeneity

No significant heterogeneity was observed in the included meta-analyses.

Mechanisms/biological plausibility

Plausible mechanisms are presented above, see Chapter 5.2.

Upgrading factors

There are no dose-response relationships described between LC n-3 FA and CVD mortality. No upgrading factors have been evaluated.

Conclusion weight of evidence LC n-3 FA intake and CVD mortality

Results from the three meta-analyses and systematic reviews of RCTs and the meta-analysis of cohort studies showed a small protective effect of LC n-3 FA intake on CVD mortality or no effect. The direction of the effect in the pooled analyses in all three meta-analyses of RCTs was towards protective, and generally consistent. Limited/no unexplained heterogeneity was observed in all studies. There is evidence for biological plausibility. In conclusion, the evidence that LC n-3 FA intake protect against CVD mortality is graded as “probable”.

5.2.3 LC n-3 FA and CHD mortality

5.2.3.1 *Results from the meta-analyses for LC n-3 FA and CHD mortality*

Below is a summary table for LC n-3 FA and CHD mortality (Table 5.2.3.1-1) based on the identified meta-analyses.

Table 5.2.3.1-1 Overview of results from meta-analyses included in the weight of evidence analysis of LC n-3 FA intake and CHD mortality (includes CHD mortality, coronary mortality, sudden cardiac mortality, and cardiac mortality).

Author, year	Study design, dose range, N	Total no studies	No of cases	Comparison	Summary RR/OR/HR (95 % CI)	Heterogeneity	Overall results
Rizos, 2021	RCT EPA+DHA: 0.27-6 g/d n=72 360	2 4 3 3	-	Placebo or open-labelled control	<1 capsule/day=<0.84 g RR 0.99 (0.73-1.33) 1 capsule/day=0.84-1.68 g RR 0.96 (0.90-1.02) 2 capsules/day=1.68-2.52 g RR 0.55 (0.33-0.90) >3 capsules/day=>2.52 g RR 0.82 (0.68-0.99)	$I^2=0\%$ $I^2=0\%$ $I^2=0\%$ $I^2=0\%$	LC n-3 FA supplementation at >2 capsules/day has a protective effect on cardiac mortality
Abdelhamid, 2020	RCT EPA+DHA: 0.5-5 g/d n=127 378	24	1728 high intake vs 1870 low intake	Placebo or open-labelled control	RR 0.90 (0.81 to 1.00)	$I^2=35.2\%$	LC n-3 FA intake has a small protective effect on CHD mortality
Casula, 2020	RCT EPA+DHA: 1-6 g/day n=72 102	13	1598 treatment vs 1724 CTR		OR 0.91 (0.85-0.98)	$P=0.28$	LC n-3 FA have a protective effect on cardiac mortality. In subgroup analysis, the protective effect was confirmed only in RCTs that enrolled patients in secondary prevention
Lombardi, 2020	RCT EPA+DHA: 0.4-4 g/d n=125 051	14	-	Placebo or open-labelled control	Incidence rate ratio 0.79 (0.65-0.96) high dose versus control Incidence rate ratio 0.92 (0.87-0.98) low dose versus control	$I^2 < 50\%$, $P > 0.05$	High dose treatment has a protective effect on cardiac mortality ($P=0.02$) Low-dose treatment has a protective effect on cardiac mortality ($P=0.009$)

Author, year	Study design, dose range, N	Total no studies	No of cases	Comparison	Summary RR/OR/HR (95 % CI)	Heterogeneity	Overall results
					Incidence rate ratio 0.83 (0.67-1.02) high dose versus control Incidence rate ratio 0.90 (0.80-1.02) low dose versus control		High dose treatment has no protective effect on sudden cardiac mortality ($P=0.08$) Low-dose treatment has no protective effect on sudden cardiac mortality
Hu, 2019	RCT (without REDUCE-IT) EPA+DHA: 0.4-1.8 g/d n=119 244	12	1405 treatment vs 1529 CTR	Placebo or open-labelled control	RR 0.92 (0.86-0.98)	$I^2=20.9\%$	LC n-3 FA have a protective effect on CHD mortality ($P=0.014$)
Balk, 2016	RCT EPA+DHA: 0.4-3.46 g/d n=61 917 Prospective cohort studies n=145 148	10	838 treatment vs 821 CTR	Placebo or open labelled 0.04 to 2.1 g/d	RR 1.04 (0.92-1.17) ES 1.09 (0.76- 1.57)	$I^2=11.04\%$	LC n-3 FA have no effect on sudden cardiac mortality No association between marine oil intake and CHD mortality

Overall, two meta-analyses of RCTs found a significant small protective effect between intake of LC n-3 FA on CHD mortality (Abdelhamid et al., 2020 and Hu et al., 2019). Three meta-analysis of RCTs found a significant protective effect between intake of LC n-3 FA on cardiac mortality and sudden cardiac mortality (Rizos 2021; Lombardi 2020; Casula 2020). Balk et al. (2016) found no effect of intake of LC n-3 FA on sudden cardiac mortality. The meta-analysis by Balk et al. from 2016 does not include the latest large RCTs performed (ASCEND, VITAL, and REDUCE-IT). The meta-analysis of prospective cohort studies by Balk et al. (2016) showed no association between marine oil intake on CHD mortality.

Rizos et al. (2021) included 12 RCTs with duration of more than 1 year and reported 3830 cardiac deaths among 72360 participants. Three studies which supplemented participants (two secondary prevention trials and one mix of primary and secondary prevention trial) with 2 capsules per day (1.68-2.52 g EPA+DHA per day), and three studies which supplemented participants (two secondary prevention trials and one mix of primary and secondary prevention trial) with more than 3 capsules per day (>2.52 g EPA+DHA per day) shows a significant protective effect of LC n-3 FA intake on cardiac mortality. Six trials with lower doses did not show any significant effect on cardiac death. The meta-analysis included the two of the latest large RCTs performed (VITAL and REDUCE-IT). In addition, seven RCTs overlapped with Lombardi et al. (2020), three overlapped with Balk et al. (2016), and six overlapped with Casula et al. (2020).

Abdelhamid et al. (2020) included 24 RCTs, and the overall effect, based on RR and 95% CI show an overall small protective effect of LC n-3 FA on CHD mortality. Among the 24 trials, 18 show decreased risk, and two out of these shows significant decreased risk of CHD mortality (DART and GISSI-P). No studies show significant increased risk. The meta-analysis included the two of the latest large RCTs performed (VITAL and ASCEND). In addition, all 12 large supplement studies that was included in Hu et al. (2019) was included. In addition, one study included fortified food, and two studies used advice to increase intake of LC n-3 FA, and several smaller studies with few events were included. Since all studies have been weighted, and up-and downgraded based on risk of bias and other criteria by the authors of the meta-analysis, the GRADE assessment suggested low-certainty evidence that LC n-3 FA intake may slightly reduce CHD mortality (downgraded once for imprecision and once for publication bias).

Lombardi et al. (2020) included 14 RCTs, and when they compared high dose LC n-3 FA supplementation (>1 g per day) studies (n=4) with control, and low dose LC-n-3 FA supplementation (≤1 g per day) studies (n=10) with control, the RR and 95% CI show that there is a protective effect of both low and high dose of LC n-3 FA intake on cardiac mortality. No effect of LC n-3 FA intake on sudden cardiac death was seen for either low or high doses. This meta-analysis included the three of the latest large RCTs performed (VITAL, REDUCE-IT and ASCEND). In addition, seven studies overlapped with Rizos et al. (2021), and nine overlapped with Casula et al. (2020), and six overlapped with Balk et al. (2016).

Casula et al. (2020) included 13 RCTs, and the overall effect, based on RR and 95% CI shows a small protective effect of LC n-3 FA intake on cardiac mortality. Among the 13 RCTs, 10 show decreased risk, and two out of these 10 shows a significant decreased risk (IEIS-4

and GISSI-P). No studies show significant increased risk. The meta-analysis included none of the latest large RCTs performed (VITAL, REDUCE-IT and ASCEND). Influence analysis showed that the significant protective effect of LC n-3 FA on the risk of cardiac mortality was no longer significant when excluding the GISSI-P study. In subgroup analysis, the risk reduction of cardiac mortality was confirmed only in the sub-analyses of RCTs that enrolled patients in secondary prevention (n=7 trials) (OR 0.79, 95% CI 0.67-0.93) compared with OR 0.95, 95% CI 0.87-1.03 for mixed prevention trials where some, but not all participants, had CVD at baseline (n=9 trials). Only the administration of more than 1 g per day of LC n-3 FA seemed to be effective in reducing risk of cardiac mortality (OR 0.65, 95% CI 0.47-0.91).

Hu et al. (2019) included 12 RCTs (without REDUCE-IT), and the overall effect, based on RR and 95 % CI, shows a small protective effect of LC n-3 FA intake on CHD mortality. Among the 12 RCTs, 10 shows decreased risk, and one of these shows a significant decreased risk (GISSI-P). No studies show significant increased risk. The meta-analysis included two of the latest large RCTs performed (VITAL and ASCEND). In the sensitivity analysis that excluded DOIT, SU.FO-L.OM3, Alpha-Omega, and OMEGA (because of considerably lower dose, duration, or size), the inverse association for CHD mortality was slightly reduced (RR 0.92, 95% CI 0.86 to 0.99). In the sensitivity analysis that excluded two open-label trials, GISSI-P and JELIS, the point estimate for CHD mortality was reduced (RR 0.94, 95% CI 0.87-1.01).

Balk et al. (2016) included ten RCTs, and the overall effect, and based on HR and 95% CI, show no effects of LC n-3 FA on sudden cardiac mortality. Among the ten studies, three show decreased risk, but none of them show significant decreased risk. No studies show significant increased risk. The meta-analysis included none of the latest large RCTs performed (VITAL, REDUCE-IT and ASCEND). They also included prospective cohort studies with more than 145 000 individuals and based on the overall effect size there seem not to be any association between marine oil intake on CHD death.

5.2.3.2 Heterogeneity LC n-3 FA intake and CHD mortality

In Rizos et al. (2021), heterogeneity was low ($I^2=0\%$) for cardiac mortality.

In Abdelhamid et al. (2020), heterogeneity was moderate ($I^2=35.22\%$, $P_{\text{heterogeneity}}=0.05$) for CHD mortality.

In Lombardi et al. (2020), heterogeneity was moderate ($I^2<50\%$, but no reported value of $P_{\text{heterogeneity}}$) for sudden cardiac mortality and cardiac mortality.

In Casula et al. (2020), the $P_{\text{heterogeneity}}$ was not significant for cardiac mortality ($P=0.28$, but no reported value of I^2).

In Hu et al. (2019), heterogeneity was low ($I^2=20.9\%$, $P_{\text{heterogeneity}}=0.238$) for CHD mortality.

In Balk et al. (2016), heterogeneity was low ($I^2=11.04\%$, $P_{\text{heterogeneity}}=0.515$) for sudden cardiac mortality.

5.2.3.3 *Dose-response relationship LC n-3 FA intake CHD mortality*

Hu et al. (2019) investigated linear dose-response relationship between LC n-3 FA supplementation, but no linear dose-response was found.

For CHD death, there were no apparent differences in association between marine oil intake dose and outcome at lower or higher dose ranges (Balk et al., 2016).

Abdelhamid et al. (2020) found no relationship between LC n-3 FA dose and risk of CHD mortality.

Lombardi et al. (2020) did not report any dose-response. Casula et al. (2020) highlight a relevant clinical benefit only when giving high dose supplementation (more than 1 g per day). Rizos et al. (2021) found association between dose-specific LC n-3 FA supplementation and cardiac mortality.

5.2.3.4 *Weight of evidence for LC n-3 FA intake and CHD mortality*

In this section, the evidence of the association between LC n-3 FA and CHD mortality is weighed according to the WCRF criteria presented in the method chapter (Box 2 in Chapter 3.1.6, but applied to evaluation of systematic reviews and meta-analyses).

Published evidence of LC n-3 FA intake and CHD mortality

Five meta-analyses and systematic reviews of RCTs (Abdelhamid et al., 2020; Casula et al., 2020; Hu et al., 2019; Lombardi et al., 2020; Rizos et al., 2021), showed that intake of LC n-3 FA has a small protective effect against either CHD mortality, coronary mortality or cardiac death or sudden cardiac death, while one meta-analysis (Balk et al., 2016) did not find any association between sudden cardiac mortality and intake of LC n-3 FA. All three meta-analysis had very similar RR and 95% CI, but the two who reported a significant protective effect included three new, large RCTs. Balk et al. (2016) also included prospective cohort studies and found no association between marine oil intake and CHD mortality.

More specifically, two meta-analyses of RCTs found a significant small protective effect between intake of LC n-3 FA on CHD mortality (Abdelhamid et al., 2020 and Hu et al., 2019). Three meta-analysis of RCTs found a significant protective effect between intake of LC n-3 FA on cardiac mortality and sudden cardiac mortality, but doses may play a role (Casula et al., 2020; Lombardi et al., 2020; Rizos et al., 2021). Balk et al. (2016) found no effect of intake of LC n-3 FA on sudden cardiac mortality. The meta-analysis of prospective cohort studies by Balk et al. (2016) showed no association between marine oil intake on CHD mortality.

Heterogeneity

No significant heterogeneity was observed in the included meta-analyses.

Mechanisms/biological plausibility

Plausible mechanisms are presented above, see Chapter 5.2.

Upgrading factors

Rizos et al. (2021) found association between dose-specific n-3 supplementation on cardiac mortality.

Conclusion weight of evidence LC n-3 FA intake and CHD mortality

Results from the five meta-analyses and systematic reviews of RCTs and the meta-analysis of cohort studies showed that intake of LC n-3 FA has a small protective effect against either CHD mortality, coronary mortality or cardiac death or sudden cardiac death (Rizos et al., 2020, Abdelhamid et al 2020, Lombardi et al 2020, Casula et al 2020, Hu et al 2019), while one meta-analysis (Balk et al., 2016) did not find any association between sudden cardiac mortality and intake of LC n-3 FA. The direction of the effect in the pooled analyses in all five meta-analyses of RCTs was towards protective, and generally consistent. Limited/no unexplained heterogeneity was observed in all studies. There is evidence for biological plausibility. In conclusion, the evidence that LC n-3 FA intake protects against CHD mortality including coronary death, and cardiac death is graded as “probable”. The evidence for sudden cardiac death may be less convincing.

5.2.4 LC n-3 FA and CVD incidence

5.2.4.1 *Results from the meta-analyses for LC n-3 FA intake and CVD incidence*

Below is a summary table for LC n-3 FA and CVD incidence (Table 5.2.4.1-1) based on the identified meta-analyses.

Table 5.2.4.1-1 Overview of results from meta-analyses included in the weight of evidence analysis of LC n-3 FA intake and CVD incidence.

Author, year	Study design, intake range, N	Total no studies	No of cases	Comparison	Summary RR/HR (95 % CI)	Heterogeneity	Overall results
Abdelhamid, 2020	RCT EPA+DHA: 0.5-5 g/d n=140 482	43	8658 high intake vs 8961 low intake	Placebo or open labelled	RR 0.96 (0.92- 1.01)	$I^2=44.08\%$	No effect of LC n-3 FA on cardiovascular events
Casula, 2020	RCT EPA+DHA: 1-6 g/day n=81 073	16	6070 treatment vs 6433 CTR	Placebo or open labelled	OR 0.90 (0.82-0.99)	$P<0.001$	LC n-3 FA have a protective effect on major adverse cardiovascular events
Lombardi, 2020	RCT EPA+DHA: 0.4-4 g/d n=125 051	14	-	Placebo or open labelled	Incidence rate ratio 0.78 (0.71-0.85) high dose versus control Incidence rate ratio 0.98 (0.94-1.01) low dose versus control Incidence rate ratio 0.79 (0.72-0.88) high dose versus low dose	$I^2<50\%$, $P>0.05$	High-dose treatment has a protective effect on major vascular event compared to control ($P<0.0001$), and versus low dose ($P<0.0001$) Low-dose treatment has no protective effect on major vascular event
Hu, 2019	RCT EPA+DHA: 0.4-4 g/d n=127 423	13	7689 treatment vs 8070 CTR	Placebo or open labelled	RR 0.95 (0.92-0.98) (included REDUCE IT)	$I^2=60.7\%$	LC n-3 FA have a protective effect on total CVD*, the effect was stronger when including REDUCE-IT
		12	7230 treatment vs 7464 CTR		RR 0.97 (0.94-0.99) (excluded REDUCE IT)	$I^2=18.2\%$	LC n-3 FA have a protective effect on total CVD*
Hu, 2019	RCT EPA+DHA: 0.4-4 g/d n=127 423	13	8048 treatment vs 8430 CTR		RR 0.95 (0.93-0.98) (included REDUCE IT)	$I^2=59.9\%$	LC n-3 FA have a protective effect on major vascular events only when REDUCE IT is included [#]
		12	7243 treatment vs 7529 CTR		RR 0.97 (0.94-1.00) (excluded REDUCE IT)		

Author, year	Study design, intake range, N	Total no studies	No of cases	Comparison	Summary RR/HR (95 % CI)	Heterogeneity	Overall results
Marston, 2019	RCT EPA+DHA: 0.4-4 g/d n=125 544	13	Total 15933	Placebo or open labelled	RR 0.93 (0.91-0.95) by EPA dose for each 1g/d	$I^2=0\%$	Each 1 g/d EPA administered was associated with a 7% relative risk reduction in major vascular events ($P<0.0001$)

*Total CVD includes non-fatal MI, non-fatal stroke, death from CVD, or hospitalization because of cardiovascular cause (except for JELIS, and ALPHA-OMEGA, which include revascularization).

#Major vascular events include non-fatal MI, non-fatal stroke, death from CVD, or revascularization.

Overall, four out of five meta-analyses of RCTs found a protective effect of LC n-3 FA intake on CVD incidence. However, one of the meta-analysis (Marston et al., 2019) included exactly the same papers as Hu et al. (2019).

Abdelhamid et al. (2020) included 43 RCTs and shows no protective effect of LC n-3 FA intake and cardiovascular events, based on RR and 95% CI. Among the studies, 22 trials show a decrease in risk, and four of these show a significant decreased risk. One trial shows significant increased risk (DART2). The meta-analysis included the three of the latest large RCTs performed (VITAL, REDUCE-IT and ASCEND). In addition, one study included supplemented food, and two studies used advice to increase intake of LC n-3 FA (DART and DART2), and several smaller studies with few events were included. Since all studies have been weighted, and up-and downgraded based on risk of bias and other criteria by the authors of the meta-analysis, the GRADE assessment suggested high-certainty evidence that LC n-3 intake makes little or no difference to risk of cardiovascular events (not downgraded).

Lombardi et al. (2020) included 14 RCTs, and based on IRR and 95% CI, a small protective effect of LC n-3 FA intake and major vascular event was found. When they compared high dose LC n-3 FA supplementation (>1 g per day) studies ($n=4$) with control, and low dose LC-n-3 FA supplementation (≤ 1 g per day) studies ($n=10$) with control, the RR and 95% CI show that there is a protective effect of both low and high dose of LC n-3 FA intake on risk of major vascular events. The risk reduction is larger when they compared studies with high dose of LC n-3 FA intake with low dose of LC n-3 FA. The meta-analysis included the three of the latest large RCTs performed (VITAL, REDUCE-IT and ASCEND). Sensitivity analysis showed that olive oil as control may lead to smaller changes between LC n-3 FA intake and control. In addition, ten studies overlapped with Casula et al. (2020), and 13 overlapped with Hu et al. (2019). All overlapped with Abdelhamid et al. (2020).

Casula et al. (2020) included 16 RCTs, and the overall effect, based on RR and 95% CI shows a small protective effect of LC n-3 FA intake on major adverse cardiovascular events. Among the trials, 11 shows a protective effect, and out of these, three shows significant protective effect. None of the trials show significant increased risk of LC n-3 FA on major adverse cardiovascular events. The meta-analysis included one of the latest large RCTs performed (REDUCE-IT). Influence analysis showed that the significant protective effect of LC n-3 FA on the risk of cardiac mortality was no longer significant when excluding the GISSI-P study. And the benefit on the risk of major adverse cardiovascular events became not statistically significant by excluding IEIS-4, the JELIS, or the REDUCE-It trial. There were some studies including only EPA, thus, it may suggest that the effect is EPA-dependent. In subgroup analysis by type of prevention (secondary or mix), the protective effect on major adverse cardiovascular events was only significant in RCTs that enrolled patients in primary/secondary prevention. Only the administration of more than 1 g per day of LC n-3 FA seemed to be protective against major adverse cardiovascular events.

In addition, ten trials overlapped with Lombardi et al. (2020), seven trials overlapped with Hu et al. (2019), and 13 trials overlapped with Abdelhamid et al. (2020).

Marston et al. (2019) included 13 RCTs, and based on RR and 95% CI, show overall small protective effect of EPA intake on major cardiovascular event. This systematic review includes the same trials as Hu et al. (2020).

Hu et al. (2019) included 13 RCTs, and based on RR and 95% CI, showed overall small protective effect of LC n-3 FA intake on total CVD incidence. Among the trials, 10 shows a protective effect, and out of these, two trials show a significant protective effect (JELIS and REDUCE-IT). No trials significantly increased the risk of CVD. Since REDUCE-IT gave a high dose of EPA (4 g/d), this study was excluded from the analysis, and even after exclusion of REDUCE-IT (4 g EPA/day) there was still an overall significant protective effect of LC n-3 FA intake on CVD incidence. In the sensitivity analysis that excluded DOIT, SU.FO-L.OM3, Alpha-Omega, and OMEGA (because of considerably lower dose, duration, or size), the protective effect of LC n-3 FA on total CVD incidence was slightly stronger (RR 0.94, 95% CI 0.91 to 0.97, and RR 0.96, 95% CI 0.93-0.98 excluded REDUCE-IT). In the sensitivity analysis that excluded two open-label trials, GISSI-P and JELIS, the point estimates for total CVD showed less protective effect (RR 0.96, 95% CI 0.93-0.99, and RR 0.98, 95% CI 0.95-1.01 excluded REDUCE-IT). Based on RR and 95% CI there was an overall protective effect of LC n-3 FA on major vascular events. Among the trials, ten shows a protective effect, two of these trials show a significant protective effect (JELIS and REDUCE-IT). No trials significantly increased the risk of major vascular events. However, the protective effect on major vascular events was only observed when REDUCE-IT was included. The meta-analysis included three of the latest large RCTs performed (REDUCE-IT, VITA and ASCEND). In the sensitivity analysis that excluded DOIT, SU.FO-L.OM3, Alpha-Omega, and OMEGA (because of considerably lower dose, duration, or size), the inverse associations for major vascular events were unchanged (RR 0.95, 95% CI 0.92-0.98, and RR 0.97, 95% CI 0.94-1.00 excluded REDUCE-IT). In the sensitivity analysis that excluded two open-label trials, GISSI-P and JELIS, the point estimates for major vascular events were unchanged (RR 0.95, 95% CI 0.92-0.98, and RR 0.97, 95% CI 0.94-1.01 excluded REDUCE-IT). In addition, all trials overlapped with Lombardi et al. (2020), nine trials overlapped with Casula et al. (2020), and all trials overlapped with Abdelhamid et al. (2020).

5.2.4.2 Heterogeneity LC n-3 FA intake and CVD incidence

In Abdelhamid et al. (2020), heterogeneity was moderate ($I^2=44.08\%$, $P_{\text{heterogeneity}}=0$) for cardiovascular events.

In Lombardi et al. (2020), heterogeneity was moderate ($I^2<50\%$) for major vascular events.

In Casula et al. (2020), the $P_{\text{heterogeneity}}$ was significant for major adverse cardiovascular events ($P_{\text{heterogeneity}}<0.001$).

In Marston et al. (2019), heterogeneity was low ($I^2=0\%$, $P_{\text{heterogeneity}}=0.55$).

In Hu et al. (2019), heterogeneity was moderate for total CVD incidence ($I^2=60.7\%$, $P_{\text{heterogeneity}}=0.002$). The heterogeneity was high for major vascular events ($I^2=59.9\%$, $P_{\text{heterogeneity}}=0.003$).

5.2.4.3 *Dose-response relationship LC n-3 FA intake and CVD incidence*

In Hu et al. (2019), there was a dose-response relationship between LC n-3 FA intake and total CVD incidence and major vascular events. Each 1000 mg/day LC n-3 FA intake lowered total CVD incidence by 17% (95% CI: 4%, 29%) and major vascular events by 17% (95% CI: 3%, 28%) without evidence of heterogeneity.

In Abdelhamid et al. (2020), meta-regression suggested reduction in cardiovascular disease risk at higher LC n-3 FA doses, as would be expected from a dose response. However, when the single outlying trial REDUCE-IT 2019 (with a large effect size and high EPA dose) was omitted, no relationship between LC n-3 FA dose and risk of cardiovascular disease events was observed.

In Lombardi et al. (2020) high-dose treatment has a protective effect on major vascular event compared to control, and versus low dose.

In Marston et al. (2019), a meta-regression by EPA-dose, for each 1 g/day EPA administered, there was a 7% relative lower risk of major vascular events. This was not observed for DHA.

5.2.4.4 *Weight of evidence for LC n-3 FA intake and CVD incidence*

In this section, the evidence of the association between LC n-3 FA and CVD incidence is weighed according to the WCRF criteria presented in the method chapter (Box 2 in Chapter 3.1.6, but applied to evaluation of systematic reviews and meta-analyses).

Published evidence of LC n-3 FA intake and CVD incidence

Overall, four out of five meta-analyses of RCTs found a protective effect of LC n-3 FA intake on CVD incidence. The meta-analysis of Abdelhamid et al found no significant overall effect. Abdelhamid included smaller trials with few events, and also trials based on dietary advice or enriched foods. The RR and 95 % CI is still very close to the results from the other meta-analysis, which showed a small protective effect of LC n-3 FA intake on CVD incidence, but these meta-analyses have smaller CI intervals. Lombardi et al compared high and low dose of LC n-3 FA supplementation (which included the same trials as Hu et al 2019), and they observed a larger effect with higher doses. When removing REDUCE-IT from the analysis, the overall effect on major vascular events was not significant in some of the meta-analysis.

Heterogeneity

One meta-analysis reported significant heterogeneity (Hu et al., 2019). Otherwise, no significant heterogeneity was observed in the included meta-analyses.

Mechanisms/biological plausibility

Plausible mechanisms are presented above, see Chapter 5.2.

Upgrading factors

There is a dose-response relationship between LC n-3 FA intake on CVD incidence and major vascular event.

Conclusion weight of evidence LC n-3 FA intake and CVD incidence

We included five meta-analysis and systematic reviews of RCTs reported effects on cardiovascular events, major vascular events or major adverse cardiovascular events after intake of LC n-3 FA. Among these, one reported no significant effect on CVD events (Abdelhamid et al., 2020), while two reported effects either with high-dose of LC n-3 FA (Lombardi et al., 2020) or only when REDUCE-IT trial was included (which supplement with 4 g/day of EPA) (Hu et al., 2019). One meta-analyses reported that intake of LC n-3 FA protects against total CVD. There is evidence for biological plausibility and no unexplained heterogeneity. In conclusion, the evidence that LC n-3 FA intake protects against risk of CVD, in particular major vascular events, is graded as “limited, suggestive” for ordinary doses that we can consume from fish (<1 g LC n-3 FA per day), and “probable” for higher doses from supplements.

5.2.5 LC n-3 FA and CHD incidence

5.2.5.1 *Results from the meta-analyses for LC n-3 FA intake and CHD incidence*

Below is a summary table for LC n-3 FA intake on CHD incidence (Table 5.2.5.1-1) based on the identified meta-analyses.

Table 5.2.5.1-1 Overview of results from meta-analyses included in the weight of evidence analysis of LC n-3 FA intake and CHD incidence.

Author, year	Study design, intake range, N	Total no studies	No of cases	Comparison	Summary RR/HR (95% CI)	Heterogeneity	Overall results
Abdelhamid, 2020	RCT EPA+DHA: 0.5-5 g/d n=134 116	32	4224 high intake vs 4553	Placebo or open labelled	RR 0.91 (0.85 to 0.97)	$I^2=36.52\%$	LC n-3 FA have a protective effect on CHD events.
Hu, 2019	RCT EPA+DHA: 0.4 -4 g/d n=127 423	13	4060 treatment vs 4375 CTR	Placebo or open labelled	RR 0.93 (0.89-0.96) (included REDUCE IT)	$I^2=54.7\%$	LC n-3 FA have a protective effect on total CHD events*. The finding was strengthened after including REDUCE-IT
		12	3668 treatment vs 3868 CTR		RR 0.95 (0.91-0.99) (excluded REDUCE-IT)	$I^2=35.4\%$	
Alexander, 2017	RCT EPA+DHA: 0.75-5.04 g/d (fatty fish, or supplement) n=93 000	18	-	Balanced diet, corn oil, no supplement, olive oil	SRRE# 0.94 (0.85-1.05)	$P=0.07$	LC n-3 FA have no protective effect on CHD events. Subgroup analysis indicated a statistically significant CHD reduction among higher risk-populations (increased TG and LDL-C)
	Prospective cohort studies	17	-	High vs low intake	SRRE 0.82 (0.74-0.92)	$P<0.001$	Inverse association between intake of EPA-DHA and any CHD event
Balk, 2016	Prospective cohort studies n=178 005			0.038 to 3.47 g/d	0.94 (0.81-1.10)		No association between marine oil intake (including fish intake) and CHD

*Total CHD includes MI, death from CHD, or coronary revascularization.

#Summary relative risk estimate (SRRE).

Overall, two out of three meta-analyses of RCTs found a small protective effect between LC n-3 FA intake and CHD incidence. The two who found a protective effect include more recent large trials (VITAL, REDUCE-IT and ASCEND) than the third who did not find a significant protective effect. Two of the meta-analyses included prospective cohort studies, and one of these found a protective association between LC n-3 FA intake on CHD incidence. The oldest meta-analysis did not find any association between marine oil and CHD.

Abdelhamid et al. (2020) included 32 RCTs and shows a small protective effect of LC n-3 FA intake and cardiovascular events based on RR and 95% CI. Among the trials, 21 shows a protective effect, and three trials (GISSI-P, VITAL and REDUCE-IT) show all significant protective effects. None of the trials show significant increased risk of cardiovascular events. The meta-analysis included the three of the latest large RCTs performed (VITAL, REDUCE-IT and ASCEND). In addition, one study included supplemented food, and two studies used advice to increase intake of LC n-3 FA (DART), and several smaller studies with few events were included. All studies have been weighted, and up-and downgraded based on risk of bias and other criteria by the authors of the meta-analysis, and the GRADE assessment performed by the authors of this meta-analysis suggested low-certainty evidence that increasing LCN3 fat intake may slightly reduce risk of coronary heart events (downgraded twice for risk of bias).

Hu et al. (2019) included 13 RCTs and shows a small protective effect of LC n-3 FA intake on CHD based on RR and 95% CI. Among the trials, 11 shows a protective effect, and three show significant protective effect (GISSI-P, VITAL and REDUCE-IT). When the REDUCE –IT (4 g EPA/day) study was excluded, there was still an overall protective effect in the meta-analysis. None of the trials show a significant increased risk of CHD. In the sensitivity analysis that excluded DOIT, SU.FO-L.OM3, Alpha-Omega, and OMEGA (because of considerably lower dose, duration, or size), the inverse associations for CHD were slightly strengthened (RR 0.92, 95% CI 0.88-0.96, and RR 0.94, 95% CI 0.90-0.98 excluded REDUCE-IT). In the sensitivity analyses that excluded two open-label trials, GISSI-P and JELIS, the point estimates for CHD were weaker (RR 0.94, 95% CI 0.90-0.98, and RR 0.96, 95% CI 0.92-1.01 excluded REDUCE-IT).

In addition, all overlapped with Abdelhamid et al. (2020), and eight studies overlapped with Alexander et al. (2017).

Alexander et al. (2017) included 18 RCTs and shows no significant protective effect of LC n-3 FA on CHD incidence based on RR and 95% CI. Among the trials, 13 shows a protective effect, and only one shows significant protective effect (JELIS which only included subgroup with no CHD participants). One trial shows a significant increased risk of CHD (DART2). In addition, 11 overlapped with Abdelhamid et al. (2020), and eight studies overlapped with Alexander et al. (2017). None of the recent large RCTs were included (VITAL, REDUCE-IT and ASCEND), but DART2 was included which was not included in Hu et al (2019) and Abdelhamid et al (2020). They also included prospective cohort studies, and overall effect of 17 prospective cohort studies showed a protective association between LC n-3 FA intake on CHD.

Balk et al. (2016), including only prospective cohort studies, showed no overall protective association between LC n-3 FA intake on CHD.

5.2.5.2 Heterogeneity LC n-3 FA intake and CHD incidence

In Abdelhamid et al. (2020), heterogeneity was moderate ($I^2=36.52\%$, $P_{\text{heterogeneity}}=0.02$) for CHD events.

In Hu et al. (2019), heterogeneity was moderate for CHD ($I^2=54.7\%$, $P_{\text{heterogeneity}}=0.009$). When excluding REDUCE-IT the heterogeneity was lower and no longer statistically significant ($I^2=35.4\%$, $P_{\text{heterogeneity}}=0.107$).

In Alexander et al. (2017), no I^2 value was reported. The $P_{\text{heterogeneity}}$ was borderline statistically significant ($P=0.07$), reflecting differences in several study characteristics, including baseline triglyceride and LDL-C levels. Also, higher doses (above 1 g/day of EPA+DHA) had a stronger impact among those with elevated triglyceride levels.

5.2.5.3 Dose-response relationship LC n-3 FA intake and CHD incidence

In Hu et al. (2019), there is a dose-response relationship when including REDUCE-IT trial without introducing significant heterogeneity. Each 1000 mg/day LC n-3 FA intake lowered CHD incidence by 7% (95% CI: 0%, 13%).

In Abdelhamid et al. (2020), the main meta-analysis suggested a 9% reduction in people experiencing CHD events with higher intake of LC n-3 FA. Meta-regression suggested reduction in CHD incidence at higher LC n-3 FA doses, as would be expected from a dose response. When REDUCE-IT trial was omitted, no relationship between LC n-3 FA dose and CHD incidence was observed.

In Alexander et al. (2017), a meta-regression did not produce a continuous dose-response effect when including data of less than 1 g/day of EPA+DHA.

For CHD incidence, there were no apparent differences in association between marine oil intake dose and outcome at lower or higher dose ranges (Balk et al., 2016).

5.2.5.4 Weight of evidence for LC n-3 FA intake and CHD incidence

In this section the evidence of the association between LC n-3 FA and CHD incidence is weighed according to the WCRF criteria presented in the method chapter (Box 2 in Chapter 3.1.6, but applied to evaluation of systematic reviews and meta-analyses).

Published evidence of LC n-3 FA intake and CHD incidence

Overall, two out of three meta-analyses of RCTs found a small protective effect between LC n-3 FA intake and CHD incidence. The two who found a protective effect include more recent large trials (VITAL, REDUCE-IT and ASCEND) than the third who did not find a significant protective effect. The direction of the effect in the pooled analyses in all the meta-analyses is generally consistent (towards protective), and the RR between the meta-analysis is very

similar, but the confidence interval differs due to differences in included studies. Two of the meta-analyses included prospective cohort studies, and one of these found a protective association between LC n-3 FA intake on CHD incidence. The oldest meta-analysis did not find any association between marine oil and CHD.

Heterogeneity

All three meta-analysis of RCTs reported significant heterogeneity (Abdelhamid et al., 2020, Hu et al., 2019, Alexander et al. 2017), although in Alexander et al. (2017), the heterogeneity was borderline significant. Heterogeneity is most probably due to different doses and study populations.

Mechanisms/biological plausibility

Plausible mechanisms are presented above, see Chapter 5.2.

Upgrading factors

There is a dose-response relationship between LC n-3 FA and CHD incidence.

Conclusion weight of evidence LC n-3 FA intake on CHD incidence

Two out of three meta-analysis of RCTs found that LC n-3 FA intake has a protective effect on CHD incidence. The direction of the effect in the pooled analyses in all the meta-analyses is generally consistent (towards protective). Two of the meta-analyses also included prospective cohort studies in separate analyses, and one of these found a protective association of LC n-3 FA intake on CHD incidence. The oldest meta-analysis did not find any association between marine oil and CHD. There is evidence for biological plausibility and there is no unexplained heterogeneity. In conclusion, the evidence is graded “probable” that intake of LC n-3 FA protect against CHD incidence.

5.2.6 LC n-3 FA and MI incidence

5.2.6.1 *Results from the meta-analyses for LC n-3 FA intake and MI incidence*

Below is a summary table for LC n-3 FA and MI incidence (Table 5.2.6.1-1) based on the identified meta-analyses.

Table 5.2.6.1-1 Overview of results from meta-analyses included in the weight of evidence analysis of LC n-3 FA intake and MI incidence.

Author, year	Study design, intake range, N	Total no studies	No of cases	Comparison	Summary RR/HR (95 % CI)	Heterogeneity	Overall results
Rizos, 2021	RCT EPA+DHA: 0.27-6 g/d	3 5 3 3		Placebo or open labelled	<1 capsule/day=<0.84 g RR 1.1 (0.75-1.34) 1 capsule/day=0.84-1.68 g RR 0.90 (0.78-1.05) 2 capsules/day=1.68-2.52 g RR 0.76 (0.28-2.05) >3 capsules/ day=>2.52 g RR 0.90 (0.54-1.48)	$I^2=0\%$ $I^2=51\%$ $I^2=54\%$ $I^2=49\%$	No effect of LC n-3 FA and non-fatal MI for any of the assessed formulations
Abdelhamid, 2020	RCT EPA-DHA: 0.5-5 g/d n=133 012	27	1877 higher intake vs 2115 lower intake	Placebo or open labelled	RR 0.88 (0.81-0.96)	$I^2=24.6\%$	LC n-3 FA has a protective effect on MI
Casula, 2020	RCT EPA+DHA: 1-6 g/d n=80 773	15	1236 treatment vs 1406 CTR	Placebo or open labelled	OR 0.83 (0.71-0.98)	$P=0.002$	LC n-3 FA has a protective effect on MI. In subgroup analysis, the risk reduction of MI was only confirmed in RCTs that enrolled patients in secondary prevention
Lombardi, 2020	RCT EPA+DHA: 0.4-4 g/d n=125 051	14	-	Placebo or open labelled	Incidence rate ratio 0.71 (0.62-0.82) high dose versus control Incidence rate ratio 0.91 (0.84-0.98) low dose versus control Incidence rate ratio 0.79 (0.67-0.92) high dose versus low dose	$I^2<50\%$, $P>0.05$	High-dose and low dose treatment has a protective effect on MI compared to control ($P<0.0001$, and $P=0.01$). High dose compared to low dose has a more protective effect on MI ($P=0.003$)

Author, year	Study design, intake range, N	Total no studies	No of cases	Comparison	Summary RR/HR (95 % CI)	Heterogeneity	Overall results
Hu, 2019	RCT EPA+DHA: 0.4-4.0 g/d n=127 423	13	4060 treatment vs 4375 CTR	Placebo or open labelled	RR 0.88 (0.83-0.94) (included REDUCE IT)	$I^2=51.2\%$	LC n-3 FA has a protective effect on MI
		12	1553 treatment vs 1580 CTR		RR 0.92 (0.86-0.99) (excluded REDUCE IT)	$I^2=25.5\%$	The effect was strengthened after including REDUCE-IT
Balk, 2016	RCT EPA+DHA: 0.34-6 g/d n=60 473	12	748 treatment vs 808 CTR	Placebo or open labelled	HR 0.88 (0.77-1.02)	$I^2=23.46\%$	LC n-3 FA have no effect on MI

Overall, four of six meta-analyses of RCTs found a protective effect of LC n-3 FA intake and risk of MI. One study which did not find a significant effect looked into different doses of LC n-3 FA intake (Rizos et al., 2021), but it included the newest RCTs VITAL, REDUCE-IT and ASCEND. The second study that did not find a significant effect of LC n-3 FA intake is the oldest meta-analysis, from 2016 (Balk et al., 2016).

Rizos et al. (2021) included 14 RCTs (13 non-fatal and one fatal/non-fatal MI) with duration of more than 1 year and reported 2483 events among 79064 participants. Based on RR and 95% CI, no protective effect of LC n-3 FA on MI was found for any of the assessed doses. Three studies which supplemented participants with <1 capsule/ day (<0.84 g EPA+DHA per day), show a small non-significant increase in risk (RR 1.01), while five studies with 1 capsule/day (0.84-1.68 g EPA+DHA per day), three studies with 2 capsules per day (1.68-2.52 g EPA+DHA per day), and three studies with more than 3 capsules per day (>2.52 g EPA+DHA per day) show all a non-significant protective effect of LC n-3 FA intake on non-fatal MI. The meta-analysis included the two of the latest large RCTs performed (VITAL and REDUCE-IT). In addition, 12 RCTs overlapped with Abdelhamid et al. (2021), 10 RCTs overlapped with Lombardi et al. (2020), four overlapped with Balk et al. (2016), and six overlapped with Casula et al. (2020), and nine overlapped with Hu et al. (2019).

Abdelhamid et al. (2021) included 27 RCTs and shows an overall protective effect of LC n-3 FA on MI based on RR and 95% CI. Among the trials, 18 show a protective effect, and three trials (VITAL and REDUCE-IT) show all significant protective effects. None of the trials show significant increased risk of MI. The meta-analysis included the three of the latest large RCTs performed (VITAL, REDUCE-IT and ASCEND). In addition, one study included supplemented food, and one study used advice to increase intake of LC n-3 FA (DART), and several smaller studies with few events were included. MI was not defined as primary outcome in the meta-analysis and no GRADE assessment was done. In addition, 12 RCTs overlapped with Rizos et al. (2021), nine RCTs overlapped with Balk et al (2016), all overlapped with Lombardi et al. (2020), 12 overlapped with Casula et al. (2020), and all overlapped with Hu et al. (2019).

Lombardi et al. (2020) included 14 RCTs, and based on IRR and 95% CI, shows a protective effect of LC n-3 FA on MI. Comparing high dose LC n-3 FA supplementation (>1 g per day) studies (n=4) with control, shows a significant protective effect (IRR of 0.71). Comparing low dose LC-n-3 FA supplementation ($\leq$ 1 g per day) studies (n=10) with control, shows a significant protective effect (IRR 0.91). When they compared high dose with low dose there was a significant effect, thus there is a protective effect of both low and high dose of LC n-3 FA intake on MI. The risk reduction is larger when they compared studies with high dose of LC n-3 FA intake with low dose of LC n-3 FA. The meta-analysis included the three of the latest large RCTs performed (VITAL, REDUCE-IT and ASCEND). In addition, all studies overlapped with Abdelhamid et al. (2020) and Hu et al. (2019), ten studies overlapped with Rizos et al. (2021), nine overlapped with Casula et al. (2020), and six overlapped with Balk et al. (2019).

Casula et al. (2020) included 15 RCTs, and based on RR and 95% CI, shows a protective effect of LC n-3 FA on MI. Among the studies, 10 show a protective effect of LC n-3 FA intake on MI, and three of these show a significant protective effect (IEIS-4, OPACH and

REDUCE-IT). No studies show significant increased risk of MI. The meta-analysis included the one of the latest large trials performed (REDUCE-IT). In addition, seven studies overlapped with Rizos et al. (2021), 13 overlapped with Abdelhamid et al. (2020), 10 overlapped with Lombardi et al. (2020), nine overlapped with Hu et al. (2019), and six overlapped with Balk et al. (2016).

Hu et al. (2019) included 13 RCTs, and based on RR and 95% CI, shows overall protective effect of LC n-3 FA intake on MI, even after exclusion of REDUCE-IT (4 g EPA/day). Among the trials, nine show protective effect of LC n-3 FA on MI, and two show a significant protective effect (VITAL and REDUCE-IT). None of the trials show significant increased risk of MI. The meta-analysis included the three of the latest large RCTs performed (VITAL, REDUCE-IT and ASCEND). In the sensitivity analysis that excluded DOIT, SU.FO-L.OM3, Alpha-Omega, and OMEGA (because of considerably lower dose, duration, or size), the protective effect of LC n-3 FA on MI was slightly improved (RR 0.87, 95% CI 0.82-0.96, and RR 0.93, 91% CI 0.85-0.98 excluded REDUCE-IT). In the sensitivity analysis that excluded two open-label trials, GISSI-P and JELIS, the point estimates for MI were unchanged for all, but weaker when excluding REDUCE-IT (RR 0.88, 95% CI 0.82-0.94, and RR 0.93, 95% CI 0.86-1.00 excluded REDUCE-IT).

In addition, nine trials overlapped with Rizos et al. (2021) and Casula et al. (2020), all overlapped with Abdelhamid et al. (2020) and Lombardi et al. (2020), and six overlapped with Balk et al. (2016).

Balk et al. (2016) included 12 RCTs, and based on RR and 95% CI, shows no protective effect of LC n-3 FA on MI. Among the trials, 10 shows a protective effect, none of the trials show significant protective effect. None of the trials show a significant increased risk of MI.

5.2.6.2 Heterogeneity LC n-3 FA intake and MI incidence

In Rizos et al. (2021), heterogeneity was low to moderate in studies with doses above 1 capsule per day (>0.84 g/d) ($I^2=49-54\%$, no P -value reported).

In Abdelhamid et al. (2020), heterogeneity was low ($I^2=24.6\%$, $P_{\text{heterogeneity}}=0.12$).

In Lombardi et al. (2020), heterogeneity (I^2) was below 50%, and the P -value was not significant ($P_{\text{heterogeneity}}>0.05$).

In Casula et al. (2020), the P -value for heterogeneity was significant ($P=0.002$), and no I^2 value was reported.

In Hu et al. (2019), heterogeneity was moderate for MI ($I^2=51.2\%$, $P_{\text{heterogeneity}}=0.017$), and lower when excluding the REDUCE-IT study ($I^2=25.5\%$, $P_{\text{heterogeneity}}=0.194$). In Balk et al. (2016), the heterogeneity was low ($I^2=23.5\%$, $P_{\text{heterogeneity}}=0.542$).

5.2.6.3 *Dose-response relationship LC n-3 FA intake and MI incidence*

In Hu et al. (2019), there is a significant dose-response relationship between LC n-3 FA intake and MI when including REDUCE-IT trial, without introducing significant heterogeneity. Each 1000 mg/day LC n-3 FA intake lowered risk of MI by 9% (95% CI: 2%, 15%).

In Lombardi et al. (2020) they show a larger risk reduction when they compared studies with high dose of LC n-3 FA intake with low dose of LC-n-3 FA.

None of the other studies included dose-response relationship for LC n-3 FA intake and MI.

5.2.6.4 *Weight of evidence for LC n-3 FA intake and MI incidence*

In this section, the evidence of the association between LC n-3 FA and MI is weighed according to the WCRF criteria presented in the method chapter (Box 2 in Chapter 3.1.6, but applied to evaluation of systematic reviews and meta-analyses).

Published evidence of LC n-3 FA intake and MI incidence

Overall, four of six meta-analyses of RCTs found a protective effect of LC n-3 FA intake and risk of MI. One study which did not find a significant effect looked into different doses of LC n-3 FA intake (Rizos et al., 2021), but they included few trials in each of the comparisons, and they only looked at non-fatal MI. The meta-analysis included the newest RCTs VITAL, REDUCE-IT and ASCEND. The second study that did not find a significant effect of LC n-3 FA intake is the oldest meta-analysis, from 2016 (Balk et al., 2016). The four meta-analysis that found a protective effect had some overlap between included studies. Most of the included RCTs show protective effect. Both lower doses (below 1 g/day) and higher doses of LC n-3 FA (above 1 g/day) seems to have a protective effect, although higher doses have a larger effect based on the finding from Lombardi et al. (2020), and also in Hu et al. (2019) since the effect was lower when REDUCE-IT was removed from the analysis.

Heterogeneity

Significant heterogeneity was observed in two of the included meta-analyses, but in one of these, the heterogeneity was lower, and no longer significant when one of the largest trials was removed (REDUCE-IT).

Mechanisms/ biological plausibility

Plausible mechanisms are presented above, see Chapter 5.2.

Upgrading factors

There is a dose-response relationship between LC n-3 FA and MI.

Conclusion weight of evidence LC n-3 FA intake and MI incidence

Four out of six meta-analysis of RCTs found a protective effect of LC n-3 FA intake on MI. The direction of the effect in the pooled analyses in all the meta-analyses is generally consistent (towards protective). There is evidence for biological plausibility and there is no unexplained heterogeneity. In conclusion, the evidence that LC n-3 FA intake protect against MI is graded “probable”.

5.2.7 LC n-3 FA and stroke incidence

5.2.7.1 *Results from the meta-analyses for LC n-3 FA intake and stroke incidence*

Below is a summary table for LC n-3 FA and stroke (Table 5.2.7.1-1) based on the identified meta-analyses.

Table 5.2.7.1-1 Overview of results from meta-analyses included in the weight of evidence analysis of LC n-3 FA intake and stroke incidence.

Author, year	Study design, intake range, N	Total no studies	No of cases	Comparison	Summary RR/HR (95 % CI)	Heterogeneity	Overall results
Abdelhamid, 2020	RCT EPA+DHA: 0.5-5 g/d n=138 888	31	1436 high intake vs 1414 low intake	Placebo or open labelled	RR 1.02 (0.94 to 1.12)	$I^2=11.4\%$	LC n-3 FA have no effect on stroke
Casula, 2020	RCT EPA+DHA: 1-6 g/day	12	948 treatment vs 936 CTR	Placebo or open labelled	OR 1.00 (0.89-1.23)	$P=0.02$	LC n-3 FA have no effect on stroke
Lombardi, 2020	RCT EPA+DHA: 0.4-4 g/d n=128 051	14	-	Placebo or open labelled	Incidence rate ratio 0.89 (0.76-1.05) high dose versus control 1.05 (0.96-1.15) low dose versus control	$I^2<50\%$, $P>0.05$	LC n-3 FA have no effect on stroke ($P=0-18$)
Campano, 2019	RCT EPA+DHA: 0.5-1 g/d n=2237 3 month or longer duration	5	119 treatment vs 118 CTR	Placebo	RR 1.02 (0.78-1.35)	16%	No effect on vascular-related death
	RCT EPA+DHA: 0.4-1 g/d n=1819 3 month or longer duration	3	10 treatment vs 14 CTR	Placebo	RR 0.69 (0.31- 1.55)	0%	No effect on recurrent events (fatal only) ischemic and hemorrhagic stroke)
Hu, 2019	RCT EPA+DHA: 0.4-4.0 g/d n=127 423	13	1358 treatment vs 1325 CTR		RR 1.02 (0.95-1.10) (included REDUCE IT)	$I^2=36.2\%$	LC n-3 FA have no effects on stroke
		12	1260 treatment vs 1191 CTR		RR 1.05 (0.98-1.14) (excluded REDUCE IT)	$I^2=6.3\%$	No effect of LC n-3 FA intake and stroke

Author, year	Study design, intake range, N	Total no studies	No of cases	Comparison	Summary RR/HR (95 % CI)	Heterogeneity	Overall results
Balk, 2016	RCT EPA+DHA: 0.84-6 g/d	8	730 treatment vs 737 CTR		HR 0.98 (0.88-1.09)	$I^2=20.59\%$	LC n-3 FA have no effect on stroke
	Prospective cohort studies n=178 249			0.025-0.6 g/d	0.68 (0.53 to 0.87)		Inverse association between intake of marine oil and total stroke

Overall, none of the meta-analysis of RCTs found an effect of LC n-3 FA intake and risk of stroke. One meta-analysis included prospective cohort studies, and this study reported a negative association between intake of marine oil and total stroke.

Abdelhamid et al. (2020) included 31 RCTs, and overall, there is no effect of LC n-3 FA intake on stroke based on RR and 95% CI. Among the trials, 12 show a protective effect, and 19 show an increased risk of stroke. One of the trials shows a significant protective effect (REDUCE-IT), and one of the trials shows a significant increased risk (Omega trial). The meta-analysis included the three of the latest large RCTs performed (VITAL, REDUCE-IT and ASCEND). In addition, one study included supplemented food, and two studies used advice to increase intake of LC n-3 FA (DART and DART2), and several smaller studies with few events were included. GRADE assessment performed by the authors of the meta-analysis suggests moderate-certainty evidence that LC n-3 FA intake probably makes little or no difference to risk of experiencing a stroke (downgraded once for imprecision). In addition, 13 of the trials overlapped with Lombardi et al. (2020), 10 of the trials overlapped with Casula et al. (2020), 13 of the trials overlapped with Hu et al. (2019), and six overlapped with Balk et al. (2016).

Lombardi et al. (2020) included 14 RCTs, and based on IRR and 95% CI, shows no overall significant protective effect of LC n-3 FA on stroke. Comparing high dose LC n-3 FA supplementation (>1 g per day) studies (n=4) with control, shows no significant protective effect (IRR of 0.89). Comparing low dose LC-n-3 FA supplementation ($\leq$ 1 g per day) studies (n=10) with control, shows no protective effect (IRR 1.05). Thus, there is no protective effect of both low and high dose of LC n-3 FA intake on stroke. The meta-analysis included the three of the latest large RCTs performed (VITAL, REDUCE-IT and ASCEND). In addition, 13 trials overlapped with Abdelhamid et al. (2020) and 13 studies overlapped with Hu et al. (2019), nine overlapped with Casula et al. (2020), and four overlapped with Balk et al. (2016).

Casula et al. (2020) included 12 RCTs, and based on RR and 95% CI, shows no overall effect of LC n-3 FA on stroke. Among the studies, six show a protective effect, and one of the trials show significant reduced effect (REDUCE-IT). One trial shows a significant increased risk of stroke after intake of LC n-3 FA (Omega trial). The meta-analysis included one of the latest large RCTs performed (REDUCE-IT). In addition, 10 of the trials overlapped with Abdelhamid et al. (2020), nine overlapped with Lombardi et al. (2020), nine overlapped with Hu et al. (2019), and four overlapped with Balk et al. (2016).

Hu et al. (2019) included 13 RCTs, and based on RR and 95% CI, shows no overall effect of LC n-3 FA on stroke both with and without inclusion of REDUCE-IT. Among the trials, three trials show protective effect, and one of these trials shows significant protective effect (REDUCE-IT). One trial shows significant increased risk of stroke after intake of LC n-3 FA (Omega trial). The meta-analysis included the three of the latest large RCTs performed (VITAL, REDUCE-IT and ASCEND). In the sensitivity analysis that excluded DOIT, SU.FO-L.OM3, Alpha-Omega, and OMEGA (because of considerably lower dose, duration, or size), no protective effect of LC n-3 FA and stroke was seen (RR 1.01, 95% CI 0.94-1.09, and RR 1.05, 95% CI 0.96-1.13 excluded REDUCE-IT). In the sensitivity analysis that excluded two

open-label trials, GISSI-P and JELIS, the point estimates for total stroke were unchanged, but weaker when excluding REDUCE-IT (RR 1.01, 95% CI 0.93-1.09, and RR 1.05 95% CI 0.96-1.14 excluded REDUCE-IT).

In addition, nine overlapped with Casula et al. (2020), all overlapped with Lombardo et al. (2020) and Abdelhamid et al. (2020), and five overlapped with Balk et al. (2016).

Campano et al. (2019) included 5 and 3 RCTs, and based on RR and 95 % CI, no overall effect of LC n-3 FA on neither vascular-related death or recurrent events (fatal only) ischemic and hemorrhagic stroke, respectively was found. Among the studies reporting vascular-related death, three of the trials show non-significant protective effect. No studies show significant increased risk of vascular-related death. Among the studies reporting recurrent events, two show non-significant protective effect. No studies show significant increased risk. In addition, four of the trials overlapped with Abdelhamid et al. (2020), three trials overlapped with Lombardo et al. (2020), three overlapped with Hu et al. (2019), two overlapped with Casula et al. (2020), and one overlapped with Balk et al. (2016). None of the latest large RCTs was included (REDUCE-IT, VITAL and ASCEND).

Balk et al. (2016) included 8 RCTS, and based on RR and 95% CI, shows no overall effect of LC n-3 FA on stroke. Among the trials, two show protective effect, and one of them show significant protective effect (JELIS with only CVD population). None of the trials show significant increased risk of stroke. The prospective cohort studies show a negative association between intake of marine oil and total stroke.

5.2.7.2 Heterogeneity LC n-3 FA intake and stroke incidence

In Abdelhamid et al. (2020), heterogeneity was low ($I^2=11.4\%$). The $P_{\text{heterogeneity}}$ was not significant ($P=0.6$).

In Lombardi et al. (2020), heterogeneity (I^2) was below 50%, and $P_{\text{heterogeneity}}$ was not significant (>0.05).

In Casula et al. (2020), the $P_{\text{heterogeneity}}$ was significant ($P=0.002$).

In Hu et al. (2019), heterogeneity was moderate for total stroke ($I^2=136.2\%$, $P_{\text{heterogeneity}}$ 0.093), and low after excluding the REDUCE-IT study ($I^2=16.3\%$, $P_{\text{heterogeneity}}=0.383$).

In Campano et al. (2019), the heterogeneity was low for vascular-related death ($I^2=16\%$, $P_{\text{heterogeneity}}=0.86$), and low for recurrent events (fatal only) ischemic and hemorrhagic stroke ($I^2=0$, $P_{\text{heterogeneity}}=0.37$).

In Balk et al. (2016), the heterogeneity was low ($I^2=20.59\%$, $P_{\text{heterogeneity}}=0.414$).

5.2.7.3 Dose-response relationship LC n-3 FA intake and stroke incidence

In Hu et al. (2019), there is a significant linear dose-response relationship only between LC n-3 FA intake and stroke when the REDUCE-IT trial was included. Each 1000 mg/day intake of LC n-3 FA reduces the risk of stroke (RR 0.89, 95% CI: 0.82-0.98).

In Abdelhamid et al. (2020), meta-regression to assess effects of LC n-3 FA dose did not find any clear dose response on risk of stroke.

In Lombardi et al. (2020) they investigated low and high dose of LC n-3 FA, and there was no effect of either low or high dose of LC n-3 FA intake on stroke.

In Balk et al. (2016), they showed that for ischemic stroke that there may be a ceiling effect (where intake above a certain level adds no further benefit) but it is unclear where this threshold is. For hemorrhagic stroke, there were no apparent differences in association between marine oil intake dose and outcome at lower or higher dose ranges (Balk et al., 2016).

None of the other studies included dose-response curves for LC n-3 FA intake and stroke.

5.2.7.4 *Weight of evidence for LC n-3 FA intake and stroke incidence*

In this section, the evidence of the association between LC n-3 FA and stroke is weighed according to the WCRF criteria presented in the method chapter (Box 2 Chapter 3.1.6, but applied to evaluation of systematic reviews and meta-analyses).

Published evidence of LC n-3 FA intake and stroke incidence

Overall, none of the meta-analysis of RCTs found an overall protective effect of LC n-3 FA intake on stroke. The direction of the effects in all meta-analysis goes in both directions, and most goes to the direction of increased risk of stroke.

Heterogeneity

One meta-analysis reported significant heterogeneity (Casula et al., 2020). Otherwise, no significant heterogeneity was observed in the included meta-analyses.

Mechanisms/biological plausibility

Plausible mechanisms are presented above, see Chapter 5.2.

Upgrading factors

One meta-analysis (Hu et al. (2019) showed a dose-response relationship between LC n-3 FA and stroke only when REDUCE-IT is included in the meta-analysis.

Conclusion weight of evidence LC n-3 FA intake and stroke incidence

None of the meta-analysis of RCTs reported an effect of LC n-3 FA intake on risk of stroke. An inverse association was observed in the prospective cohort studies. Plausible mechanisms exist, and no unexplained heterogeneity was observed. This leads to the conclusion that the evidence that intake of LC n-3 FA protects against stroke is graded "limited, no conclusion".

5.2.8 LC n-3 FA and atrial fibrillation

5.2.8.1 *Results from the meta-analyses for LC n-3 FA intake and atrial fibrillation*

Below is a summary table for LC n-3 FA and atrial fibrillation (Table 5.2.8.1-1) based on the identified meta-analyses.

Table 5.2.8.1-1 Overview of results from meta-analyses included in the weight of evidence analysis of LC n-3 FA intake and atrial fibrillation.

Author, year	Study design, intake range, N	Total no studies	No of cases	Comparison	Summary RR/HR (95 % CI)	Heterogeneity	Overall results
Kow, 2021	RCT EPA+DHA: 0.84-4 g/d n=75 120	6	1135 treat- meant vs 918 CTR	Placebo/control	Rate ratio 1.31 (1.13-1.51)	$I^2=56\%$	LC n-3 FA significantly increases risk of atrial fibrillation. Subgroup analysis stratified by dose, both low and high dose were similarly associated with a significant increased risk
Abdelhamid, 2020	RCT EPA+DHA: 0.5-5 g/d n=77 990	30	2380 high intake vs 2206 low intake	Placebo or open label	RR 0.99 (0.92-1.06)	$I^2=44.37\%$	LC n-3 FA have no effect on arrhythmia*
Lombardi, 2020	RCT EPA+DHA: 0.4-4 g/d n=128 051	14	-	Placebo or open labelled	Incidence rate ratio 1.35 (1.1-1.66) high dose versus control 1.10 (0.97-1.25) low dose versus control	$I^2 < 50\%$, $P > 0.05$	High dose LC n-3 FA increases risk of atrial fibrillation events ($P=0.004$)
Jiang, 2017	RCT n=1268	4	-	Placebo or open labelled	HR 1.13 (0.96-1.33)	$I^2=0\%$	LC n-3 FA have no effect on atrial fibrillation recurrence.
Li, 2017	Prospective cohort studies	6		Highest versus lowest intake	RR 1.03 (0.97-1.09)	$I^2=0\%$	No association between intake of LC n-3 FA and risk of atrial fibrillation. This null association persisted in subgroup and dose-response analysis

* Fatal or nonfatal, new or recurrent arrhythmia, including atrial fibrillation, ventricular tachycardia and ventricular fibrillation.

Overall, two of the four meta-analyses of RCTs found an increased risk of LC n-3 FA intake on atrial fibrillation. Two of the meta-analyses of RCTs found no effect in intake of LC n-3 FA on risk of atrial fibrillation. One meta-analysis included prospective cohort studies, and they found no association between intake of LC n-3 FA on atrial fibrillation.

Kow et al. (2021) included 6 RCTs, and based on rate ratio and 95% CI, shows that LC n-3 FA increase the risk of atrial fibrillation. Among the trials, all showed an increased risk of atrial fibrillation, and three of them which are recent trials show a significant increased risk (STRENGTH 2020, OMEMI 2020, and REDUCE-IT 2019). Three of the trials overlapped with Abdelhamid et al. (2020),

Abdelhamid et al. (2020) included 30 RCTs, and based on RR and 95% CI, shows no effect of LC n-3 FA intake and arrhythmia. Among the trials, 13 show a protective effect, and two of them show significant protective effect. Most of the studies show increased risk (17 in total), but none of them show significant increased risk. The REDUCE-IT study was also included here but the number of events reported is not exactly the same as in Kow et al. (2021). Three of the studies overlapped with Kow et al. (2021), none of the studies overlapped with Jiang et al. (2017), and 10 overlapped with Lombardi et al. (2020). This meta-analysis includes many trials with different sample sizes, doses, and durations. The authors of the meta-analysis state that there may be some harm associated with increasing LC n-3 FA intake on arrhythmia risk, and fatal arrhythmia, particularly in the longer term and in primary prevention. GRADE assessment suggested low-certainty evidence that LC n-3 FA intake may slightly increase the risk of arrhythmia (downgraded once for risk of bias and once for imprecision).

Lombardi et al. (2020) included 14 RCTs and based on incidence rate ratio (IRR) and 95% CI, LC n-3 FA increases the risk of atrial fibrillation. Comparing high dose LC n-3 FA supplementation (>1 g per day) studies (n=4) with control, shows significant increased risk of atrial fibrillation (IRR 1.35). Comparing low dose LC-n-3 FA supplementation (≤1 g per day) studies (n=10) with control, shows no protective effect (IRR 1.10). Thus, there is an increased risk of LC n-3 FA intake on atrial fibrillation, and higher doses increases the risk. Three of the trials overlapped with Kow et al. (2021), none overlapped with Jiang et al. (2017), and 10 trials overlapped with Abdelhamid et al. (2020).

Jiang et al. (2017) included 4 RCTs, and based on HR shows and 95% CI, shows that LC n-3 FA intake have no protective effect on preventing recurrence of atrial fibrillation, rather a non-significant increased risk was observed. Among the trials, all show increased risk, but none of them show significant increased risk. None of the trials overlapped with Kow et al. (2021), Abdelhamid et al. (2020), and Lombardi et al. (2020).

Li et al. (2017) included six prospective cohort studies, and based on RR and 95% CI, shows no association between intake of LC n-3 FA and atrial fibrillation. Among the cohort studies, five show an increased risk of atrial fibrillation, but none of them show significant increased risk. None of the studies show significant protective effect. Heterogeneity LC n-3 FA intake and atrial fibrillation

5.2.8.2 Heterogeneity LC n-3 FA intake and atrial fibrillation

The three most recent meta-analyses all showed some heterogeneity between studies. In Abdelhamid et al. (2020), heterogeneity was moderate ($I^2=44.37\%$, $P_{\text{heterogeneity}}=0.7$). Similarly, in Lombardi et al. (2020), heterogeneity (I^2) was moderate ($I^2 < 50\%$, $P\text{-value} > 0.05$). In Kow et al. (2021), heterogeneity was also moderate, ($I^2=56\%$).

In Li et al. (2017) and Jiang et al. (2017) there was no heterogeneity ($I^2=0\%$ for both).

5.2.8.3 Dose-response relationship LC n-3 FA intake and atrial fibrillation

In Kow et al. (2021), no dose-response relationship was observed. In Li et al. (2017), they showed a marginally significant nonlinear relationship between LC n-3 FA intake and AF.

Lombardi et al. (2020) showed that intake above 1 g per day of LC n-3 increases the risk of atrial fibrillation more than lower doses (below 1 g per day).

In Abdelhamid et al. (2020), overall, there was no effect of LC n-3 FA dose.

5.2.8.4 Weight of evidence for LC n-3 FA intake and atrial fibrillation

In this section, the evidence of the association between LC n-3 FA and atrial fibrillation is weighed according to the WCRF criteria presented in the method chapter (Box 2 in Chapter 3.1.6, but applied to evaluation of systematic reviews and meta-analyses).

Published evidence of LC n-3 FA intake and atrial fibrillation

Overall, two of the four meta-analyses of RCTs found an overall increased risk of LC n-3 FA intake on atrial fibrillation (Kow et al. 2021, Lombardi et al. (2020). Most of the trials included in these meta-analyses show increased risk and three recent new trials also show significant increased risk of atrial fibrillation. Two of the meta-analyses of RCTs found no effect in intake of LC n-3 FA on risk of atrial fibrillation, but in Abdelhamid et al. (2020), 17 out of 30 trials show increased risk, and in Jiang et al. (2017), all trials show increased risk. One meta-analysis included prospective cohort studies, and they found no association between intake of LC n-3 FA on atrial fibrillation, but also here the direction was towards increased risk in the majority of studies (five out of six).

Heterogeneity

The heterogeneity was moderate in two of the meta-analyses included (Abdelhamid et al. 2020 and Kow et al. 2021), but it was not significant.

Mechanisms/biological plausibility

No plausible mechanisms are presented.

Upgrading factors

Li et al. (2017) reported that among five of the original studies in the meta-analysis, a marginally significant association between LC n-3 FA intake and risk of atrial fibrillation for each 0.3 g/day increments in intake of LC n-3 FA.

Conclusion weight of evidence LC n-3 FA intake and atrial fibrillation

None of the four meta-analyses of RCTs found a protective effect of LC n-3 FA intake on risk of atrial fibrillation. On the contrary, two of the meta-analyses show an increased risk of atrial fibrillation overall. The direction of effects in all meta-analysis of both RCTs and cohorts is frequently toward increased risk, but with some heterogeneity. LC n-3 FA may have an adverse effect on new onset and/or recurrence of atrial fibrillation, but the mechanism remains unknown. This led to the conclusion that there is "limited, suggestive" evidence for an adverse effect of LC n-3 FA on the risk of atrial fibrillation.

5.2.9 LC n-3 FA and all-cause mortality

5.2.9.1 *Results from the meta-analyses for LC n-3 FA intake and all-cause mortality*

Below is a summary table for LC n-3 FA and all-cause mortality, which also includes all-cause death and total death (Table 5.2.9.1-1) based on the identified meta-analyses.

Table 5.2.9.1-1 Overview of results from meta-analyses included in the weight of evidence analysis of LC n-3 FA intake and all-cause-mortality.

Author	Study design, intake range, N	Total no studies	No of cases	Comparison	Summary RR/HR (95 % CI)	Heterogeneity	Overall results
Rizos, 2021	RCT EPA+DHA: 0.27-6 g/d n=72 360	4	-	Placebo or open labelled	<1 capsule/day=<0.84 g RR 1.0, (0.85-1.19)	$I^2=0\%$	No effect of LC n-3 FA on all-cause-mortality
		7			1 capsule/day=0.84-1.68 g RR 0.99 (0.94-1.04)	$I^2=9\%$	
		2			2 capsules/day=1.68-2.52 g RR 0.58 (0.31-1.07)	$I^2=0\%$	
		3			>3 capsules/day=>2.52 g RR 0.89 (0.76-1.03)	$I^2=0\%$	
Abdelhamid, 2020	RCT EPA+DHA: 0.5-5 g/d n=143 693	45	5569 higher intake vs 5728 lower intake	Placebo or open labelled	RR 0.97 (0.93 to 1.01)	$I^2=5.31\%$	No effect of LC n-3 FA on all-cause-mortality
Casula, 2020	RCT EPA+DHA: 1-6 g/day n=81 073	16	3667 treatment vs 3803 CTR	Placebo or open labelled	OR 0.96 (0.88-1.04)	$P=0.03$	No effect of LC n-3 FA and all-cause-mortality
Lombardi, 2020	RCT EPA+DHA: 0.4-4 g/d n=125 051	14	-	Placebo or open labelled	Incidence rate ratio 0.95 (0.85-1.06) high dose versus control Incidence ratio 0.98 (0.94-1.02) low dose versus control	$I^2<50\%$, $P>0.05$	No effect of LC n-3 FA on all-cause-mortality ($P=0.38$)
Balk, 2016	RCT EPA+DHA: 0.3-3.46 g/d n=80 727	17	4149 treatment vs 4331 CTR	Placebo or open labelled	HR=0.97 (0.92-1.03)	$I^2=23.74\%$	No effect of LC n-3 FA and all-cause-mortality

Author	Study design, intake range, N	Total no studies	No of cases	Comparison	Summary RR/HR (95 % CI)	Heterogeneity	Overall results
	Prospective cohort studies (n=100 767)			0.066 to 1.58 g/d	Effect size 0.62 (0.31- 1.25)		No association between LC n-3 FA intake (including from fish) and all-cause-mortality

Overall, none of the five meta-analyses of RCTs, and the meta-analysis of cohort studies found an association between LC n-3 FA intake and all-cause-mortality.

Rizos et al. (2021) included 16 RCTs with duration of more than one year and reported 7227 deaths among 83 286 participants. They included four studies which supplemented participants with less than 1 capsule per day (<0.84 g EPA+DHA per day), seven studies which supplemented participants with 1 capsule per day (0.84 - 1.68 g EPA+DHA per day), two studies which supplemented participants with 2 capsules per day (1.68 - 2.52 g EPA+DHA per day), and three studies which supplemented participants (two secondary prevention trials and one mix of primary and secondary prevention trial) with more than 3 capsules per day (>2.52 g EPA+DHA per day). No protective effect of LC n-3 FA intake on all-cause-mortality was observed for any of the doses, but the studies with the largest doses (2 capsules per day or more) show an overall decreased risk, but this is not significant. The meta-analysis included the two of the latest large RCTs performed (VITAL and REDUCE-IT). In addition, ten RCTs overlapped with Lombardi et al. (2020), nine overlapped with Balk et al. (2016), ten overlapped with Casula et al. (2020), but these trials were not all the same. All overlapped with Abdelhamid et al. (2020).

Abdelhamid et al. (2020) included 45 RCTs with 11297 deaths in more than 143 000 participants. The overall effect, based on RR and 95% CI, show no protective effect of LC n-3 FA on all-cause mortality. Among the 45 trials, 26 show decreased risk, and two of these shows significant decreased risk of all-cause mortality (DART and GISSI-P). One trial show significant increased risk of all-cause mortality (DART2). The meta-analysis included the three of the latest large RCTs performed (VITAL, REDUCE-IT and ASCEND). In addition, one study included supplemented food, and two studies used advice to increase intake of LC n-3 FA (DART and DART2), and several smaller studies with few events were included. Since all studies have been weighted, and up-and downgraded based on risk of bias and other criteria by the authors of the meta-analysis, GRADE assessment suggests that the finding of little or no effect of LC n-3 FA on all-cause mortality was supported by high-certainty evidence (not downgraded).

Lombardi et al. (2020) included 14 RCTs, and when they compared high dose LC n-3 FA supplementation (>1 g per day) studies ($n=4$) with control, and low dose LC-n-3 FA supplementation (≤ 1 g per day) studies ($n=10$) with control, the IRR and 95% CI show that there is no protective effect of both low and high dose of LC n-3 FA intake on all-cause mortality. The meta-analysis included the three of the latest large RCTs performed (VITAL, REDUCE-IT and ASCEND). In addition, ten studies overlapped with Rizos et al. (2021), eight overlapped with Casula et al. (2020), and ten overlapped with Balk et al. (2016). All overlapped with Abdelhamid et al. (2020).

Casula et al. (2020) included 16 RCTs, and the overall effect, based on RR and 95% CI shows no protective effect of LC n-3 FA intake on all-cause mortality. Among the trials, 10 shows a protective effect, and two of these shows significant reduced effect (GISSI-P and IEIS-4). No studies show significant increased effect. The meta-analysis included one of the latest large RCTs performed (REDUCE-IT). In addition, ten overlapped with Rizos et al.

(2021), eight overlapped with Casula et al. (2020), nine overlapped with Balk et al. (2016), and 12 overlapped with Abdelhamid et al. (2020).

Balk et al. (2016) included 17 RCTs, and the overall effect, based on HR and 95% CI shows no effects of LC n-3 FA and all-cause mortality. Among the trials, eight shows a protective effect, and two of these are significant (GISSI and GISSI-HF). They also included prospective cohort studies with more than 100 000 individuals and based on overall effect size there was no association between marine oil intake and all-cause mortality. In addition, nine overlapped with Casula et al. (2020) and Rizos et al. (2021), 14 overlapped with Abdelhamid et al. (2020), and ten overlapped with Lombardi et al. (2020).

5.2.9.2 Heterogeneity LC n-3 FA intake and all-cause mortality

In Rizos et al. (2021), heterogeneity was low ($I^2=0\%$ and $I^2=9\%$ depending on dose, no reported $P_{\text{heterogeneity}}$ value).

In Abdelhamid et al. (2020), heterogeneity was low ($I^2=5.31\%$, $P_{\text{heterogeneity}}=0.37$) for all-cause mortality.

In Lombardi et al. (2020), heterogeneity was moderate ($I^2=50\%$, no reported $P_{\text{heterogeneity}}$ value) for total death.

In Casula et al. (2020), the $P_{\text{heterogeneity}}$ was significant for all-cause mortality ($P_{\text{heterogeneity}}=0.03$, no reported I^2 value). In subgroup analysis by type of prevention (secondary or mix), no apparent effect modification was found by prevention status among participants in RCTs regarding the risk of all-cause mortality.

In Balk et al. (2016), heterogeneity was low ($I^2=23.74\%$, $P_{\text{heterogeneity}}=0.137$) for all-cause mortality.

5.2.9.3 Dose-response relationship LC n-3 FA intake and all-cause mortality

For all-cause-mortality, there may be a ceiling effect at about 0.2 g LC n-3 FA per day. Increasing marine oil intakes up to this level may be associated with lower all-cause-mortality but increasing intake above this level may not be associated with further decreased risk (Balk et al., 2016).

Abdelhamid et al. (2020) found no significant effect of LC n-3 FA dose in pre-planned subgrouping. They did not perform meta-regression since there was no suggestion of any effect of LC n-3 FA on all-cause mortality.

No protective effect of LC n-3 FA intake on all-cause-mortality was observed for any of the doses (Rizos 2021), and Lombardi et al (2020) did not find any protective effect of both low and high dose of LC n-3 FA intake on all-cause mortality

5.2.9.4 *Weight of evidence for LC n-3 FA intake and all-cause mortality*

In this section the evidence of the association between LC n-3 FA and all-cause mortality is weighed according to the WCRF criteria presented in the method chapter (Box 2 in Chapter 3.1.6, but applied to evaluation of systematic reviews and meta-analyses).

Published evidence of LC n-3 FA intake and all-cause mortality

Overall, none of the five meta-analyses of RCTs, and the meta-analysis of cohort studies found an association between LC n-3 FA intake and all-cause-mortality. All three meta-analysis of RCTs had very similar RR and 95% CI.

Heterogeneity

One meta-analysis reported significant heterogeneity (Casula et al., 2020). Otherwise, no significant heterogeneity was observed in the included meta-analyses.

Mechanisms/biological plausibility

Plausible mechanisms are presented for CVD and CHD mortality above, see Chapter 5.2, and these diseases are contributing to all-cause mortality.

Upgrading factors

Balk et al. (2016) found a possible ceiling effect at about 0.2 g LC n-3 FA per day. But they also state that marine oil intake above about 0.2 to 0.4 g/day may not further strengthen any association between higher marine oil intake and lower rate of all-cause death (Balk et al., 2016). None of the other meta-analysis found any dose-response effect.

No other upgrading factors have been evaluated.

Conclusion weight of evidence LC n-3 FA intake and all-cause mortality

None of the meta-analyses and systematic reviews of RCTs and prospective cohort studies showed that intake of LC n-3 FA protect against all-cause mortality.

In conclusion, the evidence that LC n-3 FA intake protects against all-cause mortality is "limited, no conclusion".

5.2.10 LC n-3 FA and neurodevelopment in children

The current chapter summarizes the epidemiological evidence of the association between LC n-3 FA intake and neurodevelopmental outcomes in children from systematic reviews and meta-analyses published between 2015 and 2020. We performed a systematic search for systematic reviews and meta-analyses (see Chapter 3.2.4 for details). The complete search strategies are given in Chapter 15, Appendix II.

Plausible mechanisms for LC n-3 FA influence on neurodevelopment in children is described in Chapter 5.2.

Nine systematic reviews and/or meta-analyses relevant for LC n-3 FA and neurodevelopment in children were read as full papers. Four of these were included to fill in knowledge about the association between LC n-3 FA and neurodevelopmental outcome in children, and five were excluded (see Table 5.2.10-1 for reasons for exclusions).

Table 5.2.10-1 Included papers and reasons for exclusion of identified papers from the search of systematic reviews and meta-analysis of LC n-3 FA intake and the neurodevelopmental outcome in children, 2015-2020.

Included papers	Excluded paper and reasons for exclusions
Emery et al., 2020 Shulkin et al., 2018 Taylor et al., 2017 Quin et al., 2016	The following were evaluated as quality C: Derbyshire et al., 2018: Study selection and data extraction not in duplicate, quality assessment not used properly, heterogeneity not assessed properly. Freedman et al., 2018: Study selection and data extraction not in duplicate, no quality assessment of included papers, heterogeneity not assessed properly. Rangel-Huerta et al., 2017: Study selection and data extraction not in duplicate, no quality assessment of included papers, heterogeneity not assessed properly. Gajos et al. 2016: Study selection and data extraction not in duplicate, no quality assessment of included papers. The following was excluded for other reasons: Ottolini et al., 2020: LC n-3 FA only part of other exposure (nutrition).

The meta-analyses are described in more detail below; first, there are main descriptions of the methods used and then main/selected results from each review.

Meta-analyses for child supplementation/intake

Emery et al. (2020) is a meta-analysis of randomized controlled trials (RCTs) investigating the effect of LC n-3 FA supplementation on cognitive test performance in children and young adults up to 25 years. The authors performed two independent systematic literature searches in PubMed, Cochrane Library, PsycARTICLES and PsycINFO (last search June 2019). Studies with intervention products containing EPA and/or DHA, including fish but no other food products were included. Only studies assessing cognitive domains through standardized tests were included. Studies on both healthy subjects and subjects with psychiatric disorders were included. Studies including subjects with severe mental dysfunctions and studies in which infants were supplemented through maternal intake were excluded. Studies supplementing arachidonic acid in the experimental compared to control group were also excluded. The quality of the eligible publications included in the meta-analysis was assessed using the Cochrane risk of bias criteria resulting in 29 publications looking into LC n-3 FA from supplementation and cognitive domains in children, four of the studies were rated low across all bias risks. Overall, the risk of bias was considered to be moderate. Overall, the 29 studies included 4,247 participants, ranging in age from birth to 20.43 years. The participants were treated with either the intervention product or the control product for four to 48 weeks. Thirteen studies used more EPA than DHA in their interventions, and 17 studies used more DHA than EPA in their interventions. One study

created two intervention groups, where one received more DHA and the other more EPA. Doses of DHA range from 84 mg to 1200 mg and for EPA from 56 mg to 1109 mg.

Meta-analyses for maternal supplementation/intake

Taylor et al. (2017) is a meta-analysis and systematic review of RCTs investigating the effect of nutritional interventions during pregnancy, on cognitive and visual development in infants and children up to age 18 years. The authors performed a systematic literature search in MEDLINE, Pre-Medline, Embase, PsychINFO and Maternity and Infant Care via Ovid, Scopus, Proquest, Web of Science and Cumulative Index to Nursing and Allied Health Literature via EBSCO. RCTs and pseudo-RCTs of any date in pregnant women with singleton pregnancy, of any age and ethnicity, with nutritional interventions and cognitive measures by cognitive assessment tests in the children were included. The types of nutritional interventions included nutrient supplements, whole foods, fortified foods, and nutrition education. The quality of the eligible publications included in the meta-analysis and systematic review was assessed by the American Dietetic Association (ADA) Quality Criteria Checklist for Primary Research. No studies were given a negative rating in this review and therefore no studies were excluded. A total of 34 publications were included in the meta-analyses, of these 14 investigated the effect of LC n-3 FA intake during pregnancy on nine cognitive outcomes (attention, behavior, crystallised intelligence, fluid intelligence, global cognition, memory, motor skills, visual processing, and problem solving) in infants and children up to age 18 years. Intervention included mainly fish or algal oil capsules compared to control with doses ranging from 80 mg to 2200 mg DHA and 38 mg to 1800 mg EPA.

Meta-analyses for maternal and child supplementation/intake

Shulkin et al. (2018) is a meta-analysis and systematic review of RCTs investigating the effect of LC n-3 FA supplementation during pregnancy, lactation or given to infants (up to two years), on cognitive and visual development in infants and children up to age 18 years. The authors performed a systematic literature search in PubMed, PsycINFO, Embase, the CochraneLibrary, and clinicaltrials.gov, without language restrictions, from the earliest indexing year through 14 April 2016. The exposure was supplementation with n-3 PUFAs, including DHA or EPA (as well as both), via supplements, fortified foods, or diet. The primary outcomes of interest were standardized measures of cognition and visual development in infants and children. Studies in generally healthy subjects were included. The quality of the eligible publications included in the meta-analysis and systematic review was assessed using the Cochrane risk of bias tool. A total of 38 publications years were included. The quality of the included papers was overall moderate. The mean (SD) supplementation duration was 21.8 (7.5) weeks for studies with maternal supplementation, 45.3 (14.5) in preterm infants and 37.2 (14.5) in infants born to term. The mean (SD) doses were 675 (547) mg/d DHA ranging from 200-2200 and 297 (512) mg/d EPA ranging from 0-1800 for maternal supplementation. For preterm infants, DHA, EPA, and arachidonic acid (AA) mean (SD) doses were 0.28 (0.13), 0.12 (0.21), and 0.34 (0.28) % FA (fatty acids) respectively, and in term infants 0.38 (0.22), 0.05 (0.14), and 0.40 (0.29) % FA, for DHA, EPA and AA respectively. Meta-analysis was done for Bayley Scales of Infant and Toddler Development, Mental Development Index (MDI) and Psychomotor development index (PDI), intelligence quotient

and visual acuity. Findings were evaluated and pooled across supplementation periods (studies by maternal (n=13), preterm (n=7), term infant (n=18)), and also explored stratified by supplementation period.

Quin et al. (2016) is a meta-analysis and systematic review of RCTs and semi-RCTs investigating the effect of LC n-3 FA supplementation maternally administered through breastmilk and in fortified formula given directly to the child on infant visual acuity, cardiovascular health, immunity, growth and neurodevelopment (language, cognition, and motor development) in infants. The authors performed a systematic literature search in Cochrane Central Register of Controlled Trials (CENTRAL), Embase, CINAHL, PubMed, Web of Science, MEDLINE. Studies on LC n-3 FA supplementation taken maternally during gestation, gestation and lactation, or lactation only, compared to a control group (placebo or no supplementation), and LC n-3 FA supplemented milk-based formula or capsule compared to a non-supplemented control were included. In total 32 publication involving infant formula and 37 involving breastmilk were included. The source of n-3 PUFA supplements were from fungal oils, fish oils, single-cell sources, or egg triglycerides. The duration of the intervention ranged from 11 weeks to 1 year. The quality of the eligible publications included in the meta-analysis and systematic review was assessed following the criteria outlined in the Cochrane Handbook for Systematic Reviews of Interventions, overall, the risk of bias was evaluated to be low.

5.2.10.1 *Results from the meta-analyses for LC n-3 FA intake and neurodevelopmental outcomes in children*

Below is a summary table for LC n-3 FA intake and neurodevelopmental outcome in children (Table 5.2.10.1-1) based on the identified meta-analyses.

Table 5.2.10.1-1 Overview of results from meta-analyses included in the weight of evidence analysis of LC n-3 FA intake and neurodevelopmental outcome from birth to young adults.

Author, year	LC n-3 FA intake, study design	Total no studies	Population	Comparison	Outcome measure	Summary estimates, standardized mean difference (95 % CI)	Heterogeneity	Overall conclusion
Emery, 2020, Intervention (suppl. or fish) containing EPA and/or DHA Dose range (mg/d): 84-1200 DHA and 56-110 EPA Age: birth-25 yrs	Child intake, RCTs	6	All	LC n-3 FA suppl vs control	Long-term memory (recall)	0.13 (-0.05, 0.32)	$I^2=0\%$, $P=0.65$	No main effect of LC n-3 FA supplementation on domain-specific cognitive test performance in youths.
	Child intake, RCTs	3	All	More DHA suppl than EPA vs control	Long-term memory (recall)	0.06 (-0.19, 0.32)	$I^2=0\%$, $P=0.98$	
	Child intake, RCTs	3	All	More EPA suppl than DHA vs control	Long-term memory (recall)	0.25 (-0.08, 0.58)	$I^2=24\%$, $P=0.27$	
	Child intake, RCTs	3	Non-clinical population	LC n-3 FA suppl vs control	Long-term memory (recall)	0.22 (-0.15, 0.58)	$I^2=0\%$, $P=0.42$	Subgroup analyses identified beneficial effects of EPA-rich but not DHA-rich formulations in the domain of working memory. In this domain, there was a beneficial effect in clinical rather than non-clinical populations
	Child intake, RCTs	3	Clinical population	LC n-3 FA suppl vs control	Long-term memory (recall)	0.10 (-0.12, 0.33)	$I^2=0\%$, $P=0.52$	
	Child intake, RCTs	7	All	LC n-3 FA suppl vs control	Working memory	0.12 (-0.05, 0.29)	$I^2=33\%$, $P=0.18$	
	Child intake, RCTs	4	All	More DHA than EPA suppl vs control	Working memory	0.01 (-0.13, 0.15)	$I^2=0\%$, $P=0.59$	
	Child intake, RCTs	3	All	More EPA than DHA suppl vs control	Working memory	0.36 (0.09, 0.63)	$I^2=0\%$, $P=0.39$	
	Child intake, RCTs	3	Non-clinical population	LC n-3 FA suppl vs control	Working memory	-0.07 (-0.26, 0.12)	$I^2=0\%$, $P=0.75$	
	Child intake, RCTs	3	Clinical population	LC n-3 FA suppl vs control	Working memory	0.23 (0.02, 0.45)	$I^2=28\%$, $P=0.24$	

Author, year	LC n-3 FA intake, study design	Total no studies	Population	Comparison	Outcome measure	Summary estimates, standardized mean difference (95 % CI)	Heterogeneity	Overall conclusion
	Child intake, RCTs	5	All	LC n-3 FA suppl vs control	Problem solving and visuospatial cognition	0.12 (-0.10, 0.34)	$I^2=51\%$, $P=0.08$	
	Child intake, RCTs	2	All	More DHA than EPA suppl vs control	Problem solving and visuospatial cognition	0.08 (-0.15, 0.32)	$I^2=18\%$, $P=0.27$	
	Child intake, RCTs	3	All	More EPA than DHA suppl vs control	Problem solving and visuospatial cognition	0.21 (-0.21, 0.64)	$I^2=71\%$, $P=0.03$	
	Child intake, RCTs	2	Non-clinical population	LC n-3 FA suppl vs control	Problem solving and visuospatial cognition	0.07 (-0.16, 0.30)	$I^2=29\%$, $P=0.24$	
	Child intake, RCTs	3	Clinical population	LC n-3 FA suppl vs control	Problem solving and visuospatial cognition	0.22 (-0.22, 0.65)	$I^2=70\%$, $P=0.04$	
	Child intake, RCTs	7	All	LC n-3 FA suppl vs control	Shifting and flexibility	0.12 (-0.08, 0.32)	$I^2=38\%$, $P=0.14$	
	Child intake, RCTs	2	Non-clinical population	LC n-3 FA suppl vs control	Shifting and flexibility	-0.02 (-0.22, 0.18)	$I^2=0\%$, $P=0.36$	
	Child intake, RCTs	5	Clinical population	LC n-3 FA suppl vs control	Shifting and flexibility	0.22 (-0.04, 0.48)	$I^2=36\%$, $P=0.18$	
	Child intake, RCTs	9	All	LC n-3 FA suppl vs control	Development	0.11 (-0.10, 0.23)	$I^2=2\%$, $P=0.42$	
	Child intake, RCTs	5	All	LC n-3 FA suppl vs control	Long-term memory (recognition)	0.11 (-0.12, 0.35)	$I^2=22\%$, $P=0.28$	
	Child intake, RCTs	7	All	LC n-3 FA suppl vs control	Processing speed	0.09 (-0.06, 0.24)	$I^2=0\%$, $P=0.97$	
	Child intake, RCTs	12	All	LC n-3 FA suppl vs control	Short-term memory	0.08 (-0.02, 0.17)	$I^2=0\%$, $P=0.92$	
	Child intake, RCTs	7	All	LC n-3 FA suppl vs control	Reading	0.07 (-0.09, 0.23)	$I^2=51\%$, $P=0.06$	

Author, year	LC n-3 FA intake, study design	Total no studies	Population	Comparison	Outcome measure	Summary estimates, standardized mean difference (95 % CI)	Heterogeneity	Overall conclusion
	Child intake, RCTs	3	All	LC n-3 FA suppl vs control	Spelling	0.05 (-0.12, 0.21)	$I^2=24\%$, $P=0.27$	
	Child intake, RCTs	13	All	LC n-3 FA suppl vs control	Attention	0.00 (-0.12, 0.12)	$I^2=0\%$, $P=0.54$	
	Child intake, RCTs	5	All	LC n-3 FA suppl vs control	IQ	0.00 (-0.14, 0.14)	$I^2=0\%$, $P=0.45$	
	Child intake, RCTs	14	All	LC n-3 FA suppl vs control	Inhibition	0.00 (-0.18, 0.19)	$I^2=37\%$, $P=0.08$	
	Child intake, RCTs	7	All	LC n-3 FA suppl vs control	Language	-0.07 (-0.25, 0.11)	$I^2=28\%$, $P=0.21$	
Shulkin, 2018	Maternal or child intake, RCT	21	All	LC n-3 FA intake vs placebo	Early child development, Bayley MDI	0.91 (0.00, 1.81)	$I^2=27\%$	LC n-3 FA intake improves childhood psychomotor and visual acuity, with potentially stronger effects with supplementation in preterm and term infants compared to maternal supplementation. Effects on early mental development is marginal, but an effect in preterm infants is suggested. No
Intervention: suppl, fortified foods, or diet	Maternal intake, RCTs	7	All	LC n-3 FA intake vs placebo	Early child development, Bayley MDI	-0.36 (-1.30, 0.58)	$I^2=0\%$, $P=0.58$	
	Child intake, RCTs	7	Preterm infants	LC n-3 FA intake vs placebo	Early child development, Bayley MDI	3.33 (0.72, 5.93)	$I^2=45\%$, $P=0.09$	
Mean (SD) doses were 675 (547) mg/d DHA and 297 (512) mg/d EPA for maternal supplementation. For preterm infants, DHA, EPA, and AA mean (SD) doses were 0.28 (0.13), 0.12 (0.21), and 0.34	Child intake, RCTs	18	Term infants	LC n-3 FA intake vs placebo	Early child development, Bayley MDI	0.99 (-0.26, 2.23)	$I^2=7.6\%$, $P=0.37$	
	Maternal or child intake, RCTs	21	All	LC n-3 FA intake vs placebo	Early child development, Bayley PDI	1.06 (0.10, 2.03)	$I^2=42.3\%$	
	Maternal intake, RCTs	7	All	LC n-3 FA intake vs placebo	Early child development, Bayley PDI	1.01 (-0.52, 2.55)	$I^2=48.5\%$, $P=0.07$	
	Child intake, RCTs	7	Preterm infants	LC n-3 FA intake vs placebo	Early child development, Bayley PDI	2.29 (-1.08, 5.67)	$I^2=58.8\%$, $P=0.02$	
	Child intake, RCTs	18	Term infants	LC n-3 FA intake vs placebo	Early child development, Bayley PDI	0.84 (-0.48, 2.16)	$I^2=34.8\%$, $P=0.07$	
	Maternal or child intake, RCTs	7	All	LC n-3 FA intake vs placebo	IQ	0.20 (-1.56, 1.96)	$I^2=0.0\%$	

Author, year	LC n-3 FA intake, study design	Total no studies	Population	Comparison	Outcome measure	Summary estimates, standardized mean difference (95 % CI)	Heterogeneity	Overall conclusion
(0.28)% FA, and in term infants 0.38 (0.22), 0.05 (0.14), and 0.40 (0.29)% FA	Maternal intake, RCTs	3	All	LC n-3 FA intake vs placebo	IQ	0.35 (-2.11, 2.81)	$I^2=0.0\%$, $P=0.95$	effect of LC n-3 FA on global IQ later in childhood
	Child intake, RCTs	5	Term infants	LC n-3 FA intake vs placebo	IQ	-0.37 (-4.24, 3.50)	$I^2=38.3\%$, $P=0.17$	
	Maternal or child intake, RCTs	24	All	LC n-3 FA intake vs placebo	Visual acuity	-0.06 (-0.08, -0.04)	$I^2=81.6\%$	
	Maternal intake, RCTs	9	All	LC n-3 FA intake vs placebo	Visual acuity	-0.02 (-0.04, 0.00)	$I^2=0.0\%$, $P=0.92$	
	Child intake, RCTs	6	Preterm infants	LC n-3 FA intake vs placebo	Visual acuity	-0.08 (-0.14, -0.01)	$I^2=67.1\%$, $P=0.01$	
	Child intake, RCTs	18	Term infants	LC n-3 FA intake vs placebo	Visual acuity	-0.08 (-0.11, -0.05)	$I^2=87.8\%$, $P<0.001$	
Taylor, 2017 Doses range: 80-2200 mg/d DHA and 38-1800 mg/d EPA Age range: from infancy to 18 years	Maternal intake, RCTs	2	955	LC n-3 FA suppl vs placebo	Attention	-0.07 (-0.17, 0.03)	$I^2=0.0\%$, $P=0.19$	No significant effect of LC n-3 FA interventions during pregnancy on child cognitive outcomes
	Maternal intake, RCTs	4	1725	LC n-3 FA suppl vs placebo	Behavior	-0.05 (-0.12, 0.03)	$I^2=0.0\%$, $P=0.83$	
	Maternal intake, RCTs	7	2265	LC n-3 FA suppl vs placebo	Motor skills	0.06 (-0.03, 0.15)	$I^2=8.9\%$, $P=0.22$	
	Maternal intake, RCTs	3	999	LC n-3 FA suppl vs placebo	Fluid intelligence	0.05 (-0.08, 0.18)	$I^2=10.1\%$, $P=0.45$	
	Maternal intake, RCTs	10	2632	LC n-3 FA suppl vs placebo	Global cognition	0.03 (-0.07, 0.13)	$I^2=21.3\%$, $P=0.247$	
	Maternal intake, RCTs	5	1941	LC n-3 FA suppl vs placebo	Crystallised intelligence	0.25 (-0.04, 0.53)	$I^2=87.8\%$, $P=0.09$	
Quin, 2016	Breastfed, RCTs	7	4553	LC n-3 FA suppl vs placebo	Visual acuity	0.011 (-0.016, 0.128)	$I^2=50.88\%$, $P=0.06$	LC n-3 FA supplementation in infants through breastmilk and
	Formula fed, RCTs	4		LC n-3 FA suppl vs placebo	Visual acuity	-0.041 (-0.498, 0.416)	$I^2=84.12\%$, $P=0.00$	

Author, year	LC n-3 FA intake, study design	Total no studies	Population	Comparison	Outcome measure	Summary estimates, standardized mean difference (95 % CI)	Heterogeneity	Overall conclusion
Age range: infancy to 8 years	Breastfed, RCTs	7		LC n-3 FA suppl vs placebo	Motor	0.098 (-0.024, 0.220)	$I^2=42.85\%$, $P=0.11$	formula does not improve visual acuity, language development, or cognition
	Formula fed, RCTs	8		LC n-3 FA suppl vs placebo	Motor	0.055 (-0.086, 0.196)	$I^2=76.38\%$, $P=0.00$	
	Breastfed, RCTs	5		LC n-3 FA suppl vs placebo	Language	-0.076 (-0.168, 0.015)	$I^2=0.0\%$, $P=0.42$	
	Formula fed, RCTs	5		LC n-3 FA suppl vs placebo	Language	0.012 (-0.165, 0.190)	$I^2=85.10\%$, $P=0.00$	
	Breastfed, RCTs	3		LC n-3 FA suppl vs placebo	Behavior	0.070 (-0.016, 0.155)	$I^2=0.0\%$, $P=0.66$	
	Formula fed, RCTs	3		LC n-3 FA suppl vs placebo	Behavior	0.020 (-0.061, 0.100)	$I^2=0.0\%$, $P=0.72$	
	Breastfed, RCTs	8		LC n-3 FA suppl vs placebo	Cognition	-0.001 (-0.070, 0.069)	$I^2=0.0\%$, $P=0.62$	
	Formula fed, RCTs	9		LC n-3 FA suppl vs placebo	Cognition	0.012 (-0.092, 0.117)	$I^2=41.78\%$, $P=0.09$	

Overall, the four meta-analyses showed negligible effects of LC n-3 FA-supplementation both during pregnancy and given directly to the child on visual and neurodevelopmental outcomes in children.

Emery et al. (2020) demonstrated no overall effect of LC n-3 FA-supplements to children on the cognitive domains, but an effect of EPA and an effect in clinical populations on working memory. In working memory, shifting and flexibility, and problem solving, clinical populations rather than non-clinical populations benefitted from the intervention, indicating a greater effectiveness of the intervention, although these associations were not statistically significant. Non-clinical participants benefitted more than clinical participants in the domain of long-term memory (recall) (also non-significant).

Taylor et al. (2017) showed no significant impact of LC n-3 FA-supplementation during pregnancy on child development. LC n-3 FA-supplementation may marginally improve child crystallized intelligence, however, the effect was not statistically significant and study heterogeneity among the trials was significant.

Shulkin et al. (2018) suggested that LC n-3 FA intake from supplements, fortified foods or diet significantly improved psychomotor and visual development overall, with a tendency of stronger effects with supplementation to term and preterm infants than in mothers. The effect on mental development is marginal overall, but with an effect in preterm infants. In subgroup analyses, there were no statistically significant differences in any findings according to world region, race, maternal education, age at outcome assessment, supplementation duration, DHA or EPA dose, DHA:AA ratio, or study quality score (P -interaction >0.05 each).

In Quin et al. (2016) there were no improvements from the LC n-3 FA supplements through breastmilk or formula on visual acuity, language development or cognition. Some aspects of motor development were significantly reduced in breastfed infants receiving LC n-3 FA, these results highlighted by the authors are based on one subgroup analysis within one of the included studies.

5.2.10.2 *Heterogeneity LC n-3 FA intake and neurodevelopmental outcomes in children*

In Emery et al. (2020) significant heterogeneity was found for the cognitive outcomes problem solving, visuospatial cognition and reading. In sensitivity analysis, the heterogeneity dropped and were no longer significant when removing two studies (i.e., Portillo-Reyes et al. (2014) for problem solving and visuospatial cognition, and Crippa et al. (2019) from reading) suggesting that these specific study data contributed to the heterogeneity in the analysis. Analyses in the remaining datasets did not alter the conclusions about the lack of effect for the two domains.

In Taylor et al. (2017), heterogeneity was low and insignificant for all except one outcome. For crystallized intelligence, observed heterogeneity was high and significant. There was no

evidence of publication bias and from the sensitivity analysis, the country-income of the studies did not significantly ($P > 0.05$) affect child crystallized intelligence.

In Shulkin et al. (2018), heterogeneity was low to moderate in analysis with the Bayley Scales of Infant and Toddlers Development as outcome. Results were not altered when excluding two studies using a different version of the scale, or by excluding one trial in infants diagnosed with phenylketonuria. In analysis with IQ as outcome, the heterogeneity was low, and with visual acuity observed heterogeneity was high. Post hoc analyses suggest that the mode of visual acuity assessment is of importance with less heterogeneity in trials using visual evoked potential as a measure compared to behavioral visual acuity measures.

Quin et al. (2016) stated that heterogeneity is expected when using random effects models and present heterogeneity measures (included in Table 2) in a supplementary table showing significant heterogeneity for three outcomes (visual acuity, motor, and language development). Heterogeneity was not addressed other than this is in the meta-analysis.

5.2.10.3 *Dose-response relationship LC n-3 FA intake and neurodevelopmental outcomes in children*

Emery et al. (2020) investigated dose trends first by post exclusion of studies with a daily dose smaller than 400 mg EPA and DHA combined, revealing a small effect size in the domains of both recall and recognition long term memory with satisfactory heterogeneity.

Shulkin et al. (2018), Taylor et al. (2018) and Quin et al. (2016) did not assess dose-response relationships.

5.2.10.4 *Weight of evidence for LC n-3 FA intake and neurodevelopmental outcomes in children*

In this section, the evidence of the association between LC n-3 FA and neurodevelopment in children is weighed according to the WCRF criteria presented in the method chapter (Box 2, in Chapter 3.1.6, but applied to evaluation of systematic reviews and meta-analyses).

Published evidence of LC n-3 FA intake and neurodevelopment in children

Overall, the four meta-analyses and systematic reviews showed no or only small effects of both maternal and child LC n-3 FA supplementation (different sources such as fish or algal oil, fortified food, and diet) on the neurodevelopmental outcome. Two meta-analyses showed no effect of maternal LC n-3 FA-supplementation (mainly fish and algal oil) (Taylor et al. 2017) and from LC n-3 FA-supplementation through breastmilk or formula (fungal oils, fish oils, single-cell sources, or egg triglycerides) (Quin et al., 2016). One meta-analysis on child LC n-3 FA-supplementation (intervention products containing DHA and/or EPA) showed an effect of EPA and an effect in clinical populations in only one of 14 included cognitive domains (working memory) (Emery et al., 2020), and one suggested an effect on psychomotor, mental and visual development in infants with the strongest effects with child

supplementation of LC n-3 FA (supplementation, fortified foods or diet) rather than maternal supplementation (Shulkin et al., 2018).

Heterogeneity

Some unexplained heterogeneities were documented for some of the neurodevelopmental outcomes.

Mechanisms/biological plausibility

Plausible mechanisms have been presented above, see Chapter 5.2.

Upgrading factors

No upgrading factors have been evaluated.

There are no dose-response relationships described between LC n-3 FA and neurodevelopment in children

Conclusion weight of evidence LC n-3 FA intake and neurodevelopmental outcomes in children

Results from the four meta-analyses and systematic reviews of RCTs showed no or marginal effect of maternal or child LC n-3 FA intake from sources such as fish or algal oil, fortified food, and diet on child neurodevelopment. Some unexplained heterogeneity was observed. In conclusion, the evidence that intake of LC n-3 FA is beneficial for child neurodevelopment is "limited, no conclusion".

5.2.11 LC n-3 FA and cognition and cognitive decline in adults

The current chapter summarizes the epidemiological evidence of LC n-3 FA intake and cognitive function in adults from systematic reviews and meta-analyses published between 2015 and 2020. We performed a systematic search for systematic reviews and meta-analyses (see Chapter 3.2.4 for details). The complete search strategies are given in Chapter 15, Appendix II.

Plausible mechanisms for LC n-3 FA influence on cognition and cognitive decline in adults is described in Chapter 5.2.

Eighteen papers were identified as relevant for LC n-3 FA intake, cognition, and cognitive decline in adults, and were read in full text. Six of these were included to fill in knowledge about the association between LC n-3 FA and cognitive function in adults, and 12 were excluded (see Table 5.2.11-1 for reasons for exclusions).

Table 5.2.11-1 Included papers and reasons for exclusion of identified papers from the search of systematic reviews and meta-analysis of LC n-3 FA intake and cognitive function in adults, 2015-2020.

Included papers	Excluded paper and reasons for exclusions
Balachandar et al., 2020 Zhang et al., 2020 Alex et al., 2019 Teo et al., 2017 Zhang et al., 2016a Forbes et al., 2015	The following were evaluated as quality C: Avallone et al., 2019: Study characteristics not provided, no quality assessment of included papers, heterogeneity not assessed properly. Marti et al., 2019: Study selection and data extraction not in duplicate, no quality assessment, heterogeneity not assessed properly. Butler et al., 2018: Study characteristics not provided, quality assessment not used properly. D'Cunha et al., 2018: Study selection and data extraction not in duplicate, no quality assessment, heterogeneity not assessed properly. McGrattan et al., 2018: Study selection and data extraction not in duplicate. Solfrizzi et al., 2018: No quality assessment of the included studies. Masana et al., 2017: Heterogeneity not assessed properly, LC n-3 FA not assessed as single exposure. Yorko-Mauro et al., 2015: No quality assessment of the included studies. Zhang et al., 2016b: The study was not adequate as meta-analysis or as a systematic review. The following were excluded for other reasons: Brainard et al., 2020: Includes studies in populations with mixed comorbidities. No intake and supplement doses given. Cooper et al., 2015: Includes studies in populations with cognitive disorders. Wu et al., 2015: Includes only overlapping studies with Zhang et al. (2016a).

The included meta-analyses are described in more detail below; first, main descriptions of the methods used and then main/selected results from each review.

Meta-analyses cognition

Alex et al. (2019) is a systematic review and meta-analysis of RCTs investigating the effect of LC n-3 FA supplementation on cognitive decline in non-demented adults. The authors performed a systematic literature search in Cochrane Library, Embase, CINAHL, Scopus, and MEDLINE databases from January 1980 until January 2019. The Jadad scale was used to assess the quality of the eligible studies. Twenty-five RCTs examining LC n-3 FA supplements (range 180-1120 mg/day DHA and 0-2187 mg/day EPA) and cognitive function were included in the meta-analysis. The quality was high in 19 of the included studies, while it was low in five of the studies. The outcome was the change in global cognitive function measured using the global cognition score from the Mini-Mental State Examination score (Folstein et al., 1975).

Systematic review cognition

Teo et al. (2017) is a systematic review of RCTs investigating the effect of LC n-3 FA supplements on cognitive brain function in both military and civilian adult population. The authors performed a systematic literature search in PubMed, CINAHL, EMBASE, PsychINFO, and Cochrane Library (Clinical Trials) databases until January 2014. The quality of the eligible papers included in the systematic review was assessed by Scottish Intercollegiate

Guidelines Network (SIGN) 50 Checklist (Healthcare Improvement Scotland, 2014). Thirteen RCTs examining LC n-3 FA intake and cognition were included. Of these, one was of high quality, while eight were of acceptable and four of low quality. The examined cognitive measures were memory, verbal fluency, attention and vigilance, simple and complex reaction time, psychomotor performance, and problem solving and reasoning.

Meta analyses cognitive decline

Balachandar et al. (2020) is a systematic review and meta-analysis of RCTs investigating the effect of DHA supplementation on age-related cognitive decline in elderly subjects without cognitive decline. The authors performed a systematic literature search in PubMed, Scopus, Cochrane Library, ProQuest, and Embase databases until June 2018. Ten RCTs examining LC n-3 FA intake (range 176-900 mg/day DHA and 0-2188 mg/day EPA) and cognitive decline were included in the meta-analysis. The risk of bias was partly low and partly unclear in nine of the included studies, while it was partly high, partly unclear and partly low in one of the studies. Memory, executive function, working memory, attention, and processing speed were endpoints in the meta-analysis.

Zhang et al. (2020) is a meta-analysis of RCTs investigating the effect of LC n-3 FA supplementation on cognitive impairment in mild cognitive decline adults aged 60 years or older. The authors performed a systematic literature search in Google Scholar, Embase, and PubMed databases from 2008 until December 12, 2018. The quality of the eligible papers was assessed by Heyland methodological quality score. Seven RCTs examining LC n-3 FA intake and cognitive impairment were included in the meta-analysis. The quality was high in all included papers. The endpoint was Mini-Mental State Examination Score.

Zhang et al. (2016) is a systematic review and meta-analysis of cohort studies investigating the association between total dietary EPA and DHA intake and risk of cognitive impairment conditions spanning from mild impairment to severe diseases in the general population. The authors performed a systematic literature search in PubMed, Embase, and Cochrane Library databases until May 2015. The quality of the eligible studies was evaluated with the Newcastle-Ottawa quality-assessment scale. Twelve papers examining EPA or DHA intake and cognitive impairment conditions were included in the meta-analysis. The quality was high in nine, and low in three of the included studies. Endpoints were mild cognitive impairment, cognitive decline, dementia, Alzheimer's disease, or Parkinson disease.

Forbes et al. (2015) is a systematic review and meta-analysis of RCTs investigating the effect of LC n-3 FA supplementation on the onset of age-associated cognitive decline and dementia in adults with normal cognition. The authors performed a systematic literature search in MEDLINE, Embase, CINAHL, and the Cochrane Database of Systematic Reviews from 2003 until June 2013. The risk of bias in the eligible papers was assessed based on criteria derived from the Cochrane Handbook for Systematic Reviews of Interventions. Six RCTs examining LC n-3 FA supplements (range 400-2200 mg/day EPA+DHA) and cognition were included. The risk of bias was high in one of the included studies, while it was moderate in one and

low in four of the studies. Cognition was measured as Mini-Mental State Examination score and Digit Span Forward.

5.2.11.1 *Results from the meta-analyses for LC n-3 FA intake and cognition and cognitive decline*

Below is a summary table for LC n-3 FA intake, cognition, and cognitive decline (Table 5.2.11.1-1) based on the identified meta-analyses.

Table 5.2.11.1-1 Overview of results from meta-analyses included in the weight of evidence analysis of LC n-3 FA intake and cognitive outcomes in adults.

Author, year	Type of studies	Total no studies	No of cases (domain)	Comparison	Summary RR/HR (95 % CI)	Hetero-geneity	Overall result
Cognition							
Alex, 2019	RCTs in non-demented adults	4	780 (global score)	LC n-3 FA supplement intake vs placebo	Hedge's g=0.01 (-0.128 to 0.151)	I ² =0%	LC n-3 FA supplements had significant effect on slight improvement in memory function, but not on global cognitive function in healthy individuals. Effect on cognitive decline was not possible to study in this material because of no observed decline in the placebo groups
		15	2524 (memory function)	LC n-3 FA supplement intake vs placebo	Hedge's g=0.31 (0.104 to 0.516)	I ² =80.57%	
		15	2022 (executive function)	LC n-3 FA supplement intake vs placebo	Hedge's g=0.19 (0.058 to 0.329)	I ² =44.69%	
		8	1802 (visuospatial effects)	LC n-3 FA supplement intake vs placebo	Hedge's g=0.22 (0.048 to 0.388)	I ² =58.07%	
Cognitive decline							
Balachandar, 2020	RCTs in elderly subjects without cognitive decline	10	2277 (incremental memory)	DHA supplement intake vs placebo	Standardized mean difference=0.22 (-0.17 to 0.61)	I ² =94.4%	No cognitive benefits by DHA supplements on cognitive ageing. DHA supplementation has insignificant/no beneficial role in slowing/improving age-related decline in memory, executive functions, working memory, and attention
		9	2226 (incremental executive function)	DHA supplement intake vs placebo	Standardized mean difference=-0.06 (-0.31 to 0.19)	I ² =88.8%	
		5	1233 (incremental attention)	DHA supplement intake vs placebo	Standardized mean difference=0.10 (-0.04 to 0.25)	I ² =32.7%	
Zhang, 2020	RCTs in elderly with mild cognitive decline	7	434 (Cognitive decline)	LC n-3 FA supplement intake vs placebo	Weighted mean difference=0.85 (0.04 to 1.67)	I ² =52.4%	LC n-3 FA supplements may have beneficial effect in older people with mild cognitive impairment, but, due to heterogeneity, the result is not robust

Author, year	Type of studies	Total no studies	No of cases (domain)	Comparison	Summary RR / HR (95 % CI)	Heterogeneity	Overall result
Zhang, 2016	Cohort studies in the general population	2	507 (risk of dementia)	Per 0.1 g/d increment in DHA intake	RR=0.86 (0.76 to 0.96)	$I^2=92.7\%$	Higher DHA intake from diet and supplements may be associated with lower risks of dementia and Alzheimer's, but without a linear dose-response relationship
		3	535 (risk of Alzheimer's)	Per 0.1 g/d increment in DHA intake	RR=0.63 (0.51 to 0.76)	$I^2=94.5\%$	
		3	659 (risk of Parkinson)	Per 0.1 g/d increment in DHA intake	RR=1.00 (0.97 to 1.03)	$I^2=0\%$	
Forbes, 2015	RCTs in adults with normal cognition	4	2713 (MMSE)	LC n-3 FA supplement intake vs placebo	Mean difference=0.06 (-0.08 to 0.19)	$I^2=0\%$	No convincing evidence of benefit for LC n-3 FA supplements on cognitive impact in non-demented middle-aged and older adults
		3	1053 (digit span forward)	LC n-3 FA supplement intake vs placebo	Mean difference=-0.02 (-0.30 to 0.25)	$I^2=0\%$	

The meta-analysis examining the association between LC n-3 FA supplementation and cognition in adults (Alex et al., 2019) found a small but significant effect of LC n-3 FA on memory and executive function in adult populations without cognitive disorders. The systematic review of RCTs (Teo et al., 2017) found no effect of LC n-3 FA supplementation on cognitive performance in healthy adult populations.

The meta-analysis examining the association between DHA supplement intake and cognitive decline in RCTs (Balachandar et al., 2020) found no effect on cognitive function in elderly subjects without cognitive decline. The meta-analysis examining the association between LC n-3 FA supplements and cognitive decline in RCTs (Zhang et al., 2020) found a slight beneficial effect on cognitive function in older people with mild cognitive impairment. The meta-analysis of cohort studies (Zhang et al., 2016) found that high total dietary DHA intake may be associated with a reduced risk of dementia and Alzheimer's disease, but not of Parkinson disease. The meta-analysis of Forbes et al. (2015) found no effect of LC n-3 supplementation on cognition in non-demented middle-aged and older adults.

5.2.11.2 *Heterogeneity LC n-3 FA intake and cognition and cognitive decline in adults*

Cognition

Alex et al. (2019) addresses heterogeneity in subgroup analysis excluding low quality studies from the meta-analysis. The authors suggest that for cognitive measures in which the heterogeneity level decreases when excluding low quality studies, publication bias is a likely reason for heterogeneity. The authors conclude that effects sizes in the study are robust, suggesting that the evidence for the slight effect of LC n-3 FA on improvement in memory function and no effect on global cognitive function in healthy individuals is solid.

Cognitive decline

There is some serious heterogeneity in the meta-analysis of Balachandar et al. (2020). The authors do not address reasons for the heterogeneity, but they conclude that the study results remain the same when stratified in sub-groups (those supplemented ≥ 750 DHA per day and those supplemented with DHA and EPA).

Zhang et al. (2020) addresses heterogeneity in the discussion, acknowledging that their findings of a beneficial effect of LC n-3 FA in older people with mild cognitive impairment may be diluted because of the heterogeneity in the study.

Reasons for the large heterogeneity found in the meta-analysis of Zhang et al. (2016) were addressed in several subgroup analyses. Heterogeneity for DHA intake results was related to study location, BMI, education, apoE $\epsilon 4$ status, and intake of other nutrients. No heterogeneity was found in the meta-analysis of Parkinson disease.

Forbes et al. (2015) did not find any heterogeneity between the included studies, which also suggests robust findings of no effect of LC n-3 FA on cognition.

5.2.11.3 *Dose-response relationship LC n-3 FA intake and cognition and cognitive decline in adults*

No dose-response assessments were provided in any of the meta-analyses.

5.2.11.4 *Weight of evidence for LC n-3 FA intake and cognition and cognitive decline in adults*

In this section the evidence of the association between LC n-3 FA intake and cognitive function in adults is weighed according to the WCRF criteria presented in the method chapter (Box 2 in Chapter 3.1.6, but applied to evaluation of systematic reviews and meta-analyses).

Published evidence of LC n-3 FA intake and cognitive function in adults

Cognition: One meta-analysis of RCTs (Alex et al., 2019) found evidence for a slight beneficial effect of LC n-3 FA intake on cognitive function.

Cognitive decline: Two meta-analysis of RCT (Balachandar et al. (2020) and Forbes et al. (2015)) found no or insignificant effect of LC n-3 FA supplements on age-related cognitive decline, while one meta-analyses of RCTs (Zhang et al., 2020) found evidence for a slight beneficial effect of LC n-3 FA supplements on cognitive health in older people with mild cognitive impairment. A meta-analysis of cohort studies (Zhang et al., 2016) concluded that DHA intake is associated with a lower risk of dementia and Alzheimer's disease based on two and three cohort studies, respectively, but not with Parkinson disease.

Heterogeneity

Significant or borderline significant heterogeneity was observed partly in the meta-analysis examining cognition in adults with normal cognition. Moderate or large heterogeneity was observed in the three meta-analyses examining cognitive decline, except for the meta-analysis on Parkinson disease (Zhang et al., 2016).

Mechanism / biological plausibility

Plausible biological mechanisms have been presented above, see Chapter 5.2.

Upgrading factors

No upgrading factors have been evaluated.

Conclusion weight of evidence for LC n-3 FA intake and cognition and cognitive decline in adults

Cognition: There was evidence from one meta-analysis of RCTs that LC n-3 FA intake has some beneficial effect on cognitive outcome in adults. Although the results were solid, there were no other meta-analyses to confirm this finding. Plausible biological mechanisms have

been presented. In conclusion, the evidence that intake of LC n-3 FA is beneficial for cognitive function is “limited, no conclusion”.

Cognitive decline: There was evidence from three meta-analyses of RCT’s that LC n-3 FA supplements have either no or a small beneficial effect on cognitive ageing in elderly subjects. There was evidence from one meta-analysis of cohort studies that intake of DHA is associated with lower risk of dementia and Alzheimer’s disease. Some heterogeneity was observed. Plausible biological mechanisms have been presented. In conclusion, the evidence that intake of LC n-3 FA is beneficial for cognitive health is “limited, no conclusion”.

5.2.12 LC n-3 FA and mental health in adults

The current chapter summarizes the epidemiological evidence of LC n-3 FA from supplements and food intake and the prevention of mental health in adults from systematic reviews and meta-analyses published between 2015 and 2020. We performed a systematic search for systematic reviews and meta-analyses (see Chapter 3.2.4 for details). The complete search strategies are given in Chapter 15, Appendix II.

Plausible mechanisms for LC n-3 FA influence on mental health in adults is described in Chapter 5.2.

Four papers were identified as relevant for LC n-3 FA intake and mental health outcomes in adults and read in full text. One of these was included to fill in knowledge about the association between LC n-3 FA and mental health outcomes in adults, and three were excluded (see Table 5.2.12-1 for reasons for exclusions).

Table 5.2.12-1 Included papers and reasons for exclusion of identified papers from the search of systematic reviews and meta-analysis of LC n-3 FA and mental health outcomes in adults, 2015-2020.

Included papers	Excluded paper and reasons for exclusions
Grosso et al., 2016	The following was evaluated as quality C: Yang et al., 2018: No quality assessment of included studies. The following were excluded for other reasons: Deane et al., 2019: Included only treatment studies. Bai et al., 2018: Included only treatment studies.

The included systematic review and meta-analysis is described in more detail below; first, main descriptions of the methods used and then main/selected results.

Grosso et al. (2016) is a systematic review and meta-analysis of results from observational studies exploring the association between fish, n-3 PUFA dietary intake, and depression. The authors performed a systematic and comprehensive search in MEDLINE, Embase, PsychINFO, and the Cochrane Database of Systematic Reviews of all observational studies evaluating the association between dietary LC n-3 PUFA intake and depression in cohorts of individuals published up to August 2014. The quality of the studies was assessed following the principles of the Newcastle-Ottawa Quality Assessment Scale. Thirty-one studies were

included and reviewed, of these seven studies evaluated the association between LC n-3 FA intake and depression in prospective cohort studies. Endpoints included physician diagnosis and clinical interviews, as well as questionnaire-based assessments such as the Hopkins Symptom Check list and Center for Epidemiological Studies Depression.

5.2.12.1 *Results from the meta-analysis for LC n-3 FA intake and mental health in adults*

Below is a summary table for LC n-3 FA intake and mental health (Table 5.2.12.1-1) based on the identified meta-analysis.

Table 5.2.12.1-1 Overview of results from meta-analysis included in the weight of evidence analysis of LC n-3 FA intake and depression outcomes in adults.

Author, year	Study design	Total no studies	Comparison	Summary RR (95 % CI)	Heterogeneity	Overall result
Grosso, 2016	Prospective cohort studies*	4	High vs low intake EPA+DHA	0.74 (0.61, 0.89)	$I^2=0\%$	LC n-3 FA intake is associated with lower risk of depression

*No of cases not available.

In Grosso et al. (2016), the main analyses included both prospective and cross-sectional studies (seven estimates in total) but estimates for prospective studies alone were presented in subgroup analyses. For EPA+DHA, four of the total seven estimates included in the analyses were from prospective studies providing evidence for a beneficial association between dietary LC n-3 FA intake and depression (Table 5.2.5.1-1). Notably, two of these estimates are on male/female from the same study (Colangelo, 2009) and one is from a study on post-partum depression (Strom, 2009). One could argue that post-partum depression and depressive symptoms not related to pregnancy and giving birth have different mechanisms and should not be handled in the same analysis. In a subgroup analysis, the authors removed studies on post-partum depression with the result that the associations were no longer significant (0.81 (0.65, 1.01), $I^2=48\%$). These analyses included both prospective and cross-sectional studies, however, and it is not known whether the associations between LC n-3 FA intake and depression would be significant for the prospective studies alone removing post-partum depression.

5.2.12.2 *Heterogeneity LC n-3 FA intake and mental health in adults*

There was no observed heterogeneity.

5.2.12.3 *Dose-response relationship LC n-3 FA intake and mental health in adults*

A dose-response analysis demonstrated a J-shaped decreased risk of depression up to an intake of 0.6 g/day of LC n-3 FA (RR 0.66, 95% CI: 0.45, 0.97). A non-significant decreased risk was evident also for further increment in intake. These analyses were not restricted to

the four prospective cohort studies but included also cross-sectional studies. Whether there is a dose-response relationship in analyses with prospective studies alone is not known.

5.2.12.4 *Weight of evidence for LC n-3 FA intake and mental health in adults*

In this section, the evidence of the association between LC n-3 FA intake and mental health is weighed according to the WCRF criteria presented in the method chapter (Box 2 in Chapter 3.1.6, but applied to evaluation of systematic reviews and meta-analyses).

Published evidence of LC n-3 FA intake and mental health

There were only one meta-analysis and systematic review that was identified reporting findings on LC n-3 FA intake and depression through three prospective cohort studies (four estimates). Findings suggested a protective association of the LC n-3 FA intake on the risk of depression. It is not known, however, whether this association stands if removing one study on post-partum depression.

Heterogeneity

There is limited unexplained heterogeneity.

Mechanism/biological plausibility

Plausible biological mechanisms have been presented above, see Chapter 5.2.

Upgrading factors

No upgrading factors have been evaluated.

Conclusion LC n-3 FA intake and mental health in adults

One systematic review and meta-analysis provided some evidence for a protective association between LC n-3 FA intake and the risk of depression in adults. There was little unexplained heterogeneity, a dose-response was suggested, and plausible biological mechanisms have been described. The systematic review and meta-analysis included evidence from three studies only, and one of these was on post-partum depression. We therefore conclude that the evidence on LC n-3 FA intake and depression is graded "limited, suggestive".

5.2.13 **LC n-3 FA and preterm birth**

The current chapter summarizes the epidemiological evidence of LC n-3 FA intake and preterm birth (PTB) from systematic reviews and meta-analyses published between 2015 and 2020. We performed a systematic search for systematic reviews and meta-analyses (see Chapter 3.2.4 for details). The complete search strategies are given in Chapter 15, Appendix II.

Plausible mechanisms for LC n-3 FA influence on PTB are presented in Chapter 5.2.

Five systematic reviews and/or meta-analyses were identified as relevant for LC n-3 FA intake and PTB and read in full text. Two of these were included to fill in knowledge about the association between LC n-3 FA and PTB, and three were excluded (see Table 5.2.13-1 for reasons for exclusions).

Table 5.2.13-1 Included papers and reasons for exclusion of identified papers from the search of systematic reviews and meta-analysis of LC n-3 FA intake and PTB, 2015-2020.

Included papers	Excluded paper and reasons for exclusions
Chen et al., 2016 Newberry et al., 2016	The following was evaluated as quality C: Kar et al., 2016: Uninsufficient quality on dose information. Saccone et al., 2015: The paper lacked clear research question and inclusion criteria, and the scientific quality of the included studies was not used appropriately in formulating conclusions. The following was excluded for other reasons: Saccone et al., 2016: Investigated high risk pregnancies.

The meta-analyses are described in more detail below; first, main descriptions of the methods used and then main/selected results from each review.

Chen et al. (2016) is a meta-analysis of RCTs investigating the effect of maternal LC n-3 FA supplement intake during pregnancy on duration of gestation, risk of preterm birth, LBW, stillbirth and infant growth measures. The authors performed a systematic literature search in PubMed, Embase and the Cochrane Library databases until February 25, 2015. The quality of the eligible papers was assessed using criteria in the Cochrane Handbook. Nine RCT examining maternal LC n-3 FA intake and risk of PTB (gestational age <37 weeks) in low-risk pregnancies, and three RCTs examining risk of early PTB (gestational age <34 weeks) in low-risk pregnancies were included. Three RCTs examining maternal LC n-3 FA intake and risk of early PTB in low-risk pregnancies, and four RCTs examining risk of early PTB in low-risk pregnancies were included. Doses of fish oil supplementation were in the range 200 and up to 4950 mg/day. The quality was assessed high in 13 of the included studies.

Newberry et al. (2016) is a systematic review and meta-analysis of RCTs and prospective cohort studies investigating the effect of maternal LC n-3 FA intake during pregnancy on birth and neurodevelopmental and immune outcomes in the offspring. This is an extensive Evidence Report/Technology Assessment updating a previous report. The authors performed a systematic literature search in MEDLINE, Embase, the Cochrane Central Register of Controlled Trials (CENTRAL), Cochrane Database of Systematic Reviews, and Centre for Agriculture and Biosciences Abstracts from 2000 until August 2015. The quality of the eligible RCTs was assessed using the Cochrane Risk of Bias tool, and the quality of the eligible prospective cohort studies was assessed using the Newcastle-Ottawa tools. Seven RCTs examining maternal DHA or DHA+EPA supplementation and risk of preterm birth in healthy pregnant women, and seven RCTs examining maternal DHA+EPA supplementation and risk of preterm birth in pregnant women at risk of preterm birth were included in the meta-analyses. Doses of DHA or DHA+EPA supplementation were in the range 130 and up to 2700 mg/day. Low risk of bias was reported in the updated included studies.

5.2.13.1 *Results from the meta-analyses for LC n-3 FA intake and preterm birth*

Below are summary tables for LC n-3 FA intake and PTB (Table 5.2.13.1-1) based on the identified meta-analyses.

Table 5.2.13.1-1 Overview of results from meta-analyses included in the weight of evidence analysis of LC n-3 FA intake and PTB.

Author, year	Study design	Total no studies	No of cases	Comparison	Summary RR/OR (95% CI)	Heterogeneity	Overall result
Preterm birth (gestational age <37 weeks)							
Chen, 2016	RCT	9	157 LC n-3 FA supplementation vs 184 control	LC n-3 FA supplements vs placebo	RR 0.86 (0.70, 1.05)	$I^2=8\%$	No statistic. sig. assoc
Newberry, 2016*	RCT	7	-	DHA supplements vs placebo	OR 0.87 (0.66, 1.15)	$I^2=0\%$	No statistic. sig. assoc
	RCT	4	-	EPA+DHA supplements vs placebo	OR 0.86 (0.65, 1.15)	$I^2=0\%$	No statistic. sig. assoc
Early preterm birth (gestational age <34 weeks)							
Chen, 2016	RCT	3	22 LC n-3 FA supplementation vs 41 control	LC n-3 FA supplements vs placebo	RR 0.55 (0.33, 0.91)	$I^2=56\%$, $P=0.10$	Reduced risk of early PTB in the LC n-3 FA group

*It should be noted that one RCT in high-risk pregnancies is included in the meta-analyses in Newberry et al. (2016).

None of the included studies found significant effects for LC n-3 FA supplementation during pregnancy and risk of PTB (gestational age <37 weeks).

One study including 3 RCTs found a significant reduced risk of early PTB (gestational age <34 weeks) in the LC n-3 FA intervention group.

5.2.13.2 *Heterogeneity LC n-3 FA intake and preterm birth*

Both Chen et al. (2016) and Newberry et al. (2016) reported low heterogeneity for the finding of no significant effect on PTB, and Chen et al. (2016), reported high heterogeneity for the finding of reduced risk of early PTB.

5.2.13.3 *Dose-response relationship LC n-3 FA intake and preterm birth*

No dose-response assessments were provided in the meta-analysis by Chen et al. (2016). Newberry et al. (2016) found no significant dose-response relationship between LC n-3 FA and risk of PTB.

5.2.13.4 *Weight of evidence for maternal LC n-3 FA intake and preterm birth*

In this section, the evidence of the association between maternal LC n-3 FA intake and birth weight in the newborn is weighed according to the WCRF criteria presented in the method chapter (Box 2 in Chapter 3.1.6, but applied to evaluation of systematic reviews and meta-analyses).

Published evidence of maternal LC n-3 FA intake and preterm birth

Two meta-analyses showed no effect of maternal LC n-3 FA supplementation during pregnancy on the incidence of PTB (gestational age <37 weeks) neither from EPA + DHA or DHA alone (Chen et al., 2016 and Newberry et al., 2016). One meta-analysis reported reduced risk of early PTB (gestational age <34 weeks) after maternal LC n-3 FA during pregnancy (Chen et al., 2016).

Heterogeneity

Generally, little unexplained heterogeneity was observed in two of the included meta-analyses (Chen et al., 2016 and Newberry et al., 2016). The strength of evidence for reduced risk of early PTB of maternal LC n-3 FA supplementation during pregnancy was, however, low because of high heterogeneity between included studies.

Mechanism/biological plausibility

Plausible biological mechanisms have been presented above, see Chapter 5.2.

Upgrading factors

No upgrading factors have been evaluated.

Conclusion weight of evidence maternal LC n-3 FA intake and preterm birth

No significant effect of LC n-3 FA was reported in the two included meta-analyses for PTB. One meta-analysis reported a lower risk of early PTB of maternal LC n-3 FA supplementation during pregnancy in low-risk pregnancies, but heterogeneity was high for this result ($I^2=56\%$). There is evidence for biological plausibility. In conclusion, the evidence that maternal LC n-3 FA supplementation during pregnancy reduces the risk of PTB is "limited, no conclusion".

5.2.14 LC n-3 FA and low birth weight and birth weight

The current chapter summarizes the epidemiological evidence of LC n-3 FA intake and birth weight and low birth weight from systematic reviews and meta-analyses published between 2015 and 2020. We performed a systematic search for systematic reviews and meta-analyses (see Chapter 3.2.4 for details). The complete search strategies are given in Chapter 15, Appendix II.

Plausible mechanisms for LC n-3 FA influence on birth weight are presented in Chapter 5.2.

Five systematic reviews and/or meta-analyses were identified as relevant for LC n-3 FA intake and birth weight and read in full text. Three of these were included to fill in knowledge about the association between LC n-3 FA and birth weight, and two were excluded (see Table 5.2.14-1 for reasons for exclusions).

Table 5.2.14-1 Included papers and reasons for exclusion of identified papers from the search of systematic reviews and meta-analysis of LC n-3 FA intake and birth weight/low birth weight, 2015-2020.

Included papers	Excluded paper and reasons for exclusions
Li et al., 2018 Chen et al., 2016 Newberry et al., 2016	The following was evaluated as quality C: Saccone et al., 2015: The paper lacked clear research question and inclusion criteria, and the scientific quality of the included studies was not used appropriately in formulating conclusions. The following were excluded for other reasons: Saccone et al., 2016: Included studies in pregnant women with previous or current high-risk pregnancies.

The meta-analyses are described in more detail below; first, main descriptions of the methods used and then main/selected results from each review.

Li et al. (2018) is a systematic review and meta-analysis of RCTs investigating the effect of maternal LC n-3 FA supplement intake during pregnancy on birth outcomes in the offspring. The authors performed a systematic literature search in PubMed, Web of Science and the Cochrane Library databases from 1997 until April 2017. The quality of the eligible papers was assessed using criteria in the Cochrane Handbook. Twenty-two RCTs examining maternal LC n-3 FA supplementation (range 200-1180 mg/day DHA and 0-1280 mg/day EPA) and birth weight were included. The risk of bias was low in 11 of the included studies, while it was moderate in eight and high in three of the studies.

Chen et al. (2016) is a meta-analysis of RCTs investigating the effect of maternal LC n-3 FA supplement intake during pregnancy on duration of gestation, risk of preterm birth, LBW, stillbirth and infant growth measures. The authors performed a systematic literature search in PubMed, Embase and the Cochrane Library databases until February 25, 2015. The quality of the eligible papers was assessed using criteria in the Cochrane Handbook. Seventeen RCTs examining maternal LC n-3 FA supplementation (range 200-2070 mg/day and 0-2880 mg/day EPA) and birth weight in normal pregnancies, and five RCTs examining LC n-3 FA supplementation (range 200-1183 mg/day DHA and 0-1280 mg/day EPA) risk of low birth weight (LBW) in normal pregnancies were included (all the five studies with LBW as outcome were included in the birth weight studies). The quality was assessed high in 13 of the included studies (including in all the five studies reporting results for LBW).

Newberry et al. (2016) is a systematic review and meta-analysis of RCTs investigating the effect of maternal LC n-3 FA intake during pregnancy on birth and neurodevelopmental and immune outcomes in the offspring. This is an extensive Evidence Report/Technology

Assessment updating a previous report. The authors performed a systematic literature search in MEDLINE, Embase, the Cochrane Central Register of Controlled Trials (CENTRAL), Cochrane Database of Systematic Reviews, and Centre for Agriculture and Biosciences Abstracts from 2000 until August 2015. The quality of the eligible RCTs was assessed using the Cochrane Risk of Bias tool, and the quality of the eligible prospective cohort studies was assessed using the Newcastle-Ottawa tools. Seventeen RCTs examining maternal DHA or DHA+EPA supplementation (range 200-2070 mg/day DHA and 0-2880 mg/day EPA) and birth weight, and four RCTs examining maternal DHA supplementation (range 400-800 mg/day) and LBW were included. The quality was high in 11 of the included studies, and medium or low in six of the included studies.

5.2.14.1 *Results from the meta-analyses for LC n-3 FA intake and low birth weight and birth weight*

Below is a summary table for LC n-3 FA intake and birth weight and LBW (Table 5.2.14.1-1) based on the identified meta-analyses.

Table 5.2.14.1-1 Overview of results from meta-analyses included in the weight of evidence analysis of LC n-3 FA intake and birth weight and risk of low birth weight.

Author, year	Study design	Total no studies	No of cases	Comparison	Summary RR/HR (95 % CI)	Heterogeneity	Overall result
Risk of low birth weight							
Chen, 2016	RCT	5	87 vs 124	LC n-3 FA supplements vs placebo	RR=0.71 (0.54-0.92)	$I^2=21\%$	Maternal LC n-3 FA supplementation during pregnancy is associated with reduced risk of LBW
Newberry, 2016	RCT	4	78 vs 106	DHA supplements vs placebo	RR=0.72 (0.43, 1.11)	$I^2=7\%$	Maternal supplementation of DHA during pregnancy may not have any significant effect on risk for delivering a LBW infant
Birth weight							
Li, 2018	RCT	22	8627	LC n-3 FA supplements vs placebo	MD*=42.55 g (21.25 to 63.85 g), $P=0.004$	$I^2=49.8\%$	Maternal LC n-3 FA supplementation during pregnancy is associated with higher birth weight of the newborn
Chen, 2016	RCT	17	5580	LC n-3 FA supplements vs. placebo	MD*=61.19 g (14.83 to 107.55 g), $P=0.01$	$I^2=44\%$	Maternal LC n-3 FA during pregnancy is associated with larger size of the newborn
Newberry, 2016	RCT	12	4525	DHA supplements vs. placebo	MD*=90.12 g (2.62 to 177.62 g)	$I^2=63.2\%$	Maternal supplementation of DHA or DHA rich fish oils may increase birth weight
	RCT	5	715	EPA+DHA supplements vs. placebo	MD*=37.89 g (-19.53 to 95.31 g)	$I^2=0\%$	Maternal supplementation of EPA+DHA may not have any significant effect on infants' birth weight compared with placebo

*MD: mean difference.

The overlap of primary studies included in the systematic reviews and meta-analyses for low birth weight and birth weight is shown in Table 5.2.14.1-2.

Table 5.2.14.1-2 List of the included primary studies in the meta-analysis of low birth weight/ birth weight showing overlap.

RCTs/ primary studies Author, year	Included in the studies		
	Li, 2018	Chen, 2016	Newberry, 2016
Asserhoj, 2009	X		
Bergmann, 2008	X	X	
Campoy, 2011	X		
Carlson, 2013	X	X	X
Catena, 2016	X		
Courville, 2011	X	X	X
Dorte Rytter, 2011	X		
Dunstan, 2003		X	
Dunstan, 2008			X
Gustafson, 2014			X
Hauner, 2012	X		X
Helland, 2001	X	X	
Helland, 2008			X
Ines Gonzalez, 2015	X		
Judge, 2007		X	X
Judge, 2012			X
Krauss-Etschmann, 2008		X	
Lauritzen, 2005	X		
Lucia Bergmann, 2007			X
Makrides, 2010	X	X	
Malcolm, 2003	X	X	
Miles, 2011			X
Mozurkewich, 2013	X	X	X
Mulder, 2014			X
Olsen, 1992	X	X	
Ramakrishnan, 2010	X	X	X
Ramakrishnan, 2015	X		
Sanjuro, 2004	X	X	
Smuts, 2003a	X	X	
Smuts, 2003b	X	X	
Stein, 2011	X		
Tofail, 2008			X
van Goor, 2010	X	X	X
Zhou, 2012			X

Five of the studies were included in all three meta-analyses. A total of 13 studies were included in both Li et al. (2018) and Chen et al. (2016). Li et al. (2018) additionally included nine studies, of which three were published after Chen's data search. Chen et al. (2016)

included three studies which were not included in Li et al.'s meta-analysis. Six of the included studies in Li et al. (2018) were also included in Newberry et al.'s meta-analysis, which additionally included 10 other studies.

LBW: In the meta-analysis by Chen et al. (2016), supplementation of LC n-3 FA during pregnancy significantly decreased the risk of LBW, RR 0.77 (95% CI 0.65-0.92). This was found to be similar for low and high risk pregnancies in the sub-analysis. No significant effect on LBW was found of DHA supplementation during pregnancy in Newberry et al. (2016), RR=0.72 (95% CI 0.43, 1.11).

Birth weight: Li et al. (2018) showed that supplementation with LC n-3 FA during pregnancy increased the birth weight of the newborn by a mean of 42.55 g (95% CI 21.25 to 63.85 g).

Chen et al. (2016) showed that supplementation with LC n-3 FA during pregnancy significantly increased the birth weight of the newborn by a mean of 61.19 g (95% CI 14.83 to 107.55 g). In the pooled analysis of 12 RCTs in Newberry et al. (2016), supplementation with DHA during pregnancy was found to significantly increase the birth weight of the newborn by a mean of 90.12 g (95% CI 2.62 to 177.62 g). However, also in Newberry et al. (2016), a pooled analysis of five RCTs found no significant effect of maternal EPA+DHA supplementation on infant birth weight.

5.2.14.2 *Heterogeneity LC n-3 FA intake and birth weight and risk of low birth weight*

Li et al. (2018) reported significant heterogeneity in the meta-analysis on birth weight, $I^2=49.8\%$ ($P_{\text{heterogeneity}}=0.004$). The authors examined heterogeneity in a sensitivity analysis by leaving one by one study out of the analysis. Leaving out one of the studies with a small size (Mozurkewich et al., 2013), heterogeneity was reduced to low, $I^2=28\%$.

In Chen et al. (2016), heterogeneity was significant for the meta-analysis of maternal LC n-3 FA intake on birth weight ($I^2=44\%$, $P_{\text{heterogeneity}}=0.03$). There was no significant heterogeneity in the meta-analysis of maternal LC n-3 FA intake on risk of LBW ($I^2=21\%$, $P_{\text{heterogeneity}}=0.28$).

Newberry et al. (2016) reported a high degree of heterogeneity of studies in the meta-analysis of maternal DHA supplementation and birth weight ($I^2=63.2\%$), but not in the meta-analysis of maternal EPA+DHA intake and birth weight or maternal DHA intake and risk of low birth weight ($I^2=0\%$ and $I^2=7\%$, respectively). The authors stated that due to the significant heterogeneity across studies, the interpretation of overall meta-analysis results was limited.

5.2.14.3 *Dose-response relationship LC n-3 FA intake and birth weight*

No dose-response assessments were provided in the meta-analyses.

5.2.14.4 *Weight of evidence for maternal LC n-3 FA intake and birth weight*

In this section, the evidence of the association between maternal LC n-3 FA intake and birth weight in the newborn is weighed according to the WCRF criteria presented in the method Chapter 3.1.6 (Box 2), but limited to evaluation of systematic reviews and meta-analyses).

Published evidence of maternal LC n-3 FA intake and birth weight

One meta-analysis including five high quality RCTs reported a lower risk of LBW in the newborn with maternal LC n-3 FA supplementation during pregnancy, but only one of the included RCTs showed a significant association (Chen et al., 2016). One meta-analysis, including 4 RCTs, investigated the association between maternal DHA supplementation alone and LBW, and found no association (Newberry et al., 2016).

Two meta-analyses showed an effect on increasing birth weight in the newborn by maternal LC n-3 FA supplementation (EPA+DHA) during pregnancy up to approximately 40-90 g (Li et al., 2018 and Chen et al., 2016). One meta-analysis reported increased birth weight for supplementation with DHA alone, but not for EPA + DHA (Newberry et al., 2016).

Heterogeneity

Significant or borderline significant heterogeneity was observed in three of the included meta-analyses (Li et al., 2018, Chen et al., 2016 and Newberry et al., 2016). In the newest of the meta-analyses (Li et al., 2018), the heterogeneity was explained by one included study with a small number of subjects.

Mechanism / biological plausibility

Plausible biological mechanisms have been presented above, see Chapter 5.2.

Upgrading factors

No upgrading factors have been evaluated.

Conclusion weight of evidence maternal LC n-3 FA intake and low birth weight and birth weight

There is some evidence that maternal LC n-3 FA during pregnancy reduces the risk of low birth weight in the newborn. This was found by one of two meta-analyses which examined the association between EPA+DHA and LBW, while (Newberry et al., 2016) investigating only the association between DHA alone and LBW did not find an association. There was no heterogeneity in any of the results on LBW. There is evidence for biological plausibility. In conclusion, the evidence that maternal LC n-3 FA supplementation during pregnancy reduces the risk of low birth weight is graded "limited, suggestive".

Overall, the included meta-analyses showed an effect on increasing birth weight in the newborn by maternal LC n-3 FA supplementation during pregnancy. There was an effect

shown in RCT's. The heterogeneity in the latest of the meta-analyses could be explained, and was reduced to non-significant after exclusion of one study. There is evidence for biological plausibility. In conclusion, the evidence that intake of maternal LC n-3 FA-supplementation during pregnancy increases birth weight of the newborn is "probable".

5.2.15 LC n-3 FA and type 2 diabetes

The current chapter summarizes the epidemiological evidence of LC n-3 FA intake and type 2 diabetes (T2D) from systematic reviews and meta-analyses published between 2015 and 2020. We performed a systematic search for systematic reviews and meta-analyses (see Chapter 3.2.4 for details). The complete search strategies are given in Chapter 15, Appendix II.

Mechanisms for LC n-3 FA influence on T2D are presented in Chapter 5.2.

Three papers were identified as relevant for LC n-3 FA intake and T2D and read in full text. Two of these were included to fill in knowledge about the association between LC n-3 FA and T2D, and one were excluded (see Table 5.2.15-1 for reasons for exclusions).

Table 5.2.15-1 Included papers and reasons for exclusion of identified papers from the search of systematic reviews and meta-analysis of LC n-3 FA intake and type 2 diabetes, 2015-2020.

Included papers	Excluded paper and reasons for exclusions
Brown et al., 2019 Chen et al., 2017	The following was evaluated as quality C: Yanai et al., 2015: Lack of duplicate study selection and data extraction, comprehensive literature search, assessment of scientific quality of studies and appropriate methods to combine studies.

The meta-analyses are described in more detail below; first, main descriptions of the methods used and then main/selected results from each review.

Brown et al. (2019) is a systematic review and meta-analysis of RCTs investigating the effect of LC n-3 FA intake on the risk of T2D and glucose metabolism in adults at any risk of T2D. The authors performed a systematic literature search in MEDLINE, Embase, Cochrane Central Register of Controlled Trials (CENTRAL), WHO International Clinical Trials Registry Platform Clinicaltrials.gov databases, and trials in relevant systematic reviews until April 2017. The ongoing trials were reassessed in December 2018. The quality of the eligible papers was assessed by GRADE. Seventeen RCTs examining LC n-3 FA intake and T2D incidence were included. The risk of bias was low in 10 of the included studies. New T2D diagnosis were used as endpoint.

Chen et al. (2017) is a pooled meta-analysis of two separate analyses in a prospective cohort study to evaluate the potential factors that influence the effect of LC n-3 FA consumption on T2D incidence. The authors performed a systematic literature search in PubMed, Cochrane Library, MEDLINE, SIGLE, Embase and National Research Register until December 2018. Quality of the eligible papers was assessed by Newcastle-Ottawa Scale Criteria. One paper

with two cohort studies examining respectively EPA and DHA intake, and the risk of T2D was included. The quality was high in the included study. The endpoint was T2D incidence.

5.2.15.1 *Results from the meta-analyses for LC n-3 FA intake and type 2 diabetes*

Below is a summary table for LC n-3 FA intake and risk and T2D (Table 5.2.15.1-1) based on the identified meta-analyses.

Table 5.2.15.1-1 Overview of results from meta-analyses included in the weight of evidence analysis of LC n-3 FA intake and type 2 diabetes.

Author	Study design	Total no studies	No of cases	Comparison	Summary RR (95% CI)	Heterogeneity	Overall result
Brown, 2019	RCTs with LC n-3 FA intake from diet and supplements in adults at any risk of T2D	17	1105 low LC n-3 FA vs 1091 high LC n-3 FA	Higher vs lower LC n-3 FA intake	RR=1.00 (0.85-1.17)	$I^2=45\%$	No statistically significant association
Chen, 2017	Prospective cohort study in general population	2	2370	Higher vs lower EPA and DHA intake	RR=1.45 (1.31 to 1.60)	$I^2=0\%$	Increased risk of T2D by higher EPA and DHA intake

Brown et al. (2019) found no association between LC n-3 FA intake from diet and supplements and risk of T2D (RR=1.00, 95% CI 0.85-1.17) with moderate quality evidence. Subgroup analyses showed no difference in this result between the dose of LC n-3 FA or the duration of supplementation.

Chen et al. (2017) examined the association between dietary intake of EPA or DHA, and T2D risk. Intake of EPA or DHA was associated with an increased risk of T2D (RR=1.45, 95% CI 1.31 to 1.60). Dose-response association between EPA or DHA intake, and T2D risk was not shown for intake level relevant for LC n-3 FA supplementation or high LC n-3 FA intake ($\approx$ 800 mg/day and above).

5.2.15.2 *Heterogeneity LC n-3 FA intake and type 2 diabetes*

Brown et al. (2019) found no serious heterogeneity between the studies in the meta-analysis (all $I^2 < 50\%$).

There was no heterogeneity in the pooled analysis in Chen et al. (2017).

5.2.15.3 *Dose-response relationship LC n-3 FA intake and type 2 diabetes*

The dose-response curves in Chen et al. (2017) showed a consistent dose-response association between increasing intake of EPA/DHA and T2D risk up to at least 0.25 g/day intake. Dose-response association between EPA/DHA intake and T2D risk was not shown for intake level relevant for LC n-3 FA supplementation or high LC n-3 FA intake ($\approx$ 800 mg/day and above).

5.2.15.4 *Weight of evidence for LC n-3 FA intake and risk of type 2 diabetes*

In this section, the evidence of the association between LC n-3 FA intake and T2D risk is weighed according to the WCRF criteria presented in the method chapter (Box 2 in Chapter 3.1.6, but applied to evaluation of systematic reviews and meta-analyses).

Published evidence of LC n-3 FA intake and risk of type 2 diabetes

Overall, the meta-analyses show either no association or a non-beneficial association between LC n-3 FA intake on T2D risk. One meta-analysis of RCTs (Brown et al., 2019) concluded that LC n-3 FA supplementation does not affect the risk of T2D, while a pooled meta-analysis of prospective cohort studies (Chen et al., 2017) found evidence for an increased risk of T2D by increasing intake of EPA and DHA. However, the estimate of increased T2D risk by increasing EPA and DHA intake by Chen et al. (2017) was only based on one cohort with two separate analyses and could not conclude on association at LC n-3 FA intake higher than 200 mg per day.

Heterogeneity

No serious heterogeneity was observed for the results from the two meta-analyses described above.

Mechanism/biological plausibility

Plausible biological mechanisms have been presented above, see Chapter 5.2.

Upgrading factors

Dose-response gradient of EPA and DHA intake and risk of T2D was examined by Chen et al. (2017), and a linear positive relationship was found up to intake level of 200 mg per day. No dose-response gradient/curve was shown at higher intakes.

Conclusion weight of evidence LC n-3 FA intake and type 2 diabetes

There is evidence from one meta-analysis of RCTs that LC n-3 FA intake from diet and supplements has no effect on the risk of T2D, while one pooled meta-analysis including two analyses in one prospective cohort study showed increased risk by intake of EPA and DHA. No serious heterogeneity was observed for these results. There is not strong evidence for biological plausibility of increased T2D risk by high LC n-3 FA intake. In conclusion, the evidence that intake of LC n-3 FA is associated with risk of T2D is "limited, no conclusion".

5.2.16 LC n-3 FA and rheumatoid arthritis and multiple sclerosis, prevention

Our systematic review of literature described in Chapter 3.2.4 did not result in inclusion of any studies. The complete search strategies are given in Chapter 15, Appendix II.

5.2.17 LC n-3 FA and semen quality

The current chapter summarizes the epidemiological evidence of LC n-3 FA intake and parameters of male fertility from systematic reviews and meta-analyses. The search was performed without any limitations in time. We performed a systematic search for systematic reviews and meta-analyses (see Chapter 3.2.2 for details). The complete search strategies are given in Chapter 15, Appendix II.

Plausible mechanisms for LC n-3 FA influence on semen quality are presented in Chapter 5.2.

Our systematic review of literature on nutrients and semen quality/male fertility resulted in inclusion of two meta-analyses investigating the association between LC n-3 FA and various parameters of semen quality.

List of included and excluded studies and reason for exclusion is presented in Table 5.2.17-1.

Table 5.2.17-1 Included studies and reasons for exclusion of identified papers from the search of systematic reviews and meta-analysis of LC n-3 FA intake and parameters of male fertility.

Included papers	Excluded paper and reasons for exclusions
Smits et al., 2019 Salas-Huetos et al., 2018	The following was evaluated as quality C: Hosseini et al., 2019: No quality assessment of included papers. The following was excluded for other reasons: Showell et al., 2011: Updated in Smits et al. (2019).

The meta-analyses are described in more detail below; first, main descriptions of the methods used and then main/selected results from each review.

Smits et al. (2019) is a Cochrane systematic review and meta-analysis of RCTs investigating the effectiveness and safety of supplementary oral antioxidants in subfertile men. The authors performed a systematic literature search in The Cochrane Gynaecology and Fertility (CGF) Group trials register, CENTRAL, MEDLINE, Embase, PsycINFO, CINAHL, and two trials registers until February 2018. Risk of bias in the eligible papers was assessed by the Risk of bias assessment tool and Cochrane Handbook for Systematic Reviews of Interventions. One to four RCTs are included in the meta-analyses for semen quality parameters. The risk of bias in the included studies varies, but generally, the risk of bias was moderate to low in the studies with the highest impact on the pooled analysis. The men included in the analysis were subfertile and were part of couples who had been referred to a fertility clinic. The endpoints included for DHA and EPA were sperm DNA fragmentation, motility, and concentration.

Salas-Huetos et al. (2018) is a systematic review and meta-analyses of RCTs investigating the effect of nutrients from supplements or foods on semen quality parameters in fertile and infertile men. The authors performed a systematic literature search in MEDLINE until October 2017. The quality of the eligible papers was assessed by ROB index based on 7 categories (O'Connor et al., 2008). Two RCTs are included in the meta-analyses for semen quality parameters. The risk of bias was low or unclear in the included study. The endpoints included for LC n-3 FA were sperm count, concentration, motility, and morphology.

5.2.17.1 Results from the meta-analyses for LC n-3 FA intake and semen quality parameters

Below is a summary table for supplemental LC n-3 FA and semen quality parameters (Table 5.2.17.1-1) based on the identified meta-analyses.

Table 5.2.17.1-1 Overview of results from meta-analyses included in the weight of evidence analysis of LC n-3 FA intake and semen quality parameters.

Author	Type of studies included	Total no studies	No of cases	Comparison	Summary RR/MD (95 % CI)	Heterogeneity	Overall result
Sperm motility							
Smits, 2019	RCTs Total sperm motility	3	40 subfertile in the intervention groups	EPA+DHA supplementation<3 mo vs placebo	MD -8.35% (-17.40, 0.69)	$I^2=0\%$, $P=0.77$	No significant effect
	Progressive sperm motility	1	21 subfertile men in the intervention groups	EPA+DHA supplementation<3 mo vs placebo	MD 6.40% (4.83, 7.97 95% CI)		Significant increase in progressive motility ($Z=8.00$, $P<0.00001$)
	Progressive sperm motility	1	23 subfertile men in the intervention groups	DHA supplementation<3 mo vs placebo	MD -6.60% (-8.57, -4.63 95% CI)		Significant decrease in progressive motility ($Z=6.57$, $P<0.00001$)
Salas-Huetos, 2018	RCTs Total sperm motility	2	138 in the intervention groups	Supplementation with 855-2110 mg/day EPA+DHA, 10-32 weeks vs placebo	MD 7.55% (7.09, 8.01)	$I^2=94\%$, $P<0.001$	Significant increase in sperm motility ($Z=32.12$, $P<0.001$)
Sperm concentration							
Smits et al, 2019	RCTs	4	63 subfertile men in the intervention groups	EPA+DHA supplementation<3 mo vs placebo	MD 3.44×10^6 spz/mL (1.70, 5.17)	$I^2=0\%$, $P=0.93$	Significant increase in sperm concentration, $Z=3.89$, $P=0.0001$
Salas-Huetos et al, 2018	RCTs	2	138 fertile and subfertile in the intervention groups	Supplementation with 855-2110 mg/day EPA+DHA, 10-32 weeks vs placebo	MD 10.98×10^6 spz/mL (10.25, 11.72)	$I^2=94\%$, $P<0.001$	Significant increase in sperm concentration ($Z=29.37$, $P<0.001$)
Sperm count							
Salas Huetos et al, 2018	RCTs	2	138 fertile and subfertile in the intervention groups	Supplementation with 855-2110 mg/day EPA+DHA, 10-32 weeks vs placebo	MD 18.70×10^6 spz (16.89, 20.51)	$I^2=97\%$, $P<0.001$	Significant increase in sperm count, $Z=20.25$, $P<0.001$

The overlap of primary studies in the two meta-analyses included for sperm concentration and motility is listed in Table 5.2.17.1-2.

Table 5.2.17.1-2 Overlap of primary studies in the two meta-analyses included for sperm concentration and motility.

RCTs/ primary studies Author, year	Included in the meta-analyses	
	Smits, 2019)	Salas-Huetos, 2018
Sperm concentration		
Conquer, 2000a	X	
Conquer, 2000b	X	
Haghighian, 2015	X	
Martinez-Soto, 2010	X	
Martinez-Soto, 2016		X
Saferinejad, 2011		X
Sperm motility		
Conquer, 2000a	X	
Conquer, 2000b	X	
Haghighian, 2015		X
Martinez-Soto, 2010	X	
Martinez-Soto, 2016		X
Saferinejad, 2011		X

Sperm motility

Sperm motility is the ability of sperm to move efficiently. Salas-Huetos et al. (2018) found a significant increase in total sperm motility (MD=7.55%, 95% CI 7.09, 8.01) in their meta-analysis including both fertile and infertile men. Smits et al. (2019) found no such significant effect on total sperm motility, but found significant increase in progressive motility for EPA+DHA supplementation (MD=6.40%, 95% CI 4.83, 7.97) and significant decrease in progressive motility for DHA supplementation alone (MD=-6.60%, 95% CI -8.57, -4.63) in subfertile men. However, only one study and few study participants were included for the EPA+DHA and DHA alone analyses, respectively.

Sperm concentration

Sperm concentration is the number of spermatozoa per mL in a semen sample. According to WHO 5th edition, the lower reference limit for normal sperm concentration is 15×10^6 spermatozoa/mL (Cooper et al., 2005). Both Smits et al. (2019) and Salas-Huetos et al. (2018) found significant increased sperm concentration (MD= 3.44×10^6 spermatozoa/mL, 95% CI 1.70, 5.17 in Smits et al., and MD= 10.98×10^6 spermatozoa/mL, 95% CI 10.25, 11.72 in Salas-Huetos et al.).

Other sperm parameters

Sperm count is the total number of sperm in the entire ejaculation. To determine the sperm count, sperm concentration is multiplied by the total volume of the sample submitted. The lower reference limit for sperm count is 39×10^6 spermatozoa per ejaculate. Salas-Huetos et al. (2018) also found significant increase in sperm count (MD= 18.70×10^6 spermatozoa/mL, 95% CI 16.89, 20.51), and percentage of normal form spermatozoa (MD=0.91%, 95% CI 0.69, 1.13).

5.2.17.2 Heterogeneity LC n-3 FA intake and semen quality parameters

Salas-Huetos et al. (2018) found high heterogeneity between the studies in all the meta-analyses for the various semen quality parameters ($I^2=94-97\%$), whereas there was no heterogeneity in the meta-analyses in Smits et al. (2019) ($I^2=0\%$).

5.2.17.3 Dose-response relationship LC n-3 FA intake and semen quality parameters

No dose-response assessments were provided in the meta-analyses.

5.2.17.4 Weight of evidence for LC n-3 FA intake and semen quality parameters

In this section the evidence of the association between LC n-3 FA intake and semen quality parameters is weighed according to the WCRF criteria presented in the method chapter (Box 2 in Chapter 3.1.6, but applied to evaluation of systematic reviews and meta-analyses).

Published evidence of LC n-3 FA intake and semen quality parameters

Results from two meta-analyses and systematic reviews of RCTs showed either a beneficial or no effect of LC n-3 FA supplementation on semen quality parameters such as sperm concentration, sperm motility, and sperm count in populations of predominantly infertile or sub-fertile men. However, there are few included RCTs with limited numbers of participants in the intervention groups. Studies in men without fertility problems are lacking. For DHA supplementation alone, one study found a negative association for progressive sperm motility (Smits et al., 2019). This analysis, however, included only one RCT and few participants.

Heterogeneity

Serious heterogeneity was observed in one of the meta-analyses for various semen quality parameters, but in the third meta-analysis (Smits et al., 2019), there was no heterogeneity for beneficial findings on sperm concentration.

Mechanism/ biological plausibility

Plausible biological mechanisms have been presented, see Chapter 5.2.

Upgrading factors

No upgrading factors have been evaluated.

Conclusion weight of evidence LC n-3 FA intake and semen quality parameters

There was evidence from two meta-analyses of RCT's that LC n-3 FA supplements have either a beneficial effect or no effect on semen quality parameters in populations of predominantly infertile or subfertile men. High heterogeneity was observed in one meta-analysis. Plausible biological mechanisms have been presented. In conclusion, the evidence that there is a beneficial association between LC n-3 FA and semen quality parameters such as sperm concentration and sperm motility in men experiencing fertility problems is "limited, suggestive".

5.2.18 LC n-3 FA and other evaluated outcomes

Prevention of allergy during pregnancy and cancer have been evaluated in previous systematic reviews (see Table 5.2-1), but the evidence for an association between LC n-3 FA and these outcomes are weak. However, fish intake in infancy seems to reduce the risk of eczema and allergic rhinitis in children but it seems like it is the intake of fish per se in infancy, not specially n-3 LC-PUFAs that may have an allergy protective effect (Zhang et al., 2017). We have not conducted a literature search to reevaluate the evidence for these outcomes.

5.2.19 LC n-3 FA and cancer

Evidence for the association between intake of LC n-3 FA and cancer was not summarised in the Third Expert Report of WCRF/AICR in 2018. The lack of summary indicates lack of studies to conclude on the association for any cancer type on at least a "limited, suggestive" level (WCRF, 2018).

5.3 Vitamin D

In the revision of NNR (2012), Lamberg-Allardt et al. (2013) conducted an evidence-based systematic literature review of vitamin D and associated health effects. The overall aim was to review recent scientific data on requirements and health effects of vitamin D. The review by Lamberg-Allardt et al. (2013) covered the following health outcomes; pregnancy outcomes and growth, bone health (all fractures, hip fractures, vertebral fractures, bone mineral density/osteoporosis, bone mass, bone quality, rickets, osteomalacia, dental health), muscle strength, falls; all cancers, breast cancer, colorectal cancer, prostate cancer, type 1 diabetes, type 2 diabetes, multiple sclerosis, obesity, total mortality, hypertension/blood pressure, cardiovascular disease (CVD) clinical outcomes, and infections. The evidence for associations between vitamin D and bone health (including falls) and mortality were concluded to be “probable” by Lamberg-Allardt et al. (2013). None of the other health outcomes relevant for this benefit and risk assessment was judged to be “probable” or “convincing”.

For associations between vitamin D and prevention of dental health, muscle function, type 2 diabetes, multiple sclerosis, body weight, hypertension and blood pressure, cardiovascular outcomes, cancer, and infections, Lamberg-Allardt et al. (2013) concluded that the evidence was “limited and inconclusive”.

In the current benefit and risk assessment, all health outcomes from the literature search for fish were considered, and expert judgements were performed to decide on outcomes relevant for specific nutrients (see Chapter 5.7). Based on this we have updated the information on the association between vitamin D intake and the following outcomes; birth weight, and respiratory tract infections. For birth weight we conducted an updated systematic literature review. These literature searches are described in more details in Chapter 3.2. For respiratory tract infections, it was decided to use the systematic reviews from the British Scientific Advisory Committee on Nutrition (SACN, 2020).

5.3.1 Vitamin D and bone health, including fall

Lamberg-Allardt et al. (2013) concluded that there is “probable” evidence for an association between vitamin D and bone health, including fractures and falls. Furthermore, the authors stated that the effect was often only seen in persons with low baseline concentration of 25-hydroxyvitamin D (25OHD), and that most intervention studies leading to the conclusion reported that intervention with vitamin D combined with calcium and not vitamin D alone gave these benefits. Umbrella reviews published after NNR (2012) strengthen the conclusion in Lamberg-Allardt et al. (2013) that no fracture preventing effect of vitamin D alone has been shown in RCTs (Autier et al., 2017; Mateussi et al., 2017).

5.3.2 Vitamin D and mortality

Also, the evidence for associations between vitamin D and mortality was concluded to be “probable” by Lamberg-Allardt et al. (2013). However, it was not clear if co-supplementation

with calcium was necessary as the difference between trials intervening with vitamin D alone and trial intervening with vitamin D and calcium was not significant. Umbrella reviews published after NNR (2012) are in line with Lamberg-Allardt et al. (2013) concerning the effect of vitamin D on total mortality. In addition, a beneficial effect of vitamin D on cancer mortality has emerged (Autier et al., 2017; Mateussi et al., 2017; Rejnmark et al., 2017; Sluyter et al., 2020;).

5.3.3 Vitamin D and respiratory tract infection

In the NNR (2012) revision, Lamberg-Allardt et al. (2013) concluded that 'The evidence for an effect of vitamin D on infections is scarce and trials were very heterogeneous. In a meta-analysis of 25 RCTs published in 2017 it was concluded that acute respiratory tract infections could be reduced by vitamin D supplementation (Martineau et al., 2017). The current chapter summarizes updated evidence concerning vitamin D and the risk of respiratory tract infections (RTI).

5.3.3.1 *Systematic reviews, introduction*

During the Covid-19 pandemic, there was a substantial focus on the potential role of vitamin D in the prevention and treatment of respiratory tract infections in general and Covid-19 in particular. Based on this, the British Scientific Advisory Committee on Nutrition (SACN) performed a rapid review in June 2020 entitled *Rapid review: Vitamin D and acute respiratory tract infections* (SACN, June 2020). This report was based on the extensive SACN report on *Vitamin D and Health* published in 2016 (SACN 2016). The rapid review was later updated by SACN, and in the following we will describe the findings from the *Update of rapid review: Vitamin D and acute respiratory tract infections*, published in December 2020 (SACN, December 2020). It is important to emphasise that this report builds on conclusions from the two preceding SACN opinions, which first will be described briefly.

5.3.3.2 *SACN reviews on vitamin D and respiratory tract infection*

In SACN (2016) it was concluded that the evidence on vitamin D supplementation was inconsistent and generally did not show a beneficial effect of vitamin D supplementation on infectious disease risk (acute respiratory tract infections and tuberculos). This was in line with the conclusion in the systematic literature review by Lamberg Allardt et al. (2013) stating that 'the evidence for an effect of vitamin D on infections is scarce and trials were very heterogeneous' (Lamberg-Allardt et al., 2013).

In the SACN Rapid review: Vitamin D and acute respiratory tract infections from June 2020 (SACN, June 2020), the evidence concerning vitamin D and the risk of RTI published since the SACN (2016) report was assessed. The rapid review included a comprehensive literature search to identify systematic reviews, meta-analyses and pooled analyses of RCTs and controlled trials on vitamin D supplementation and incidence of RTIs published between 1 January 2016 and 22 April 2020. It was concluded that evidence did not support recommending vitamin D supplementation to prevent acute respiratory tract infections and tuberculos in the general UK population.

In the updated SACN report from December 2020, the systematic literature search was updated until 26 October 2020 using a similar strategy as in the previous search. However, preprints were included, and Web of Science was not searched. The update of the rapid review was done according to SACN's Framework for the Evaluation of Evidence. However, due to the very short time frame it was not possible to grade the quality of evidence.

Three studies were included:

- 2 systematic reviews with meta-analyses (Jolliffe et al. 2020; Wang et al., 2020)
- 1 RCT (Ganmaa et al., 2020)

The evidence concerning vitamin D and RTI was assessed in the systematic review and meta-analysis by Jolliffe et al (2020). This was an update of the systematic review and meta-analysis by Martineau et al. (2017). Both reviews were performed by the same group. A trial-level approach was used in Jolliffe et al. (2020), and data from 42 trials (46 331 participants) was included. In contrast, the Martineau et al. (2017) review used Individual Participant Data (IPD) in their analysis. Both trials in healthy population groups and trials in person with pre-existing disease were included. The trials were done in diverse populations (low, middle, and higher income) from a variety of countries. There were also difference concerning vitamin D (doses and intervals) and in the reporting and assessment of RTIs across trials. As in Martineau et al. (2017), vitamin D-supplementation showed an overall protective effect on RTI (OR 0.91, 95% CI 0.84 to 0.99). Although the effect was statistically significant, it should be noted that the effect estimate was small (as the disease endpoint (RTI) is common, OR overestimates RR substantially). Hence, the risk reducing effect is even smaller than that indicated by the OR (RR is closer to 1.00 than 0.91). There was heterogeneity across trials ($I^2=37.2\%$; $P=0.014$). In subgroup analyses, significant effects of vitamin D supplementation were found when vitamin D was given daily, but not weekly or monthly. In addition, there was a significant effect of vitamin D at doses of 10-25 µg/day, but not below 10 µg/day or above 25 µg/day and in participants aged 1-15.9 years but not in other age groups. In contrast to Martineau et al. (2017) who found the clearest effect in those with baseline 25OHD <25 nmol/L, a protective effect of vitamin D was not found in subgroups based on baseline serum 25OHD concentrations (although the point estimate was most prominent for studies with baseline 25OHD concentration <25 nmol/L).

The authors identified evidence of publication bias suggesting that the overall effect estimate might have been overestimated. The quality of the evidence was therefore downgraded to 'moderate'.

The systematic review and meta-analysis by Wang et al. (2020) included only healthy populations restricted to ages 18-65 years. They concluded that vitamin D supplementation did not reduce the incidence of colds. The included RCTs differed with respect to populations, vitamin D dose and regimens and assessment of outcomes. All the RCTs in Wang et al. (2020) were included in Jolliffe et al. (2020).

In the RCT performed by Ganmaa et al. (2020) in Mongolia, no effect of weekly vitamin D supplementation (350 µg which is equivalent to 14,000 IU) on RTI risk in children was found. The trial is included in Jolliffe et al. (2020).

5.3.3.3 *Weight of evidence, vitamin D and respiratory tract infection*

Conclusion vitamin D intake and risk of respiratory tract infections

The extensive SACN report, last updated autumn 2020, conclude that vitamin D *may* reduce the risk of respiratory tract infection, but that the size of any potential benefit of vitamin D in reducing acute RTI risk may be small (SACN, December 2020). The wordings 'may reduce' and 'potential benefit' in addition to the identified risk of publication bias led us to conclude that although there are promising indications, the evidence is "limited, suggestive" for the notion that vitamin D lowers the risk of respiratory tract infections.

5.3.4 Vitamin D and low birth weight and birth weight

No firm conclusion was given in NNR (2012) and in IOM (2011) for the association between birth weight and vitamin D intake. In a systematic review published in BMJ in 2017, it was, however, concluded that vitamin D supplementation was associated with increased birth weight (Sun et al., 2016).

Low concentration of 25OHD has been related to LBW in observational studies. Vitamin D might be of importance for the development of placenta via effects on the expression of human chorionic gonadotropin (Shin et al., 2010) and the synthesis of placental sex steroids.

We performed a systematic search for systematic reviews and meta-analyses (see Chapter 3.2 for details). The complete search strategies are given in Chapter 15, Appendix II.

The current chapter summarizes the evidence of vitamin D status risk of low birth weight (LBW) and birth weight from systematic reviews and meta-analyses published between 2015 and 2020. All the included reviews and meta-analysis have defined LBW as birth weight <2500 g. We have considered risk of LBW and birth weight as relevant for vitamin D as these outcomes have been related to low maternal concentration of 25OHD during pregnancy. As IOM and Lamberg-Allardt et al. (2013), we used 25 (OH)D to assess vitamin D status as it reflects the sum of the vitamin D produced due to sun exposure and that obtained from foods and supplements.

Eleven systematic reviews and/or meta-analyses relevant for vitamin D and risk of LBW and/or birth weight were read as full text papers. Eight of these were included to fill in knowledge about the association between vitamin D and risk of LBW and/or birth weight, and three were excluded (see Table 5.3.4-1 for reasons for exclusions).

Table 5.3.4-1 Overview of results from meta-analyses included in the weight of evidence analysis of vitamin D intake and birth weight and risk of low birth weight.

Included papers	Excluded paper and reasons for exclusions
Tous et al., 2020 Gallo et al., 2019 Maugeri et al., 2019 Palacios et al., 2019a Palacios et al., 2019b Santamaria et al., 2018 Roth et al., 2017 Perez-Lopez et al., 2015	The following was evaluated as quality C: Bi et al., 2018: Scientific quality in included papers not considered properly. The following were excluded for other reasons: De-Regil et al., 2013: Cochrane review updated in Palacios et al. (2019a) Fang et al., 2019: Included cross-sectional studies

The meta-analyses are described in more detail below; first, main descriptions of the methods used and then main/selected results from each review.

Maugeri et al. (2019) is a systematic review and meta-analysis of RCTs to evaluate the effects of oral vitamin D supplementation during pregnancy on several outcomes, including LBW and birth weight as primary outcomes. The authors performed a systematic literature search in the PubMed/MEDLINE, Embase, and Cochrane Central Register of Controlled Trials (CENTRAL) databases to up May 2017. Quality of the studies was assessed by the GRADE system. Based on eight different quality criteria, both LBW and birth weight studies were classified as moderate. The effect of vitamin D supplementation alone on birth weight was assessed by 10 RCTs published between 1980 and 2016. A total of seven studies were from Asian countries and three from UK. The duration of vitamin D intervention was 8–20 weeks. In general, the doses of vitamin D were high. Women in five intervention groups were supplemented with daily dose of vitamin D in the range of 15 to 100 µg and in five intervention groups women were supplemented with vitamin D in the range of 875 to 5000 µg. The high doses were given as single dose, in two doses or as weekly doses.

Palacios et al. (2019a) is a Cochrane review of RCTs evaluating the effects of vitamin D supplementation alone or in combination with calcium or other vitamins and minerals given to women during pregnancy on neonatal health outcomes including LBW (primary outcome) and mean birth weight (secondary outcome). The authors searched the Cochrane Pregnancy and Childbirth's Trials Register which contains regularly updates from the Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE, Embase, CINAHL and hand searches of 30 journals and the proceedings of major conferences up to July 2018. In general, the doses of vitamin D were high, ranging from 25 µg/day to single doses of 15 000 µg. The start of the supplementation was at week 20 or more weeks gestation in four of five of the LBW studies and the trials were conducted in UK, Bangladesh, Pakistan, and India. Quality of the studies was assessed by the GRADE system and the evidence was moderate for LBW studies meaning that the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.

Palacios et al. (2019b) is a Cochrane review of RCTs assessing the effects and safety of different doses of vitamin D supplementation (alone or in combination with calcium or other vitamins, minerals or nutrients during pregnancy) on several maternal and neonatal/infant outcome, including LBW (primary outcome) and birth weight (secondary outcome). The authors searched the Cochrane Pregnancy and Childbirth's Trials Register, ClinicalTrials.gov, the WHO International Clinical Trials Registry Platform (ICTRP) (12 July 2018), and the

reference lists of retrieved studies. Studies comparing doses of >15 µg/day versus <15 µg/day (comparison 1) and studies comparing >150 µg/day versus <150 µg/day (comparison 2) were included. The quality of the studies was assessed using the GRADE approach as outlined in the GRADE handbook and the quality of evidence relating to LBW was very low certainty for comparison 1 meaning that the true effect is likely to be substantial different from the estimate of effect, and low certainty for comparison 2 meaning that the true effect may be substantially different from the estimate of the effect.

Roth et al. (2017) is a systematic review and meta-analysis of 43 RCTs trials to estimate the effects of vitamin D supplementation during pregnancy on several maternal and neonatal/infant outcomes, including LBW and birth weight. The authors performed a search in the electronic databases in MEDLINE, Medline in process, Embase, PubMed, the Cochrane Database of Systematic reviews, and the Cochrane Central Register of Controlled Trials (CENTRAL). Searches were initially done in July 2016 and most recently updated in September 2017. The methodological quality of each study was evaluated using the Cochrane Collaboration tool for assessing risk of bias to independently assign quality scores to the trials. Eight of 43 trials had an overall low risk of bias.

Perez-Lopez et al. (2015) is a systematic review and meta-analysis of RCTs trials to assess the effects of vitamin D supplementation during pregnancy on obstetric outcomes and birth variables, inclusive LBW and birth weight. The authors performed a search in PubMed, MEDLINE, Embase, Scopus, Web of Science, Cochrane Library, and www.clinicaltrials.gov up to March 2014. This systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The Cochrane Collaboration tool for assessing risk of bias in RCTs was used. Of the 13 included studies, three had a low risk of bias, two had a high risk of bias, and the remaining eight had an unclear risk of bias.

Tous et al. (2020) is a systematic review and meta-analysis of observational studies assessing the association between low maternal concentrations of 25OHD by using three different cut-off levels (<30 and ≥30 nmol/L, <50 and ≥50 nmol/L, <75 and ≥75 nmol/L) and birth weight in offspring. The authors performed a systematic literature search in PubMed/MEDLINE, Scopus, and the Cochrane Library databases up to April 2018. Twenty-one of totally 54 studies had birth weight as outcome. The quality of the eligible papers included in the meta-analysis was assessed by using the STROBE criterion for observational studies and was rated as high in 2/3 of the studies.

Santamaria et al. (2018) is a systematic review and meta-analysis of observational studies aiming to better understand the role of prenatal vitamin D status in child growth, adiposity, and metabolic health, including birth weight. The authors performed a search on electronic databases of the human literature in PubMed, up to July 2017. The methodological quality of each study was evaluated using the Newcastle–Ottawa Scale (NOS) and only high-quality observational studies with a score of at least 7 out of 9 were included in this meta-analysis. Among the finally selected 30 articles, 16 cohort studies reported birth weight involving 18 096 participants.

Gallo et al. (2019) is a systematic review and meta-analysis of RCTs to evaluate associations between maternal vitamin D supplementation and maternal and infant health outcomes, including birth weight. The authors conducted a comprehensive literature search of PubMed from 2000 to 2016. Quality Criteria Checklist were used to assess risk of bias tool to assess the quality of each study, which includes 10 domains on scientific soundness. Positive rating means risk of bias in that study is very low, negative rating means that the study has high risk of bias, and neutral rating means that the study has moderate risk of bias. Eleven studies examined the effects of maternal vitamin D supplements on birth weight, eight with positive quality and three with neutral quality.

5.3.4.1 *Results from the meta-analyses for vitamin D intake and low birth weight and birth weight*

Below is a summary table for vitamin D status and birth weight/low birth weight (Table 5.3.4.1-1) based on the identified meta-analyses.

Table 5.3.4.1-1 Summary of results from meta-analyses on vitamin D and risk of low birth weight (LBW) and birth weight.

Author, year	Study design	Total no studies	No of cases	Comparison	Summary MD or RR (95 % CI)	Heterogeneity	Overall result
Low birth weight (LBW)							
Maugeri, 2019	RCT	3	267	Vitamin D supplements vs placebo or no intervention	RR 0.47 (0.23, 0.74)	$I^2=0\%$	Maternal vitamin D supplementation reduced the risk of LBW
Palacios, 2019a	RCT	5	366	Vitamin D supplements vs placebo or no intervention	RR=0.55 (0.35-0.87) (<2500g)	$I^2=36\%$	Supplementing pregnant women with vitamin D may probably reduce the risk of LBW
Palacios, 2019b	RCT	4	210	Vitamin D supplements >15 µg vs ≤15 µg alone or with any other nutrient	RR=0.90 (0.66, 1.24)	$I^2=3\%$	Supplementation during pregnancy may make little or no difference to the risk of LBW
		2	190	Vitamin D supplements >100 µg vs ≤100 µg alone or with any other nutrient	RR=0.92 (0.49, 1.70)	$I^2=6.9\%$	
Roth, 2017	RCT	7	1156	Vitamin D supplements vs placebo or no intervention	RR 0.74 (0.47, 1.16)	$I^2=47\%$	Prenatal vitamin D supplementation did not reduce the risk of LBW
Perez-Loopez, 2015	RCT	4	244	Vitamin D supplements vs placebo or no intervention	RR 0.72 (0.44, 1.16)	$I^2=0\%$	Maternal vitamin D supplementation did not prevent the risk of LBW
Birth weight as continuous variable							
Tous, 2020	Observational studies	21	4691	Vitamin D status at three different cut-offs compared with birth weight	MD -87.92g (-119.73, -55.91) Cut off: <30 and ≥30 nmol/L	$I^2=58\%$	Maternal vitamin D deficiency (<30 nmol/L) during pregnancy is associated with lower birth weight
			6394		MD -19.27g (-63.34, 24.80) Cut off: <50 and ≥50 nmol/L	$I^2=84\%$	
			3856		MD -15.15g (-12.73, 43.04) Cut off: <75 and ≥75 nmol/L	$I^2=27\%$	

Author, year	Study design	Total no studies	No of cases	Comparison	Summary MD or RR (95 % CI)	Heterogeneity	Overall result
Gallo, 2019	RCT	11	723	Vitamin D supplements vs placebo or no intervention	MD -114.2g (63.4, 165.1)	$I^2=0\%$	Fair evidence that maternal vitamin D supplementation significantly increases infant birth weight
Maugeri, 2019	RCT	10	674	Vitamin D supplementats vs placebo or no intervention	MD -118.46g (70.47, 166.45)	$I^2=13\%$	Maternal vitamin D supplementation increased the birth weight
Palacious, 2019a	RCT	17	1504	Vitamin D supplements vs placebo or no intervention	MD -80.3 g (-14.4, 175.0)	$I^2=92\%$	Supplementing pregnant women with vitamin D probably makes little or no difference
Palacious, 2019b	RCT	14	2012	Vitamin D supplements >15 µg vs ≤15 µg alone or with any other nutrient	MD 51.57g (1.07 to 102.07)	$I^2=42\%$	Supplementing pregnant women with vitamin D may favor increased birth weight
		13	1574	Vitamin D supplements >100 µg vs ≤100 µg alone or with any other nutrient	MD 46.00g (-8.99 to 101.00)	$I^2=56\%$	
Santamaria, 2018	Prospective cohort studies	16	3651	Vitamin D deficiency vs vitamin D non-deficiency	MD -100.69g (-162.25, -39.13)	$I^2=92\%$	Low prenatal vitamin D status was associated with lower birth weight
Roth, 2017	RCT	30	5273	Vitamin D supplements vs placebo or no intervention	MD - 58.33g (18.88 to 97.78)	$I^2=43\%$	Prenatal vitamin D supplementation was associated with increased infant mean birth weight
Perez-Loopez, 2015	RCT	10	752	Vitamin D supplements vs placebo or no intervention	MD -107.60g (59.9- 155.3)	$I^2=0\%$	Vitamin D supplementation during pregnancy was associated with increased infant BW

MD; mean difference weight, RR; relative risk.

Tables 5.3.4.1-2 and 3 show overlap of primary studies in the included systematic reviews and meta-analyses for birth weight and risk of low birth weight.

A total of 14 different studies were included in the four meta-analyses regarding the risk of low birth weight. The overlap of studies was small as ten of the studies only were used in one of the meta-analyses. Only the study by Brooke et al. (1980) was included in all four meta-analyses. Three of the studies in Perez-Lopez et al. (2015) were also included in Maugeri et al. (2019).

A total of 66 different studies were included in the seven meta-analyses regarding birth weight. A total of 21 studies were included in Tous et al. (2020) and Santamaria et al. (2018) (observational studies) and ten of these studies were included in both meta-analyses. Regarding the RCTs Maugeri et al. (2019) included 10 studies and seven of these were also included in Perez-Lopez et al. (2015) and in Roth et al. (2017) and six were included in Palacios et al. (2019a). Roth et al. (2017) included 28 studies and nine of these were included in Palacios et al. (2019a), seven in Gallo et al. (2019) and six in Perez-Lopez et al. (2015).

Table 5.3.4.1-2 Overlap table for primary studies included in the meta-analyses for low birth weight.

RCTs/ primary studies Author, year	Included in the meta-analyses				
	Maugeri et al., 2019	Roth et al., 2017	Palacios et al., 2019a	Palacios et al., 2019b	Perez-Lopez et al., 2015
Brooke, 1980	X	X	X		X
Brough, 2010	X				
Bhutta, 2011			X		
Hashemipour, 2013					X
Hossain, 2014		X			
Karamali, 2015		X		X	
Khan, 2016		X			
Marya, 1988		X	X		
Mojibian, 2015		X		X	
O'Brien, 2013				X	
Roth, 2010			X		
Roth, 2013		X		X	
Sablök, 2015			X		
Yu, 2009a	X				X
Yu, 2009b	X				X

Table 5.3.4.1-3 Overlap table for primary studies included in the meta-analyses for birth weight.

RCTs/ primary studies Author, year	Included in the meta-analyses							
	Tous, 2020	Maugeri, 2019	Gallo, 2019	Santamaria, 2018	Roth, 2017	Palacios, 2019a	Palacios, 2019b	Perez-Lopez, 2015
Abotorabi, 2017					X		X	
Asemi, 2013a			X		X	X		
Asemi, 2013b			X					
Asemi, 2014a			X					
Asemi, 2014b			X					
Ates, 2016	X			X				
Aydogmus, 2015	X							
Bacqui, 2009							X	
Bhatia, 2012							X	
Bhutta, 2011						X		
Bowyer, 2009	X			X				
Brook, 1980		X			X	X		X
Burris, 2012	X			X				
Charandabi, 2015		X			X			
Chen, 2015				X				
Cooper, 2016					X			
Dalgard, 2016	X			X				
Dawodu, 2013					X		X	
Eckardt, 2015	X			X				
Eggermoen, 2017	X							
Farrant, 2009	X							
Gale, 2008	X			X				
Gernand, 2013	X							
Goldring, 2013a		X						X
Goldring, 2013b		X						X
Grant, 2013						X		
Harvey 2012						X		
Hashemipour, 2014			X		X		X	X
Hollis, 2011			X		X			X
Hossain, 2014		X	X		X			X
Josefson, 2016				X				
Kalra, 2012							X	
Karamali, 2015			X		X		X	
Kaur, 1991					X	X		
Khan, 2016					X			
Leffelaar, 2010	X			X				
Litonjua, 2016					X			
Mallet, 1986					X	X		
March, 2015			X					
Marya, 1981							X	
Marya, 1988		X			X	X		X
Mirghafourvand, 2013						X		
Mojibian, 2015					X		X	
Morley, 2006	X			X				
Mutlu, 2014					X		X	
Naghshineh, 2016		X			X	X		

RCTs/ primary studies Author, year	Included in the meta-analyses							
	Tous, 2020	Maugeri, 2019	Gallo, 2019	Santamaria, 2018	Roth, 2017	Palacios, 2019a	Palacios, 2019b	Perez-Lopez, 2015
O'Brien, 2013							X	
Ong, 2016				X				
Reichetzeder, 2014	X			X				
Rostami, 2017							X	
Roth, 2010						X		
Roth, 2013		X	X		X		X	X
Sabet, 2012		X				X		X
Sabllok, 2015		X			X	X		
Sahoo, 2016					X			
Shahgheibi, 2016					X	X		
Singh, 2015						X		
Soheilykhah, 2013			X					
Song, 2012	X			X				
Stephensen, 2011							X	
Thiele, 2014							X	
Thiele, 2016					X		X	
Valizadeh, 2016					X			
Vaziri, 2016					X	X		
Wagner, 2006a							X	
Wagner, 2006b							X	
Weiler, 2005				X				
Weinert, 2016				X				
Weiss, 2009							X	
Yap, 2014			X		X			
Yesiltepe, 2014			X					
Yu, 2008						X		
Yu, 2009					X			
Zerofsky, 2014					X			
Zhou, 2014				X				
Zhu, 2015	X							

Low birth weight:

In Maugeri et al. (2020), the effect of vitamin D supplementation on incidence of LBW was assessed by 3 RCTs and 4 group comparisons and the risk of LBW was lower in the intervention groups (RR=0.47, 95% CI 0.23–0.74; $P=0.003$). Supplementation of vitamin D alone to women (omission of one study) significantly reduced the risk of LBW than controls (RR=0.47, 95% CI 0.23-0.97; $P=0.040$).

In Palacios et al. (2019a), the effect of vitamin D supplementation on LBW was studied in five RCTs including 679 women. Risk of LBW was RR=0.55 (95% CI 0.35-0.87) in infants of mothers supplemented with vitamin D compared to placebo/no intervention. The finding for LBW was downgraded from high certainty to moderate certainty due to two studies being at unclear risk of selection bias, one study being at high risk of bias for allocation concealment, and three studies being at high risk of attrition bias.

In Palacios et al. (2019b), the effect of vitamin D supplementation on LBW was studied in four RCTs involving in total 1550 women (210 cases) and suggested a similar risk between those taking more than 15 µg vitamin D and those taking 15 µg or less (RR 0.90, 95% CI 0.66 to 1.24). The certainty for this comparison 1 was very low meaning that the true effect is likely to be substantial different from the estimate of effect. Subgroup analysis did not appear to show an effect by nutrients included in the supplementation. Following the planned sensitivity analysis, after excluding one study (O'Brien 2013), which was classified as low quality, the effect changed very slightly to RR 0.88, 95% CI 0.67 to 1.15. Two RCTs studies involving 1099 women (190 cases) suggested little or no difference in risk of LBW for the comparison between those taking more than 100 µg and those taking 100 µg or less of vitamin D (RR 0.92, 95% CI 0.49 to 1.70). Subgroup analyses were not conducted due to the low number of trials. The low certainty for comparison 2 meaning that the true effect may be substantially different from the estimate of the effect.

In Roth et al. (2017), the risk of LBW was studied in seven RCTs including 1,156 participants and the available evidence did not indicate a significant effect on LBW with a risk ratio of 0.74 (95% CI 0.47, 1.16).

In Perez-Lopez et al. (2015), vitamin D supplementation did not prevent risk of LBW (RR 0.72, 95% CI 0.44-1.16) in four RCTs in neonates, and were not different for the vitamin D intervention groups.

Birth weight:

In Tous et al. (2020), 21 studies were included for birth weight. The meta-analysis of maternal vitamin D concentrations <30 nmol/L showed significantly lower mean birth weight of 87.82 g (−119.73 to −55.91) compared to mothers with vitamin D concentrations ≥30 nmol/L (15 studies). There were no significant differences in birth weight between infants born to vitamin D-insufficient mothers (<50 nmol/L) compared to infants born to vitamin D-sufficient (≥75 nmol/L) mothers (13 studies). Maternal 25OHD concentrations ≥75 nmol/L were not observed to be associated with birth weight (five studies).

In Santamaria et al. (2018), 16 studies reported birth weight involving 18 096 participants. Cut-off of vitamin D deficiency varied and was <30 nmol/L in 12 studies and 50 nmol/L in four studies. The overall summary mean difference of birth weight was −100.69 g (95% CI −162.25, −39.13) and significant. Subgroup analysis shows a significant association between prenatal 25OHD <30 nmol/L and a lower birth weight (MD −111.26; 95% CI −139.60, −82.92). Subgroup analysis also shows significant association between prenatal 25OHD <25 nmol/L and a lower birth weight (g) (MD −212.43; 95% CI −408.90, −15.96).

In Maugeri et al. (2020) the effect of vitamin D supplementation on birth weight was assessed by 13 RCTs and 15 group comparisons. Compared to controls, birth weight was significantly higher in the intervention groups (mean difference: 103.17 g, 95% CI 62.29–144.04 g; $P<0.001$). Looking at vitamin D supplementation alone without the combination with other micronutrients assessed by 10 RCTs, the birth weight increased significantly (mean difference: 118.46 g, 95% CI 70.47–166.45 g, $P<0.001$; mean difference: 62.76 g.

Subgroup analysis by regimen showed that both daily and single-intermittent high dose supplementation of vitamin D significantly increased birth weight (mean difference: 74.66 g, 95% CI 18.80–130.52 g, $P<0.001$; mean difference: 136.02 g, 95% CI 76.05–195.98 g, $P<0.001$, respectively).

In Palacios et al. (2019a), the effect of vitamin D supplementation on birth weight was studied in 17 RCTs including 2828 women. Mean difference in birth weight was non-significantly 80.3 g higher (95% CI -4.4 to 175.0) in the vitamin D group compared to control.

In Palacios et al (2019b), the effect of vitamin D supplementation on birth weight was studied in 14 trials involving 3300 women and suggested a greater birth weight among infant's from women taking more than 15 µg vitamin D compared to women receiving 15 µg or less (mean difference 51.57g, 95% CI 1.07 to 102.07; $P=0.05$). Thirteen RCTs studies involving 3710 women suggest little or no difference in birthweight among infants from women for the comparison between those taking more than 100 µg and those taking 100 µg or less of vitamin D (mean difference 46.00g, 95% CI -8.99 to 101.00).

In Gallo et al. (2019), the effects of maternal dietary supplements of vitamin D on birth weight were mixed, and the overall mean difference (MD) in birth weight in the pooled analysis was significant. Forrest plot showed that the pooled MD was +114.2 g (95% CI=63.4 to 165.1 g) with insignificant heterogeneity ($I^2=0\%$, $P_{\text{heterogeneity}}=0.66$). The overall strength of the available evidence was scored as fair to suggest that maternal vitamin D supplementation increases infant birth weight.

In Roth et al. (2017), the effect of maternal vitamin D supplementation on birth weight was studied in 30 RCTs of regular or bolus regimen vitamin D at any dose. Pooling of 37 comparisons indicated that prenatal vitamin D supplementation (versus low dose, no vitamin D, or placebo), significantly increased mean birth weight by an average of 58.33 g (95% CI 18.88, 97.78), but findings were not robust in sensitivity and subgroup analysis.

In Perez-Lopez et al. (2015), birth weight was significantly greater for neonates in the vitamin D groups (MD: 107.6 g, 95% CI 59.9-155.3 g). Although the improvements in birth weight found in eight RCTs were rather small, they suggest indirectly that vitamin D supplementation exerts a positive effect on foetal cell mass and function, skeletal mineralization, and metabolism.

5.3.4.2 Heterogeneity vitamin D intake and low birth weight and birth weight

Low birth weight:

Most of the meta-analyses of LBW examined potential sources of heterogeneity in sub-group analyses. A list of factors which could introduce between-study variation, was most often but not always specified a priori as part of the methods section.

Maugeri et al. (2019) found no significant heterogeneity across RCT studies of LBW ($P_{\text{heterogeneity}} > 0.1$; $I^2 = 0\%$). Subgroup analysis by regimen showed that daily maternal vitamin D supplementation significantly reduced the risk of LBW (RR=0.40, 95% CI 0.21–0.78; $P_{\text{heterogeneity}} = 0.007$).

Palacios et al. (2019a) reported no significant heterogeneity across RCT studies of LBW ($P_{\text{heterogeneity}} > 0.18$; $I^2 = 36\%$). The heterogeneity was not significant when testing for start of supplementation in pregnancy, supplement regimen (single dose versus daily or weekly/monthly), by latitude or by season at the start of the pregnancy ($P_{\text{heterogeneity}} = 0.37$; $I^2 = 0\%$). For the comparison LBW and pre-gestational BMI the heterogeneity was not significant either ($P_{\text{heterogeneity}} = 0.75$; $I^2 = 0\%$).

Palacios et al (2019b) reported significant heterogeneity across studies of LBW ($P_{\text{heterogeneity}} = 0.38$; $I^2 = 3\%$) when the dose of vitamin D was $>15 \mu\text{g}$ versus $\leq 15 \mu\text{g/day}$ alone or with other nutrients. For the comparison of a dose of vitamin D $>100 \mu\text{g}$ versus a dose $\leq 100 \mu\text{g/day}$ or with any other nutrient on birth weight the heterogeneity was not significant ($P_{\text{heterogeneity}} = 0.3$; $I^2 = 7\%$) across studies of LBW.

Roth et al. (2017) considered carefully the heterogeneity across all trials and reported substantial clinical and methodological heterogeneity between trials, however not specified particularly for LBW.

Perez-Lopez et al. (2015) reported no significant heterogeneity between trials on LBW.

Birth weight:

All the meta-analyses of birth weight examined potential sources of heterogeneity in sub-group analyses. A list of factors which could introduce between-study variation, was most often but not always specified a priori as part of the methods section.

Tous et al. (2020) investigated heterogeneity in observational studies by excluding studies causing asymmetry in the funnel plots. The heterogeneity decreased significantly (from $I^2 = 58\%$ to 38%) when one study was excluded, maintaining the mean difference in birth weight: (-98.33 g , 95% CI -125.74 to -70.92 g) (vitamin D <30 vs $\geq 30 \text{ nmol/L}$). In meta-regression analysis, ethnic group did not explain the observed heterogeneity although there was a tendency of lower effects in the Asian ethnic group. Regarding birth weight and vitamin D <50 vs $\geq 50 \text{ nmol/L}$, although there was significant heterogeneity ($I^2 = 84\%$), it decreased when the two studies, which caused asymmetry in the funnel plot were excluded ($I^2 = 40\%$).

Santamaria et al. (2018) found significant heterogeneity across the studies ($I^2=92\%$; $P_{\text{heterogeneity}} < 0.001$) for the birth weight overall, but not in the subgroup analysis of prenatal 25OHD < 30 nmol/L and a lower birth weight.

Maugeri et al. (2019) found no significant heterogeneity across RCTs of birth weight ($P_{\text{heterogeneity}} > 0.1$; $I^2 = 7.0\%$) or LBW ($P_{\text{heterogeneity}} > 0.1$; $I^2 = 0\%$). Subgroup analysis by regimen showed that daily maternal vitamin D supplementation significantly reduced the risk of LBW (RR=0.40, 95% CI 0.21–0.78; $P_{\text{heterogeneity}} = 0.007$).

Palacios et al. (2019a) found substantial heterogeneity between trials ($I^2 = 92\%$). Exclusion of one trial (Mallet et al., 1986) from the analysis, heterogeneity was reduced from 92% to 84% and results show that vitamin D supplementation probably results in higher birthweight (MD 99.27 95% CI 16.22 to 182.32). Exclusion of one more trial (Singh et al., 2015), reduced heterogeneity further to 67%.

Palacios et al (2019b) found that the response to supplementation of a dose of vitamin D > 15 µg versus a dose ≤ 15 µg/day alone or with other nutrients on birth weight was heterogeneous ($I^2 = 42\%$ $P_{\text{heterogeneity}} = 0.05$) and that this result should be interpreted with caution. For the comparison of a dose of vitamin D > 100 µg versus a dose ≤ 100 µg/day or with any other nutrient on birth weight the heterogeneity was significant $P_{\text{heterogeneity}} = 0.01$; $I^2 = 56\%$.

Gallo et al. (2019) noted low heterogeneity ($I^2 = 0\%$, $P_{\text{heterogeneity}} = 0.66$) in the pooled birth weight increase of 114.2 g. The authors suggest therefore that vitamin D may play a role in fetal growth.

Roth et al. (2017) reported substantial clinical and methodological heterogeneity between trials, including wide variation in baseline maternal vitamin D status. The magnitude of the pooled effect on increased birth weight remained relatively stable in sensitivity analyses and was unaffected by the removal of single outlier trials. There was a significant heterogeneity between trials that might have been partly explained by the greater effects on birth weight in groups that received bolus doses of vitamin D3 and in trials that were conducted in South Asia.

Perez-Lopez et al. (2015) reported no significant heterogeneity between trials on LBW and birth weight.

5.3.4.3 *Dose-response relationship vitamin D intake and low birth weight and birth weight*

No dose-response assessments were provided in the meta-analyses for risk of LBW.

In Maugeri et al. (2019), meta-regression analyses did not reveal a dose-depending effect of vitamin D supplementation alone on birth weight, probably due to the limited number of studies in this analysis.

Roth et al. (2017) showed that populations without vitamin D deficiency might have little to gain regarding birth weight from any dose of vitamin D, but deficient populations might require relatively high doses to raise vitamin D status to an optimal range associated with clinical benefits. In a post hoc analysis of the effect on birth weight that considered both effective dose and baseline vitamin D status, there was a significant dose-response effect in trials in which mean baseline 25OHD was 30-50 nmol/L but no association in trials with mean 25OHD <30 nmol/L.

5.3.4.4 *Weight of evidence for maternal vitamin D intake and low birth weight and birth weight*

In this section, the evidence of the association between vitamin D and birth weight and risk of LBW is weighed according to the WCRF criteria presented in the method chapter 3.1.6 (Box 2), but applied to evaluation of systematic reviews and meta-analyses).

Published evidence vitamin D and low birth weight and birth weight

Five systematic reviews and meta-analysis of RCTs reported on the risk of LBW with maternal vitamin D supplementation during pregnancy. Two of these studies (Maugeri et al., 2019; Palacios et al., 2019a), reported a significant reduction in the risk of LBW after maternal vitamin D supplementation during pregnancy and the other three studies reported no influence of vitamin D supplementation on risk of LBW (Roth et al., 2017; Perez-Lopez et al., 2015; Palacios et al 2019b). The dose of vitamin D supplementation was high in most RCTs and therefore the relevance for normal intake of vitamin D is unclear. Only one comparison in Palacios 2019b study reported significant heterogeneity, all other studies reported not significant heterogeneity, and no dose-response curves were provided in the included meta-analysis of RCTs.

Two systematic reviews and meta-analysis of observational studies (Tous et al., 2020; Santamaria et al., 2018) reported an association of maternal vitamin D status during pregnancy with infant birth weight, however, one of the meta-analyses found this association only in vitamin D deficient women (<30 nmol/L). Six systematic review and meta-analyses of RCTs (Maugeri et al., 2019; Gallo et al., 2019; Palacios et al., 2019a; Palacios 2019b; Roth et al., 2017; Perez-Lopez et al., 2015) reported all increased mean difference of birth weight in the range of 46g to 118g after maternal vitamin D supplementation during pregnancy, and the findings were significant in three (Maugeri et al 2019; Perez-Lopez et al 2015) of the six systematic reviews. Overall, the heterogeneity was high, but not significant in all studies. Only two of seven studies mentioned something about a dose-response. In one of these studies a significant dose-response effect was reported in only trials with mean baseline 25OHD concentrations of 30-50 nmol/L.

Mechanism / biological plausibility

Plausible biological mechanisms have been presented, see Chapter 5.3.

Upgrading factors

No upgrading factors have been evaluated for risk of LBW and for birth weight.

Conclusion weight of evidence vitamin D and low birth weight and birth weight

The evidence for the effect of maternal vitamin D supplementation on risk of LBW is demonstrated in several observational studies, however in combination with the evidence reported in the included meta-analysis of RCTs, we conclude that the effect of vitamin D supplementation in pregnancy on risk of LBW is graded "limited, suggestive".

Although both observational studies and RCTs reported a positive association of maternal vitamin D status during pregnancy with infant birth weight, the findings were not significant in all systematic reviews and the mother's baseline status was not clearly reported. More trials were included in the birth weight estimates compared to the risk of LBW trials; however, the dose of vitamin D supplementation was high in most RCTs and therefore the relevance for normal intake of vitamin D is unclear. In conclusion, the effect of vitamin D supplementation in pregnancy on birth weight is graded as "limited, suggestive".

5.3.5 Vitamin D and other health outcomes

Preeclampsia, gestational diabetes, multiple sclerosis, cancer (incidence), CVD and asthma have been evaluated in previous systematic reviews, but the evidence for an association between vitamin D and these outcomes were weak. We have not conducted literature searches to reevaluate the evidence for these outcomes.

Our systematic review of literature on nutrients and semen quality/male fertility did not retrieve any findings for potential associations between vitamin D and semen quality.

5.3.6 Vitamin D and cancer

Evidence for the association between vitamin D (foods containing, serum levels and supplements containing vitamin D) and cancer was summarised in the Third Expert Report of WCRF/AICR in 2018. The report concludes that there is evidence on the level "limited, suggestive" for that vitamin D decreases the risk of colorectal cancer. There was not found any evidence for association between vitamin D and any other cancer type (WCRF, 2018).

5.4 Iodine

Table 5.4-1 gives an overview of all the evaluated health outcomes related to iodine. These are based on the previous systematic literature review of health effects related to mild or moderate iodine deficiency in a recent benefit and risk assessment of iodization in household salt and salt used in bread and bakery products (VKM, 2020).

Our systematic review of literature on nutrients and semen quality/male fertility did not retrieve any findings for potential associations between iodine and semen quality.

Table 5.4-1 Overview of evaluated health outcomes for iodine in this benefit and risk assessment.

Health outcomes	Included in literature search	Argument for Yes/No	Comment
Neuro-development	No	No need to see if there is new evidence.	Limited, suggestive evidence in VKM (2020)
Goiter	No	No need to see if there is new evidence.	Established knowledge
Thyroid function	No	No need to see if there is new evidence.	Limited evidence in VKM (2020) (but effect on neurodevelopment is through thyroid function)
Birth outcomes and fertility	No	No need to see if there is new evidence.	Limited evidence in VKM (2020)

5.4.1 Iodine and neurodevelopment, thyroid function, and birth outcomes

In VKM (2020), a systematic literature review was performed to summarise the evidence for effect of mild to moderate iodine deficiency and health outcomes specifically relevant for iodine. Many studies described an inverse association between urinary iodine concentration (UIC) and different adverse neurodevelopmental outcomes. Negative health outcomes of mild to moderate iodine deficiency cannot be excluded but based on the existing literature and the use of guidelines for grading the evidence, the VKM Panel concluded that there is “limited, suggestive” evidence to support that mild to moderate iodine deficiency during pregnancy is associated with reduced neurodevelopment in children. The VKM Panel also concluded that there is “limited, suggestive” evidence to support that mild to moderate iodine deficiency in schoolchildren is associated with poorer neurodevelopmental outcomes (VKM, 2020). The weight of evidence was conducted in a similar manner in the VKM (2020) benefit and risk assessment of iodization of salt and bread as in this benefit and risk assessment of fish.

After carefully reviewing the articles on thyroid function and birth outcomes, the VKM Panel concluded that there is “limited, no conclusion” evidence to support that mild to moderate iodine deficiency is associated with thyroid dysfunction or has negative effects on birth outcomes (VKM, 2020).

5.4.2 Iodine and cancer

Evidence for the association between intake of iodine and cancer was not summarised in the Third Expert Report of WCRF/AICR in 2018. The lack of summary indicates lack of studies to conclude on the association for any cancer type on at least a “limited, suggestive” level (WCRF, 2018).

5.5 Selenium

There was no systematic review of selenium and health outcomes in the latest revision of NNR (2012). A review of observational studies and randomized controlled trials included in the Scientific Opinion on Dietary Reference Values for Selenium from EFSA that investigated the relationship between selenium and health outcomes did not provide evidence for additional benefits associated with selenium intake beyond that required for the levelling off of selenoproteins.

The NNR (2012) mention selenium supplementation for the primary prevention of cardiovascular disease and the potential effects of selenium on type 2 diabetes. According to NNR (2012), there were no statistically significant effects of selenium supplementation on all-cause mortality, CVD mortality, non-fatal CVD events or all CVD events (fatal and non-fatal). There was a small increased risk of type 2 diabetes with selenium supplementation, but this did not reach statistical significance. Selenium supplementation reduced total cholesterol, but this was not statistically significant.

Even though fish is one of the most important sources for selenium intake from the diet, and contribution from fish will be important to achieve selenium intakes in accordance with the recommended intakes, we have not encountered good or consistent evidence for any specific health outcome related to selenium (except for semen quality, see below). The following outcomes were evaluated: CHD/CVD, mortality, type 2 diabetes, immune function, cognitive function, preeclampsia, and lipid profile.

Based on previous work with dietary reference values and the health outcomes relevant for fish consumption, we have evaluated inclusion of associations between several health outcomes and selenium, but judged that it was not necessary to conduct updated systematic literature search and weight of evidence for associations between any specific health outcome and selenium. It was however conducted a literature search for all included nutrients and semen quality.

5.5.1 Selenium and semen quality

The current chapter summarizes the epidemiological evidence of selenium intake and parameters of male fertility from systematic reviews and meta-analyses. The search was performed without any limitations in time. We performed a systematic search for systematic reviews and meta-analyses (see Chapter 3.2.2 for details). The complete search strategies are given in Chapter 15, Appendix II.

Selenium is different than other antioxidant nutrients because they are involved in the mechanisms of cellular antioxidant defence by increasing the activity of the antioxidant enzyme glutathione peroxidase, and not by directly reacting with oxidant molecules (Burk 2002; Yavuz 2013 in Smits et al., 2019). It is suggested that selenium deficiency would make humans more susceptible to oxidative injury. Selenium is furthermore essential for normal spermatogenesis (Boitani 2008 in Smits et al., 2019).

Our systematic review of literature on nutrients and semen quality/male fertility resulted in inclusion of two meta-analyses investigating the association between selenium and various parameters of semen quality.

List of included and excluded studies and reason for exclusion is presented in Table 5.5.1-1.

Table 5.5.1-1 Included studies and reasons for exclusion of identified papers from the search of systematic reviews and meta-analysis of selenium intake and parameters of male fertility.

Included papers	Excluded paper and reasons for exclusions
Smits et al. (2019) Salas-Huetos et al. (2018)	Showell et al. (2011) – updated in Smits et al. (2019)

The meta-analyses are described in more detail below; first, main descriptions of the methods used and then main/selected results from each review. The included meta-analyses for selenium are the same as for LC n-3 FA (described in chapter 5.2.17) as they analysed several nutrients with antioxidant characteristics.

Smits et al. (2019) is a Cochrane systematic review and meta-analysis of RCTs investigating the effectiveness and safety of supplementary oral antioxidants in subfertile men. The authors performed a systematic literature search in The Cochrane Gynaecology and Fertility (CGF) Group trials register, CENTRAL, MEDLINE, Embase, PsycINFO, CINAHL, and two trials registers until February 2018. Risk of bias in the eligible papers was assessed by the Risk of bias assessment tool and Cochrane Handbook for Systematic Reviews of Interventions. One RCT is included in the meta-analyses for semen quality parameters. The risk of bias in the included studies varies, but generally, the risk of bias was moderate to low in the studies with the highest impact on the pooled analysis. The men included in the analysis were subfertile and were part of couples who had been referred to a fertility clinic. The endpoints included for selenium were sperm motility and concentration.

Salas-Huetos et al. (2018) is a systematic review and meta-analyses of RCTs investigating the effect of nutrients from supplements or foods on semen quality parameters in fertile and infertile men. The authors performed a systematic literature search in MEDLINE until October 2017. The quality of the eligible papers was assessed by ROB index based on 7 categories (O'Connor et al., 2008). Three RCTs are included in the meta-analyses for semen quality parameters. The risk of bias was low or unclear in the included study. The endpoints included for selenium were sperm concentration, motility, and morphology.

5.5.1.1. Results from the meta-analyses for selenium intake and semen quality parameters

Below is a summary table for supplemental selenium and semen quality parameters (Table 5.5.1.1-1) based on the identified meta-analyses.

Table 5.5.1.1-1 Summary of results from meta-analyses on selenium and semen quality parameters.

Author, year	Study design	Total no studies	No of cases	Comparison	Summary MD (95 % CI)	Heterogeneity	Overall result
Sperm motility							
Smits, 2019	RCT	1	16 subfertile in the intervention group	Selenium supplements <3 mo vs placebo	MD 14.90% (1.14, 28.66)		Significant increase in sperm motility $Z=2.12$, $P=0.03$
Salas-Huetos, 2018	RCT	3	143 in the intervention groups	Selenium supplements 100-300 µg/day, 3-11 mo vs placebo	MD 3.30% (2.95, 3.65)	$I^2=20\%$, $P=0.29$	Significant increase in sperm motility ($Z=18.59$, $P<0.001$)
Sperm concentration							
Smits, 2019	RCT	1	16 subfertile in the intervention group	Selenium supplements <3 mo vs placebo	MD 21.20×10^6 spz/mL (-11.43, 53.83)		No significant effect $Z=1.27$, $P=0.20$
Salas-Huetos, 2018	RCT	3	143 in the intervention groups	Selenium supplements 100-300 µg/day, 3-11 mo vs placebo	MD 3.91×10^6 spz/mL (3.08, 4.73)	$I^2=0\%$, $P=0.95$	Significant increase in sperm concentration ($Z=9.29$, $P<0.001$)

Sperm motility

Both Smits et al. (2019) and Salas-Huetos et al. (2018) found a significant increase in total sperm motility (MD=14.90%, 95% CI 1.14, 28.66 and MD=3.30%, 95% CI 2.95, 3.65, respectively) in their meta-analyses including both fertile and infertile men (the one RCT included in Smits et al was also included in Salas-Huetos et al).

Sperm concentration

Salas-Huetos et al. (2018) found significant increased sperm concentration (MD=3.91 x 10⁶ spermatozoa/mL, 95% CI 3.08, 5.4.73), whereas Smits et al. (2019) found no such effect. The one RCT included in Smits et al. (2019) was also included in Salas-Huetos et al. (2018)

Other sperm parameters

Salas-Huetos et al. (2018) found significant increased percentage of normal form spermatozoa (MD 1.87%, 95% CI 1.50, 2.24) in intervention group compared to placebo.

5.5.1.2 Heterogeneity selenium intake and semen quality parameters

The heterogeneity was low in the pooled analyses in Salas-Huetos et al. (2018) for the various semen quality parameters ($I^2=0-20\%$).

5.5.1.3 Dose-response relationship selenium intake and semen quality parameters

No dose-response curves were provided in the meta-analyses.

5.5.1.4 Weight of evidence for selenium intake and semen quality parameters

In this section the evidence of the association between selenium intake and semen quality parameters is weighed according to the WCRF criteria presented in the method chapter (Box 2 in Chapter 3.1.6, but applied to evaluation of systematic reviews and meta-analyses).

Published evidence of selenium intake and semen quality parameters

Overall, two meta-analyses showed significant increased sperm motility from supplemental selenium in populations of predominantly infertile or subfertile men (Smits et al., 2019 and Salas-Huetos et al., 2018). One meta-analyses showed significant increased sperm concentration from supplemental selenium (Salas-Huetos et al., 2018). However, there are few included RCTs with limited numbers of participants in the intervention groups. Studies in exclusively men without fertility problems are lacking.

Heterogeneity

No or low heterogeneity was observed in the meta-analysis for beneficial findings for various semen quality parameters.

Mechanism / biological plausibility

Plausible biological mechanisms have been presented.

Upgrading factors

No upgrading factors have been evaluated.

Conclusion weight of evidence selenium intake and semen quality parameters

Results from the two meta-analyses and systematic reviews of RCTs showed a beneficial effect of selenium supplementation on sperm motility, and one meta-analysis showed beneficial effect on sperm concentration in populations of predominantly infertile or subfertile men. One meta-analysis reported no effect on sperm concentration. However, there are few included RCTs with limited numbers of participants in the intervention groups. There is no unexplained heterogeneity. In conclusion, the evidence that there is a beneficial association between selenium and semen quality parameters such as sperm concentration and sperm motility in men experiencing fertility problems is "limited, suggestive".

5.5.1 Selenium and cancer

Evidence for the association between plasma selenium concentrations and cancer was summarised in the Third Expert Report of WCRF/AICR in 2018. The report concludes that there is evidence on the level "limited, suggestive" for that low plasma selenium concentrations increase the risk of prostate cancer. There was not found any evidence for association between selenium and any other cancer type (WCRF, 2018).

5.6 Vitamin B₁₂

There was no systematic review of vitamin B₁₂ and health outcomes in the latest revision of NNR (2012). IOM published Dietary Reference Intake values for vitamin B₁₂ in 1998 and EFSA published Dietary Reference Values for vitamin B₁₂ in 2015. The opinion from EFSA is not a systematic review.

Even though fish is an important source for vitamin B₁₂ intake from the diet, and contribution from fish will be important to achieve vitamin B₁₂ intakes in accordance with the recommended intakes, we have not encountered good or consistent evidence for any specific health outcome. The following outcomes were evaluated: cognitive function, CHD/CVD, bone health, colorectal cancer (Table 5.7-1). Based on previous work with dietary reference values and the health outcomes relevant for fish consumption, we have evaluated inclusion of associations between several health outcomes and vitamin B₁₂, but judged that it was not necessary to conduct updated systematic literature search and weight of evidence for associations between any specific health outcome relevant for fish consumption and vitamin B₁₂.

Our systematic review of literature on nutrients and semen quality/male fertility did not retrieve any findings for potential associations between vitamin B₁₂ and semen quality.

Evidence for the association between intake of vitamin B₁₂ and cancer was not summarised in the Third Expert Report of WCRF/AICR in 2018. The lack of summary indicates lack of studies to conclude on the association for any cancer type on at least a “limited, suggestive” level.

5.7 Chapter summary on LC n-3 FA, Vitamin D, iodine, selenium, and vitamin B₁₂

Health effects associated with the nutrients in this benefit and risk assessment are identified from published systematic reviews or meta-analyses.

All health outcomes from the literature search for fish were considered, and brief initial searches and expert judgements were performed to decide on outcomes relevant for specific nutrients. For the health outcomes that may be relevant for fish consumption but was not judged to be “probable” or “convincing” in the reviews from NNR (2012), we conducted systematic literature searches for systematic reviews and meta-analyses in the period 2015 - 2021, except for the search for semen quality, which was performed without any limitations in time.

The quality of the systematic reviews and meta-analyses identified as relevant was evaluated using an adapted version of AMSTAR tool for systematic reviews (see Chapter 3.1.3.2). The systematic reviews and meta-analyses judged to have quality A or B were included as input for the weight of evidence for the association between the specific nutrients and health outcomes. The WCRF criteria were used for the weight of evidence, see Chapter 3.1.6 for details about the WCRF criteria.

We considered evidence for the general population, including patient groups with type 2 diabetes, obesity, and musculoskeletal disorders.

The conclusions from the evaluation of associations between health outcomes relevant for fish consumption and the included nutrients are summarized in Table 5.7-1. All the conclusions for “probable” or “limited, suggestive” associations are protective/beneficial, except from the conclusion for atrial fibrillation.

Table 5.7-1 Summary of the conclusions for evidence for associations between included nutrients and health outcomes relevant for fish consumption.

Health outcome	Probable	Limited, suggestive	Limited, no conclusion	Established knowledge, basis for AR*
CVD mortality	LC n-3 FA (protective)			
CHD mortality	LC n-3FA (protective)			
All-cause mortality	Vitamin D (protective)		LC n-3 FA	
CVD incidence	LC n-3 FA (>1 g/day supplements) (protective)	LC n-3 FA (<1 g/day supplements) (protective)		
CHD incidence	LC n-3 FA (protective)			
MI incidence	LC n-3 FA (protective)			
Stroke incidence			LC n-3 FA	
Atrial fibrillation		LC n-3 FA (adverse)		
Type 2 diabetes			LC n-3 FA	
Child neurodevelopment (maternal exposure)		Iodine (beneficial)	LC n-3FA	

Health outcome	Probable	Limited, suggestive	Limited, no conclusion	Established knowledge, basis for AR*
Child neurodevelopment (exposure in child)			LC n-3FA	
Cognition in adults			LC n-3FA	
Cognitive decline in adults			LC n-3FA	
Mental health in adults (depression)		LC n-3FA (protective)		
PTB			LC n-3FA	
LBW		LC n-3FA (protective) Vitamin D (protective)		
Birth weight	LC n-3FA (protective)	Vitamin D (protective)		
Respiratory tract infection		Vitamin D (protective)		
Female fertility			Iodine	
Sperm concentration or quality		LC n-3 FA (beneficial) Selenium (beneficial)		
Bone fracture/fall	Vitamin D (protective)			
Goiter				Iodine (protective)
Keshan disease				Selenium (protective)
Pernicious anemia				Vitamin B ₁₂ (protective)
Colorectal cancer		Vitamin D (protective)		
Prostate cancer		Selenium (protective)		

*AR=average requirement.

5.8 References

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6 Adverse effects of contaminants where fish is an important contributor to the total dietary exposure

The focus in this benefit and risk assessment is health outcomes related to fish as such, described in Chapter 3. However, as illustrated in Figure 6-1, the health outcomes associated with fish consumption may be mediated through nutrients and contaminants. In this section, we have described contaminants where fish is an important source, and the adverse health effects associated with these contaminants.

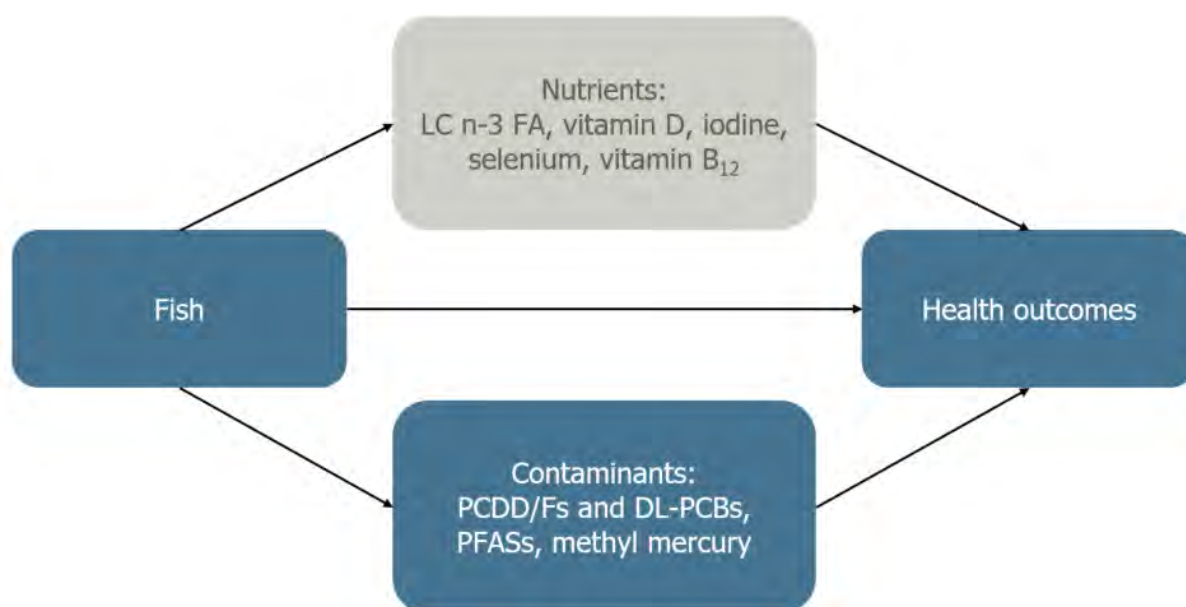


Figure 6-1 Illustration of how beneficial or adverse health effects from fish can be mediated through nutrients, contaminants or through unknown modes of action only ascribed to fish as such. This chapter covers contaminants in fish and associated adverse effects. Nutrients (illustrated in grey) are covered in Chapter 5.

6.1 Identification and characterisation of adverse health effects associated with exposure to the included contaminants

The considerations around selection of contaminants to include or exclude in the present benefit and risk assessment of fish consumption in Norway are described in Chapter 2 and

appendix IV, Chapter 17. The contaminant groups PCDD/Fs and DL-PCBs, and PFASs were listed in the mandate from the NFSA and are therefore to be included. Based on the evaluation described in Chapter 2, methylmercury is also included.

The identification of health effects related to the included contaminants are based on risk assessments from EFSA, in accordance with the procedure described in the protocol. The inclusion or exclusion of health effects are based on the criteria given in Table 3.2.1-1 in the protocol (VKM, 2020). In essence, VKM includes only health effects that are considered causal or critical in the previous risk assessments by EFSA and with exposures that are in a range that can be achieved by dietary intakes with present-day concentrations in regularly consumed foods in Norway. Further, the dose-response of the included effects are identified where possible.

6.1.1 Health effects from exposure to PCDD/Fs and DL-PCB

EFSA set a TWI of 2 pg TEQ/kg bw per week for PCDD/Fs and DL-PCBs in 2018 (see Chapter 2.3.1.) The critical effect was decreased sperm concentration at the age of 18–19 years in men that had been exposed to these substances prenatally, via breastfeeding and in childhood via food. It was based on a serum NOAEL of 7 pg PCDD/F TEQ_{WHO2005}/g lipid (median in the lowest quartile) when the men participating in the critical study (Minguez-Alarcon et al., 2017) were 9 years old, and back-calculation of the dietary intake in their mothers that would lead to this serum concentration at the age of 9. This back-calculation was performed by use of a pharmacokinetic model. The model took into account that the boys were breastfed for 12 months in their infancy with breast milk containing 5.9 pg TEQ/g lipid, which is the concentration in breastmilk resulting from a steady state exposure equal to the TWI. The model also took into consideration that children have two-fold higher intake than adults on a body weight basis, due to their higher energy requirement on a body weight basis (EFSA, 2018a).

The critical effect was based on overall weight of evidence considerations, including results from animal studies and mode of action. A decrease in sperm concentration has been reported in two other prospective cohort studies where boys had been exposed in infancy or pre-puberty to elevated levels of TCDD in addition to the background exposure to PCDD/Fs and DL-PCBs present in food in Italy at the time when the studies were conducted (Moccarelli et al., 2008; 2011). Effect on male reproduction measured as a decrease in daily sperm production (Faqi et al., 1998) was also considered as critical effect of TCDD exposure in mice (and was the basis for the previous TWI for PCDD/Fs and DL-PCBs set by SCF in 2001 (SCF, 2001). EFSA indicated that if the TWI would have been set based on animal data on sperm production, it would have been in similar range as the one from 2018 based on human studies (2 pg TEQ/kg bw per week) due to the need for additional uncertainty factors for extrapolation between animals and humans and to account for inter-individual differences.

Other effects that were also considered causal due to PCDD/F exposure were chloracne and other dermal effects, lower sex ratio at birth (boys:girls), developmental effects on teeth and

increased thyroid-stimulating hormone (TSH) in newborns (EFSA 2018). The effect on development of teeth (enamel hypomineralization) after exposure via breastmilk was estimated by EFSA to be associated with a concentration in breast milk of around 9.2 pg PCDD/F-TEQ/g fat. DL-PCBs were not considered in the studies addressing these effects. Data on breastmilk from first time mothers in Uppsala, Sweden indicate that concentrations in this range is present in some women, although the majority has much lower concentrations (Gyllenhammar et al., 2021). The situation is expected to be similar in Norway, based on geographical and cultural similarities, and that concentration in breastmilk in Norway in 2006 was in similar range as it was in Uppsala (VKM 2014). Given that the effects on sperm concentrations occur at lower exposures, possible effects on teeth development are not considered in the present benefit and risk assessment. The other effects considered causally related occur at substantially higher exposure levels and are not relevant at current exposure from food.

The critical study by Minguez-Alarcon et al. (2017), "The Russian Children's Study", included participants in 2003-2005 in Chapaevsk in Russia when the boys were 8-9 years of age. Pubertal development was assessed in the boys by yearly examination to age 17-18 (sperm samples taken one year later). Chapaevsk is a city with former production of chlorinated pesticides that ceased in 1987, thus, 7-9 years before the boys were born. Persistent chlorinated pesticides (HCB, β HCH and DDE) have been analysed and controlled for in the study, in addition to BMI, smoking status, alcohol consumption, season and abstinence time.

Serum concentration of 2,3,7,8-TCDD alone (p trend 0.005) and total PCDD TEQ (p trend 0.02) at age of 8–9 years was associated with a decreased sperm concentration at age 18-19 (n=133 participants delivering 256 semen samples). This was not the case for PCDF-TEQ (p-trend 0.78). However, the association was observed for the sum of PCDD/Fs (p-trend 0.04). DL-PCBs or total TEQ was not associated (p-trend 0.73 and 0.61, respectively). EFSA noted that the lack of association could be due to uncertainties connected to the TEFs, in particular for non-ortho PCBs (See 2.3.2.1).

NDL-PCBs were not associated with sperm concentration, and adjustment for NDL-PCBs slightly strengthened the association. Adjustment for chlorinated pesticides did not alter the associations.

With increasing TCDD concentration, there was a linear decrease in sperm concentration across the quartiles, reaching 40% decrease in the highest quartile. For the sum of PCDD/F-TEQ, there was a 36% decline in the second quartile and the sperm concentration did not decrease further. The mean sperm concentration in the lowest quartile of PCDD/F-TEQ was 64 million/mL and the mean sperm concentrations in quartile 2–4 was about 40 million/mL. This difference was considered biologically relevant by EFSA (EFSA, 2018).

Infertility affects about 15% of all couples worldwide. Male factors such as decreased semen quality contribute to around 40% of the cases (Falsig, Glerup and Knudsen, 2019). VKM notes that there are many environmental and genetic factors that can lead to decreased semen quality and exposure to PCDD/Fs and DL-PCBs above the TWI of 2 pg TEQ/kg bw per

week is regarded as a contributing factor but not sufficient by itself to result in male infertility.

The exposure to PCDD/Fs and DL-PCBs has shown a strong decline since the 1980s. From 1986 to 2005 the concentration of PCDD/Fs, DL-PCBs and NDL-PCBs in breastmilk from first-time mothers in Norway decreased by approximately 70% (VKM, 2013). The decline worldwide has been documented by WHO-coordinated monitoring of pooled breast milk samples from first time mothers (Van den Berg et al., 2017, EFSA 2018). Recent data from both WHO and Swedish mothers indicate that the decrease may be levelling off (EFSA 2018, Gyllenhammar et al., 2021). According to EFSA 2018 the pooled samples collected by WHO across European countries in 2014/2015 had concentrations of 2.4-5.7 pg WHO₂₀₀₅-TEQ/g fat for PCDD/Fs and 4.8–9.6 pg WHO₂₀₀₅-TEQ/g fat or the sum of PCDD/Fs and DL-PCBs (EFSA 2018a).

The CONTAM Panel noted that breastfed infants are known to have a higher exposure than toddlers (from 1 to < 3 years) and other children (from 13 to <10 years). The TWI was set to prevent a level in breast milk that would result in serum levels in children that have been associated with adverse effects. This issue was taken into consideration when setting the TWI, and therefore the exposure of breastfed infants should not be compared to the TWI. If the mother until, and during, the pregnancy has had a dietary intake that is lower than the TWI, it will prevent that the concentration of PCDD/Fs and DL-PCBs in the breast milk will reach a level which can increase the risk of health effects in the breastfed child later in life. EFSA also took into consideration that children, due to their higher energy demands relative to the body weight, have two-fold higher intake of dioxins and DL-PCBs from food than adults. Because higher intake in childhood (in both mothers and children) was used in back-calculations of the dietary intake leading to the critical concentration in boys aged 8-9 years, children (< 8 years) can have dietary intake of PCDD/Fs and DL-PCBs two-fold the TWI (after being breastfed for 12 months by a mother with life-long dietary intake equal to the TWI) before they will reach the critical serum concentration. A two-fold exceedance of the TWI for children is therefore not associated with higher risk than adult exposure equal to the TWI.

6.1.2 Health effects from exposure to PFAS

In 2020, EFSA CONTAM Panel set a new TWI for the sum of the four PFASs PFOA, PFNA, PFHxS, and PFOS at 4.4 ng/kg bw per week, assuming equal potencies (EFSA, 2020). It was derived from back-calculation of concentrations of these compounds in serum in children in the critical human study to the corresponding chronic dietary maternal intakes, taking breastfeeding into consideration, as described below. The four PFASs are those that are present at highest concentrations in human serum. The data available to EFSA were insufficient to derive potency factors for the different PFASs. In absence of evidence, equal potencies were assumed as a conservative approach. The TWI for the sum of the four PFASs replaces the previous temporary TWIs set for PFOS and PFOA separately in 2018 (EFSA 2018b).

The present RBA is restricted to the same four PFASs covered by the TWI: PFOA, PFNA, PFHxS, and PFOS. The basis for focusing on these substances can be found in Appendix IX, Chapter 22. EFSA concluded that effects on the immune system, which were observed at the lowest serum PFAS levels in both animals and humans, is the critical effect.

In human studies, various associations between serum levels of PFOS and PFOA and a number of outcomes have been reported and particular interest was focussed on (i) increased serum total and LDL cholesterol (risk factor for cardiovascular disease), (ii) increased ALT levels (indicating effects on liver cells), (iii) reduced birth weight and (iv) effects on the immune system in the EFSA risk assessment of PFOS and PFOA (EFSA 2018b).

In the updated EFSA opinion from 2020, other outcomes of PFAS exposure than effects on the immune system were considered less relevant due to possible confounding factors, small effect sizes, or other uncertainties. Furthermore, EFSA noted that the new TWI is protective for these other potential critical endpoints (EFSA, 2020).

Other effects than those on the immune system are therefore not considered in the present RBA.

The TWI set by EFSA on effects on the immune system was based on an overall consideration and derived from human studies, but with support from similar effects from animal experimental studies. The mode of action (MoA) behind the observed immune effects are unknown.

In experimental animal studies reviewed by EFSA (EFSA 2020), a decrease in T-cell dependent antibody response in mice was the most sensitive effect after PFOS exposure. The most sensitive mouse study had a no observable adverse effect concentration (NOAEC) of PFOS in serum of 17.8 ng/mL. Effects on the immune response has also been shown in animals after PFOA exposure. Effects were also observed in rats and also with other PFASs, but the studies available did not allow a formal comparison of potential differences in potencies.

Results from six vaccination response studies in children and adults were identified in the EFSA 2020 opinion (Grandjean et al., 2012; Granum et al., 2013; Looker et al., 2014; Kielsen et al., 2016; Stein et al., 2016b; Abraham et al., 2020). Three of these studies show, for several PFASs, relatively strong inverse associations with antibody response following vaccination to tetanus and diphtheria in children (Abraham et al., 2020; Grandjean et al., 2012) and adults (Kielsen et al., 2016). One study showed an inverse association between maternal PFAS levels and antibodies to rubella in children (Granum et al., 2013) and one study showed some, but more modest inverse associations with antibody titres to influenza in adults (Looker et al., 2014). The null findings by Stein et al. (2016b) on influenza vaccination do not contradict these results, as most subjects did not respond to the vaccination. Associations with antibody titres falling below protective levels were also reported (Grandjean et al., 2012; Looker et al., 2014).

A decrease in vaccination response is considered a good indicator of suppression of the immune system function and is seen as adverse by the scientific community, as summarised by WHO/IPCS (2012) in the Guidance for immunotoxicity risk assessment for chemicals. This may in particular apply to vulnerable population groups, i.e. infants and the elderly, considering their higher infection risk.

Two of the cohort studies on vaccination responses in children were considered potentially most critical in the EFSA opinion on PFASs, a prospective study from the Faroe Islands (Grandjean et al., 2012) and a cross sectional study from Germany (Abraham et al., 2020). The study on children in the Faroe Islands (Grandjean et al., 2012) showed several inverse associations between serum levels of PFOA, PFNA, PFHxS and PFOS, as well as the sum of PFOA, PFNA, PFHxS and PFOS at five years of age, before booster vaccination, and antibody titres against diphtheria and tetanus at both the age of 5, shortly after booster vaccination, and at 7.5 years. The dose-response could not be benchmark modelled with acceptable certainty and a NOAEC serum level at the age of 5 years for the sum of PFOA, PFNA, PFHxS and PFOS of 27.0 ng/mL, based on decreased diphtheria antibody titres at the age of 7 years, was identified.

In the study on children from Germany (Abraham et al., 2020), an inverse association was observed between serum levels of PFOA, but also the sum of PFOA, PFNA, PFOS and PFHxS, and antibody titres from vaccination against haemophilus influenzae type b (Hib), diphtheria and tetanus in serum sampled from 1-year-old children predominantly breastfed for a median duration of 7.4 months (Abraham et al., 2020; EFSA, 2020). A lowest BMDL₁₀ of 17.5 ng/mL at the age of 1 year was derived for the sum of PFOA, PFNA, PFOS, and PFHxS based on an association with reduction in antibody titres against diphtheria (EFSA, 2020). For PFOS, PFHxS and PFNA alone, no significant associations were observed in this study.

The possible confounding by a number of contaminants like PCBs, dioxins, organochlorine pesticides, lead and mercury was examined in both these studies, but adjustments for other contaminants had no effect on the observed associations.

According to EFSA (2020), at the highest quintile the mean antibody titres for Hib, diphtheria and tetanus were 63%, 42% and 49% lower, respectively, than those in the first quintile in the study from Germany. At the LOAEC in the study from the Faroe Islands, the diphtheria antibody titres were around 50% lower than at the NOAEC. Furthermore, the proportion of children having vaccination titres below the protective limit after vaccination was also increased at higher PFAS levels in that study. It was noted by EFSA that such decreases in antibody responses are clearly adverse on a population level, not only in terms of protection against the pathogen to which the vaccine is directed, but also in terms of general immunologic defence against other pathogens. There are some data suggesting that PFAS exposure is associated with increased infection risk, and also with decreased specific antibody formation after virus exposure in infants.

The study by Abraham et al. (2020) was considered by EFSA to be the most sensitive study and therefore, the BMDL₁₀ of 17.5 ng/mL was used to estimate the daily intake by mothers that would result in this critical serum concentration at 1 year of age in breastfed children.

This daily intake was subsequently used to derive a HBGV for the sum of PFOA, PFNA, PFHxS and PFOS. Using a toxicokinetic model, and assuming 12 months of breastfeeding, it was estimated that the BMDL₁₀ in infants corresponds to an intake by the mother of 0.63 ng/kg bw per day for the sum of the four PFASs. Such intake would result in a serum level in the mother at 35 years of age of 6.9 ng/mL. The CONTAM Panel decided to use the daily intake of 0.63 ng/kg bw per day as the starting point and established a group tolerable weekly intake (TWI) of $7 \times 0.63 = 4.4$ ng/kg bw per week for the sum of PFOA, PFNA, PFHxS and PFOS.

Since a decreased vaccination response is regarded as a risk factor for disease rather than a disease, and since the study was based on infants, which are expected to be a vulnerable population group, no additional uncertainty factors for potential intraindividual differences in toxicokinetics and toxicodynamics were deemed necessary.

This TWI should prevent that mothers reach a body burden that results in levels in milk that would lead to serum levels in the infant, associated with decreases in vaccination response. As a result, the higher exposure of breastfed infants is taken into account in the derivation of the TWI and the intake by infants should therefore not be compared with this TWI.

6.1.2.1 *Serum concentrations of PFASs in EU and in Norway*

The calculated dietary exposure of most part of the European population exceeds the TWI for the sum of four PFASs, according to EFSA (2020). There are large uncertainties associated with the exposure calculations due to limitations in the available occurrence data and sensitivity of the analytical methods applied. However, exceedance of the TWI is also indicated by the concentrations in human blood.

In the risk assessment in 2020, EFSA summarized data on concentrations of PFASs in blood in Europe. Several studies from Norway were included in the summary and more have been published after that (Poonthong et al., 2017; Haug et al., 2011; Berg et al., 2014; Gützow et al., 2012; Granum et al., 2013; Hansen et al., 2016; Papadopoulou et al., 2016; Averina et al., 2018; Averina et al., 2019; Averina et al., 2020). The PFAS concentrations in Norway are in similar range as those in the rest of Europe. PFOS shows the highest concentration in adults, followed by PFOA. In children, the concentration of PFOA is approximately similar as in adults. In both adults and children, the concentrations of PFHxS and PFNA are lower than for PFOS and PFOA. According to EFSA (2020), the median concentration of PFOS, PFOA, PFHxS and PFNA were respectively 7.7, 1.9, 0.67 and 0.61 ng/mL in European adults. In children, the respective concentrations were 3.2, 3.3, 0.79, and 0.60 ng/mL. The concentration of these four PFASs covers approximately 90% of the total of all PFASs that have been analysed in human serum (EFSA, 2020).

6.1.3 Health effects from exposure to methylmercury

Unborn children constitute the most vulnerable group for developmental effects of methylmercury exposure. EFSA in 2012 reduced the tolerable weekly intake (TWI) for methylmercury from 1.6 (set by WHO in 2004) to 1.3 µg/kg bw per week, expressed as

mercury, based on neurodevelopmental effects in prenatally exposed children. The reason for lowering the TWI was evidence from a more recent nutrition cohort from the Seychelles (Lynch et al., 2011) in which the possible effect of fish consumption on neurodevelopment had been taken into consideration, providing evidence that the beneficial effect of fish consumption observed levelled off at very high maternal methylmercury intake. The TWI was derived from an apparent NOEL in this cohort in combination with a BMDL₀₅ on neurodevelopment in children from the Faroe Islands.

EFSA explained how the TWI for methylmercury was derived as following: *"The mean of the apparent NOEL from the Seychelles nutrition cohort at 9 and 30 months (11 mg/kg maternal hair) and the BMDL₀₅ from the Faroese cohort 1 at age seven years (12 mg/kg in maternal hair), resulting in 11.5 mg/kg maternal hair, was used as the basis for derivation of a health-based guidance value. By application of a maternal hair to maternal blood ratio of 250, the maternal hair mercury concentration with no appreciable adverse effect was converted into a maternal blood mercury concentration of 46 µg/L. Using a one-compartment toxicokinetic model, the value of 46 µg/L in maternal blood was converted to a daily dietary mercury intake of 1.2 µg/kg b.w. A data-derived uncertainty factor of 2 was applied to account for variation in the hair to blood ratio. In addition, a standard factor of 3.2 was applied to account for interindividual variation in toxicokinetics, resulting in a total uncertainty factor of 6.4. A tolerable weekly intake (TWI) for methylmercury of 1.3 µg/kg b.w. expressed as mercury, was established."*

Methylmercury accumulates in the body and crosses the placental and blood-brain barriers. Total mercury in hair and blood are routinely used as biomarkers of methylmercury exposure. Of note, hair and nails contain almost exclusively methylmercury, whereas blood contains both inorganic mercury and methylmercury. Most methylmercury is present in red blood cells, whereas serum contains a higher proportion of inorganic mercury than whole blood. However, in fish-eating populations the blood methylmercury concentration is much larger than the inorganic mercury concentration and therefore total mercury concentration in whole blood serves as a good biomarker of methylmercury exposure. Total mercury in urine is a marker of inorganic mercury exposure.

A methylmercury concentration in hair of 11.5 mg/kg corresponds to 23 µg/L blood when applying a hair:blood ratio of 1:250 and taking into consideration an uncertainty factor of 2 in order to account for the variability in this ratio, in accordance with parameters used by EFSA (2012).

The mean concentration of total mercury in whole blood from 2982 pregnant women participating in The Norwegian Mother, Father and Child cohort study was 1.2 µg/L, whereas the 95-percentile concentration was 2.8 µg/L. The highest concentration reported among these women was 14 µg/L (Caspersen et al., 2019).

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7 Background data and methods used in exposure estimation of fish, nutrients, and contaminants

In this chapter we present the concentration of the included nutrients in fish, and the occurrence of the included contaminants both in fish and other foods. We also describe the food consumption surveys that were used and methods for how the fish intake was calculated in this assessment. Furthermore, methods for weighting the survey data to increase national representativity and for estimating the habitual intake of fish and nutrients and exposure to contaminants, from a limited number of survey days, are described.

7.1 Content of nutrients in fish

Fish is a source of several nutrients in the human diet, including vitamin D, vitamin B₁₂, iodine, selenium, and the marine long chain n-3 fatty acids (LC n-3 FA) eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA). The concentrations of these nutrients in the fish species that constitute the majority of fish eaten in the Norwegian diet (please refer to Chapter 8 for calculations of intake of fish in Norway) are presented in this chapter. The values presented here, and thus used in the present benefit and risk assessment, originate from the food composition database in the food and nutrient database and calculation system KBS at the Department of Nutrition, University of Oslo (Nordberg et al., 2018; Rimestad et al., 2000). The KBS food composition database is an extended version of the Norwegian food composition table (www.matvaretabellen.no). The KBS food composition database version AE-18 was used in the present assessment, which was updated in 2017-2018. When participants in the national dietary surveys reported intake of food items not included in the AE-18 database, food composition data from the databases AE-14 or N3 were used. These were updated in 2013-2014 and 2009-2010, respectively.

The nutrient values in fish used in this assessment originate from the Norwegian food composition table (www.matvaretabellen.no) and the food composition database in KBS at the University of Oslo. Both food composition databases compile food composition data according to the standard methods for food composition data compiling, as described in Greenfield and Southgate (2003) and the food composition guidelines from EuroFIR/FAO guidelines (EuroFIR 2006; EuroFIR2009). Food nutrient values are updated on a regular basis. The food composition data in any food composition database is a mix of data from different compiling methods. The main methods for compiling food composition data are analytical projects (sample collection and analyses in food samples) and indirect methods, when analytical data are not available. Indirect methods include nutrient estimates from recipes and borrowing data from other food composition databases. The nutrient values used in the present assessment are mainly analytical values derived from the Institute for Marine

Research, Bergen, Norway. The exceptions were 1) values for tuna and cod roe that were partly derived from analytical reports from the Swedish food composition table (Livsmedelsverket.se) and the Finnish food composition table (www.fineli.fi); 2) values for cod liver supplements that were derived from the declared product information; 3) iodine values for farmed trout that were estimated based on analytical values for farmed salmon. The representability of the food composition data is evaluated, and quality assessed through the standard procedures for food composition compiling described above. These procedures include evaluation of in which foods the original food composition data was analyzed or compiled, and weighing of samples with regard to season and/or market shares. Thus, VKM consider the representability of food composition data used in the present assessment valid.

In the description of nutrient content, the fish species are divided into lean and fatty fish (www.matvaretabellen.no). Only fish species relevant to this assessment are described. Fatty and lean fish differ in the total amount of fat in the fish, and where in the fish the fat is stored. Lean fish store fat in the liver and fatty fish store fat in muscles, i.e., in the fish fillet, and under the skin. Traditionally, lean fish was defined as fish with less than 2 g fat per 100 g fish fillet, medium fatty fish was defined as fish with between 2 and 8 g fat per 100 g fish fillet, and fatty fish as fish with more than 8 grams of fat per 100 g fish fillet. For simplicity, today, lean fish is defined as having less than 5 g fat per 100 g fish fillet, and fish with fat content above this level is defined as fatty fish (www.matvaretabellen.no; VKM, 2006). This definition is used in the present exposure assessment. Total fat and LC n-3 FA

The fatty acid content of fish was updated in KBS, and in the Norwegian food composition table in the years 2017-2018, as the result of a compiling project conducted by the University of Oslo (Norberg et al., 2018). Values for total fat and the sum of the fatty acids EPA, DPA and DHA are given in Table 7.1-1 for typically eaten fish species in the dietary surveys. The highest level of sum EPA, DPA and DHA was found in mackerel.

Table 7.1-1 Mean concentrations of total fat, and sum of EPA, DPA and DHA, in lean and fatty fish, and fish offal, per 100 grams raw fish fillet/food¹.

Food item	Total fat g/100 g	Sum EPA+DPA+DHA mg/100 g
Lean fish (<5 % fat)		
Atlantic cod	1.1	250
Haddock	0.2	52
Plaice	2.6	598
Saithe	0.3	94
Tuna, canned	1.0	295
Fatty fish (≥5% fat)		
Farmed Atlantic salmon	16	2869
Mackerel ²	25	4755
Herring	20	2969
Atlantic halibut	6.1	879
Farmed trout	10	2033
Roe and liver		
Cod roe	2.9	1051
Cod liver	59	10930

¹Norwegian Food composition table (www.matvaretabellen.no), Norwegian Food Safety Authority; KBS, AE-18 University of Oslo.

²Mackerel is autumn mackerel, since this variety is used in products like mackerel in tomato sauce.

The total fat content of farmed Atlantic salmon has decreased the last decades. These changes are reflected in database version AE-18.

7.1.1 Vitamins and minerals

The concentrations of vitamin D, vitamin B₁₂, iodine and selenium in raw fish and fish offal are given in table 7.1.1-1.

Table 7.1.1-1 Concentration of vitamin D, vitamin B₁₂, iodine and selenium in lean and fatty fish and fish offal, given per 100 grams raw fish fillet/food¹.

Food item	Vitamin D µg/100 g	Vitamin B ₁₂ µg/100 g	Iodine µg/100 g	Selenium µg/100 g
Lean fish (<5 % fat)				
Atlantic cod	2.0	1.5	279	22
Haddock	0.5	2.0	400	30
Plaice	6.0	1.2	14	30
Saithe	0.8	4.0	272	30
Tuna, canned	1.6	4.8	17	200
Fatty fish (≥5% fat)				
Farmed Atlantic salmon	10	3.5	6	30
Mackerel ²	5.4	7.4	20	60
Herring	11.5	12.0	16	50
Atlantic halibut	9.7	0.7	21	60
Farmed trout	6.9	4.8	7	30
Roe and liver				
Cod roe	12	10	195	9
Cod liver	90	43	355	80

¹Norwegian Food composition table (www.matvaretabellen.no), Norwegian Food Safety Authority; KBS, AE-18 University of Oslo.

²Mackerel is autumn mackerel, since this variety is used in products like mackerel in tomato sauce.

7.1.1.1 Vitamin D

The concentrations of vitamin D in fish and fish offal are given in table 7.1.2-1. The content of vitamin D (as D₃) is highest in the fatty fish species and varies in fatty fish between 5.4 to 11.5 µg/100 g in raw fish fillet. The content of vitamin D varies independently of fillet lipid content. In lean fish, concentrations range between 0.5 and 6.0 µg/100 g raw fish fillet. The highest concentration of vitamin D is, by far, in cod liver.

7.1.2.2 Vitamin B₁₂

Vitamin B₁₂ is found in both lean and fatty fish, in the range 0.7 to 12 µg/100 g raw fish fillet. The concentrations of vitamin B₁₂ in fish and fish offal are given in table 7.1.2-1. The highest concentrations of vitamin B₁₂ are found in herring, mackerel, and cod liver.

7.1.2.3 Iodine

Fish, and in particular lean seawater fish, is an important dietary source for iodine. The concentrations of iodine in fish and fish offal are given in table 7.1.2-1. For the lean seawater fish, Atlantic cod and haddock, two frequently eaten lean fish species in the Norwegian diet, have iodine concentrations of 279 and 400 µg/100 g of raw fish fillet, respectively. Salmon and mackerel, two frequently eaten fatty fish species in the Norwegian diet, have iodine concentrations of 6 and 20 µg/100 g of raw fish fillet, respectively.

7.1.2.4 Selenium

Selenium is found in all fish species in the Norwegian food composition table, ranging from 22 to 200 µg/100 g raw fish fillet. The selenium concentrations in salmon, mackerel, Atlantic cod and haddock, among others, is presented in table 7.1.2-1.

7.2 Concentrations of contaminants in fish and other food

7.2.1 PCDD/Fs and DL-PCBs

The available occurrence data for PCDD/Fs and DL-PCBs in food produced in Norway (VKM database), as well as the available information reported to EFSA and published by EFSA (2018) is described in detail in "Risk assessment of dioxins, furans and dioxin-like PCBs in food in Norway" (VKM, 2022). Analytical results from suspect sampling or from area with particular pollution were not included in the VKM database. EFSA did not include samples resulting from suspect sampling in the database. The exposure in the present assessment is based on the data that combines occurrence data from Norwegian food and data from the EFSA database, when data from Norwegian food was lacking. This was seen as most appropriate because it is expected to be more representative of the actual exposure in Norway, given that the mean occurrence levels seem lower in food produced in Norway than the mean concentration in food reported to EFSA, and given that the Norwegian degree of self-sufficiency of fish, meat, eggs, and dairy products is high (VKM 2022). The number of samples from Norwegian farm animals (sheep, cattle, pork, and chicken) is low and thus associated with higher uncertainty than samples from milk and egg (see Table 7.2.1-1 below and uncertainties, Chapter 11). For fish, the number of Norwegian samples is high, although different for different species.

As a conservative approach, only upper bound (UB) occurrences and exposures are included in the present benefit and risk assessment (at UB, all values reported below LOQ, level of quantification, were replaced by the LOQ). For PCDD/Fs and dl-PCBs, the mean UB dietary exposure was on average 1.9 times higher than the LB exposure, see VKM (2022).

The contribution from fruits and vegetables to exposure of PCDD/Fs and dl-PCBs is uncertain, in particular for UB estimates (see Chapter 11.2.4, and VKM 2022, Chapter 3.1.1). For this reason, in the present opinion, the contribution from fruits, vegetables, and potatoes was not included in the exposure calculations (Chapter 8 and 9).

For a more detailed description on occurrence in food, including lower bound (LB) concentrations (at LB, all values reported below LOQ, were replaced by 0). See also VKM (2022).

For each food item eaten in the national dietary surveys, a PCDD/F and DL-PCB concentration value was calculated. The concentration in the given food in whole weight (ww) was based on the occurrence data per gram of fat (Table 7.2.1-1) and the fat percentage in the food composition table (www.matvaretabellen.no). For instance, the PCDD/F and DL-PCB concentration in milk fat was multiplied by the fat percentage in different cheeses to find the PCDD/F and DL-PCB concentrations in cheese.

Table 7.2.1-1 Mean and P95 upper bound concentration of PCDD/Fs and DL-PCBs in food used as basis for the exposure assessment (pg TEQ_{WHO 2005}/g).

Food	pg/g ww ^c or fat	Sum of PCDD/Fs (17 congeners)			Sum of PCDD/ Fs and DL- PCBs (29 congeners)			Data source ^b
		n ^h	Mean	P95 ^a	n	Mean	P95 ^a	
Lean fish (<5 % fat)								
Atlantic cod	ww	60	0.04	0.06	60	0.064	0.117	VKM
Cod and whiting	ww	384	0.08	0.21	375	0.284	0.88	EFSA
Plaice	ww	54	0.12	0.315 ^d	54	0.521	1.031 ^d	VKM
Plaice	ww	63	0.218	0.736	61	0.505	1.617	EFSA
Rose fish	ww	746	0.207	0.436	746	0.594	1.604	VKM
Saithe	ww	51	0.03	0.05 ^d	51	0.093	0.162 ^d	VKM
Wolffish	ww	38	0.048	0.081 ^d	38	0.090	0.192 ^d	VKM
Sea catfish and wolf-fish	ww	69	0.087	0.491	69	0.155	0.643	EFSA
Fatty fish (≥5% fat)								
Farmed Atlantic salmon	ww	1074	0.242	0.360	1074	0.555	0.829	VKM
Trout, farmed	ww	24	0.234	0.325 ^d	24	0.488	0.682 ^d	VKM
Salmon and trout	ww	907	0.33	1.95	857	0.94	5.82	EFSA
Mackerel (autumn)	ww	541	0.389	1.011	541	1.002	2.905	VKM
Mackerel	ww	322	0.43	1.24	317	1.44	4.78	EFSA
Herring	ww	150	0.465	0.713	150	0.895	1.404	VKM
Herring	ww	401	1.25	1.95	399	2.39	6.36	EFSA
Atlantic halibut	ww	389	0.375	0.991	389	1.392	3.449	VKM
Halibut	ww	466	0.35	0.94	466	1.16	3.36	EFSA
Other seafood								
Crab, brown meat	ww	435	2.057	4.774	435	3.617	8.063	VKM
Crab, brown and white	ww	275	0.63	2.28	274	1.27	4.18	EFSA
Roe and liver								
Cod liver	ww	1207	3.30	7.06	1207	16.09	38.31	VKM
Fish offal	ww	911	4.89	13.1	911	22.0	60.5	EFSA
Cod roe-liver pâté	ww	2	3.1	na ⁱ	2	0.55	na	VKM
Cod roe ^e	ww	4	na	na	4	na	na	VKM
Marine oils (supplement)								
Cod liver oil	fat	12	0.247	0.502 ^d	12	1.080	4.066 ^d	VKM
Cod liver oil	fat	7	0.631	na	7	3.093	na	EFSA

Food	pg/g ww ^c or fat	Sum of PCDD/Fs (17 congeners)			Sum of PCDD/ Fs and DL- PCBs (29 congeners)			Data source ^b
		n ^h	Mean	P95 ^a	n	Mean	P95 ^a	
Fish oil	fat	25	1.130	4.552 ^d	25	5.135	23.192 ^d	VKM
Fish oil	fat	21	0.244	na	21	1.336	na	EFSA
Meat								
Cattle	fat	19	0.232	0.417 ^d	19	0.387	0.804 ^d	VKM
Cattle	fat	869	0.61	1.68	866	2.23	6.08	EFSA
Beef liver	ww	183	0.07	0.19	181	0.15	0.41	EFSA
Chicken	fat	5	0.333	na	5	0.576	na	VKM
Chicken	fat	573	0.26	0.58	565	0.43	1.09	EFSA
Pork	fat	7	0.130	0.160 ^d	7	0.173	0.227 ^d	VKM
Pork	fat	459	0.162	0.36	454	0.236	0.52	EFSA
Liver pâté ^e	fat	3	na	na	3	na	na	VKM
Pâté, pork liver	fat	24	0.27	na	24	0.30	na	EFSA
Pork liver	ww	5	0.140	na	55	0.13	na	EFSA
Reindeer ^f	fat	19	3.10	na	19	6.89	na	VKM
Sheep	fat	7	0.365	0.696 ^d	7	0.592	0.964 ^d	VKM
Sheep	fat	241	0.57	1.43	240	1.05	2.56	EFSA
Milk								
Cow milk	fat	62	0.279	0.632	60	0.413	0.786	VKM
Cow milk	fat	948	0.449	0.98	935	0.916	2.01	EFSA
Egg								
Whole egg, chicken	fat	146	0.468	1.251	143	0.579	1.359	VKM
Whole egg, chicken	fat	2328	0.582	1.79	2312	1.31	4.32	EFSA
Grain								
Wheat bread and rolls	ww	2	0.018	na	0	na	na	EFSA
Fruit, vegetables, and potatoes								
Apple	ww	3	0.151	na	3	0.160	na	EFSA
Brussel sprouts	ww	1	0.008	na	1	0.012	na	EFSA
Courgettes	ww	12	0.018	na	5	0.019	na	EFSA
Tomatoes	ww	2	0.019	na	2	0.023	na	EFSA
Main crop potatoes	ww	1	0.005	na	1	0.005	na	EFSA

Food	pg/g ww ^c or fat	Sum of PCDD/Fs (17 congeners)			Sum of PCDD/ Fs and DL- PCBs (29 congeners)			Data source ^b
		n ^h	Mean	P95 ^a	n	Mean	P95 ^a	
Vegetables and vegetable products	ww	164	0.05	0.21	136	0.08	0.28	EFSA
Olive oil	fat	43	0.105	na	43	0.172	na	EFSA
Rapeseed oil	fat	15	0.055	na	15	0.063	na	EFSA
Sunflower oil	fat	88	0.131	na	88	0.158	na	EFSA
Foods for infants and young children	ww	500	0.01	0.04	472	0.02	0.07	EFSA

na: Not available.

^a P95: 95th percentile.

^b EFSA: data from EFSA (2018); VKM: analytical results in foods from Norway.

^c Whole weight.

^d The number of samples is low, and gives more uncertainty to the P95 values.

^e Cod roe, and liver pâté from Kvaalem et al., 2009. Liver pâté concentrations were calculated from ww based on 22.1% fat as given by the authors.

^f Mono ortho-PCB missing in 9 samples.

^g Other food groups are composite foods that are not assigned any other category in the KBS, food oils (except for marine oils and butter), drinks, sweets, spices, and food for infants and young children.

^hn: number of samples

ⁱna: not available

7.2.2 PFAS

Occurrence values for the four PFASs: PFOS, PFOA, PFNA and PFHxS, are obtained from three data sources: 1) Annex A, Table A4 in EFSA (2020); 2) data on Norwegian fish provided by the Institute of Marine Research; 3) data from land-based food from Haug et al. (2010) and data from the EU project PERFOOD (which included data for Norwegian foods, supplementary data in Papadopoulou et al. (2017)).

For estimating LB and UB concentrations, VKM used a similar approach as applied for the dataset in EFSA (2020). To limit the impact of the non-detected values on exposure estimates and thus to limit the uncertainty, all samples with level of quantification (LOQ) or level of detection (LOD) above 1 µg/kg in the data from the Institute of Marine Research were excluded for the present assessment. In the data from the other sources, no samples had LOQ or LOD above this cut-off value. For LB, all samples where the analysis results were below the LOQ or LOD (non-detected values), were assigned a value of 0. For UB, all samples where the analysis results were below the LOQ or LOD, were assigned the value that represent the LOQ or LOD.

One of the aims of PERFOOD was to improve the analytical tools for the determination of PFASs in food. The limits (LOD/LOQ) obtained in PERFOOD and in Haug et al. (2010) were low compared to the limits applicable for most of the PFAS occurrence data available in EFSA (2020) or in the data on fish from the Institute of Marine Research.

The occurrence data used to calculate exposure estimates for PFASs are shown in Table 7.2.2-1. Information from the EFSA database and from the VKM database (data from Institute of Marine Research, Haug et al. (2010) and from PERFOOD) are shown together for similar food to facilitate comparison.

For PFASs, there is a high percentage of concentrations reported below LOQ. The difference between the mean LB and UB concentrations is large and is larger for PFNA and PFHxS than for PFOS and PFOA.

Most samples from Norway were available for fish, and there are few samples available for other foods from Norway. The EFSA database has a higher number of samples, but not necessarily for specific foods consumed in the Norwegian dietary surveys. Analytical results from suspect sampling or from area with known pollution were not included in the VKM database. EFSA did not include samples resulting from suspect sampling in the database.

Given the degree of self-sufficiency of fish, milk, eggs, and meat in Norway and the lower LOQ in the occurrence data based on Haug et al. (2010) and PERFOOD, but also the low number of Norwegian samples available for land-based food, two exposure assessments were made. One was based solely on data in the EFSA database (denoted EFSA dataset). This assessment is presented in Chapter 8 and used in the semi-quantitative risk assessment in Chapter 9.4.2. The other was based on the PFAS concentration data in fish (except fish liver and roe) provided by the Institute of Marine Research and data on land-based food from Haug et al. (2010) and PERFOOD, in combination with the data from EFSA, when information was otherwise missing (denoted VKM dataset). This assessment is presented in

Appendix IX, Chapter 22. The exposure obtained with the EFSA dataset was considered to be more robust than the VKM dataset (see explanation in Chapter 8.4.2).

For exposure assessment, each food item eaten in the national dietary surveys was assigned a concentration from similar foods/food group (based on expert judgement). For food items with no concentration data, the exposure from this food was set at zero.

Grain and grain products can serve as an example. The EFSA database has one mean occurrence value for the whole food group «grain and grain products», and this value was assigned to all food items in the food group while constructing the EFSA dataset. In the VKM database, there were occurrence values for rye, oat, wheat, and bread, in the VKM dataset, these values were assigned to the food items containing these specific grains and grain products. If the product/composite dish had a recipe in the food composition database (KBS), ingredient amounts were calculated based on the list of ingredients in the KBS, and the PFAS content of the dish is based on its ingredients. While constructing the VKM dataset, for products/composite dishes where there were no VKM occurrence values or recipes, like breakfast cereals (e.g., corn flakes) or biscuits (without recipes), the EFSA occurrence value for «grain and grain products» was used. Similarly, drinking water PFAS values in the EFSA dataset were used only for drinking water as such, whereas in the VKM dataset drinking water PFAS values were used for several beverages such as coffee, tea in addition to drinking water as such.

In EFSA (2020), concentration in the food group «food for infants and small children» was available for 11 (PFOS and PFOA) or 10 (PFNA and PFHxS) samples. None of the PFASs had levels above the LOQ, except for PFNA, which was detected at relatively high concentration in a single sample. This led to a LB concentration of PFNA in this food group of 0.13 µg/kg, which is higher than in other food. This was assessed as a large uncertainty by EFSA (2020). VKM considers this high PFNA concentration in foods for infants and small children unlikely, and the results for this food group were not used for exposure assessment.

Table 7.2.2-1 Mean concentrations (µg/kg) of PFOS, PFOA, PFNA and PFHxS in foods and food groups used for the exposure assessments.

		PFOS				PFOA				PFNA				PFHxS			
	Data-base*	n ^a	% <LOQ ^b	Lower bound	Upper bound	n	% <LOQ	Lower bound	Upper bound	n	% <LOQ	Lower bound	Upper bound	n	% <LOQ	Lower bound	Upper bound
Fish and other seafood																	
Anchovy (Engraulis)	EFSA	5	0	0.58	0.99	13	61.5	0.044	0.12	na ^c	na	na	na	na	na	na	na
Cod and whiting (Gadus spp.)	EFSA	174	67.2	0.47	1.05	145	93.1	0.012	0.74	130	91.5	0.016	0.78	27	100	0	0.53
Cod	VKM	155	32.3	0.43	0.96	71	100	0	0.92	89	100	0	0.64	40	100	0	0.64
Crab (Cancer spp.)	EFSA	16	43.8	0.69	0.93	13	46.2	0.38	0.54	16	50	0.35	0.50	20	85	0.30	0.78
Fish offal	EFSA	208	83.5	3.38	5	208	100	0	3.51	204	99.3	0.011	2.41	202	100	0	1.65
Fish products	EFSA	1	100	1.49	1.88	1	100	0	0.69	1	100	0.0049	0.66	1	100	0	0.57
Fish roe	EFSA	1	0	3.38	5	1	100	0	3.51	1	100	0	2.41	1	100	0	1.65
Haddock	VKM	94	99	<0.01	0.4	94	100	0	0.6	94	100	0.075	0.43	94	100	0	0.8
Halibut (Hippoglossus spp.)	EFSA	487	71.3	0.26	0.81	106	99.1	0.003	0.30	487	100	0	0.77	487	99.8	0.002	0.69
Halibut	VKM	406	81	0.85	1.16	406	100	0	2.55	406	100	0.04	0.12	401	100	0	0.13
Herring (Clupea)	EFSA	288	73.8	0.32	0.62	290	96.1	0.016	0.38	243	90.1	0	0.38	237	99.2	0.023	0.38
Herring	VKM	548	97.3	0.1	0.33	548	99.6	0.001	0.95	548	100	0	0.34	551	100	0	0.56
Mackerel (Scomber)	EFSA	125	78.8	0.36	0.93	136	81	0.31	0.88	129	96.3	0.004	0.74	122	98.7	0.004	0.74
Mackerel	VKM	375	82.1	0.02	0.52	228	98.3	0.005	0.27	378	100	0	0.53	378	99.7	<0.01	0.5
Mussel (Mytilus edulis)	EFSA	55	21	0.08	0.17	58	100	0	0.14	53	100	0	0.15	33	100	0	0.08
Plaice (Pleuronectes)	EFSA	39	46.2	2.95	3.29	39	97.4	0.09	0.72	28	100	0	0.85	5	100	0	0.51
Salmon and trout (Salmo spp.)	EFSA	574	87.9	0.31	0.83	521	94.5	0.13	0.63	522	99.9	0.003	0.70	365	100	0	0.63
Salmon	VKM	906	99.6	<0.01	0.49	906	100	0	0.60	906	100	0	0.57	906	100	0	0.84
Sea catfish and wolf-fish (Anarhichas)	EFSA	20	70	3.04	3.46	16	93.8	0.11	0.80	13	100	0	0.79	13	100	0	0.73

		PFOS				PFOA				PFNA				PFHxS			
Shrimps (Crangon crangon)	EFSA	39	25.1	0.74	0.76	38	76.34	0.019	0.090	34	93.4	0.024	0.12	19	100	0	0.1
Tuna (Thunnus)	EFSA	21	39.1	0.16	0.26	34	100	0	0.12	17	100	0	0.13	17	100	0	0.11
Drinks																	
Alcoholic beverages	EFSA	6	100	0	0.002	6	84.1	0.010	0.014	6	100	0	0.005	6	84.1	0.0056	0.007
Beer	VKM	1	100	0	0.002	1	100	0	0.005	1	100	0	0.005	1	100	0	0.002
Coffee ^b	VKM	12		0.0006	0.0006	12		0.004	0.004	12		0	0.0001	na	na	na	na
Drinking water	EFSA	451	87.5	0.0006	0.003	452	78.2	0.001	0.003	449	99.3	<0.0001	0.0022	449	85.2	0.0017	0.0037
Soda drinks ^b	VKM	6	100	0	0.0003	6	100	0	0.003	6	100	0	<0.0001	6	100	0	0.0006
Tea	VKM	1	0	0.00003	0.0003	1	0	0.0095	0.010	1	100	0	0.0002	1	100	0	0.00006
Drinking water ^a	VKM	5		0.00009	0.0002	5		0.001	0.001	5	100	0	0.0002	5		0.00005	0.0001
Egg																	
Eggs and egg products	EFSA	174	91.6	0.27	0.35	177	91.8	0.11	0.21	124	100	0	0.098	107	97.3	0	0.057
Hen eggs ^a	VKM	1	0	0.080	0.080	1	0	0.015	0.018	1	100	0	0.006	1	100	0	0.004
Fats																	
Animal and vegetable fats and oils	EFSA	38	90.2	0.004	0.11	38	90.2	0.002	0.11	36	100	0	0.12	53	97.5	0.0003	0.102
Butter	VKM	1	100	0	0.006	1	100	0	0.013	1	100	0	0.025	1	100	0	0.006
Margarine ^a	VKM	1	0	0.001	0.004	1	0	0.006	0.012	1	100	0	0.019	1	0	0.001	0.002
Olive oil	VKM	1	100	0	0.006	1	100	0	0.013	1	100	0	0.025	1	100	0	0.003
Fruit and fruit products																	
Apples	VKM	1	0	0.0008	0.0008	1	0	0.007	0.007	1	100	0	0.001	1	100	0	0.004
Banana	VKM	1	0	0.007	0.007	1	100	0	0.001	1	0	0.003	0.003	1	0	0.008	0.008
Fruit and fruit products	EFSA	143	76.7	0.027	0.25	144	62.5	0.009	0.26	98	72.8	0.011	0.168	94	83.8	0.022	0.156
Fruit and vegetable juices	EFSA	1	100	0	1	1	100	0	1.000	1	100	0	1.000	1	100	0	1.000
Grapefruits	VKM	1	0	0.006	0.006	1	0	0.014	0.014	1	0	0.025	0.025	1	0	0.019	0.019
Grapes ^a	VKM	1	0	0.019	0.0019	1	0	0.0045	0.004	1	0	0.004	0.004	1	100	0	0.004
Melons	VKM	1	0	0.006	0.006	1	0	0.012	0.012	1	0	0.010	0.010	1	0	0.004	0.004

		PFOS				PFOA				PFNA				PFHxS			
Peach	VKM	1	0	0.0065	0.0075	1	0	0.007	0.0075	1	0	0.006	0.006	1	0	0.008	0.01
Pears	VKM	1	100	0	0.002	1	100	0	0.001	1	100	0	0.001	1	100	0	0.004
Plums ^a	VKM	1	0	0.0015	0.0025	1	0	0.001	0.0015	1	100	0	0.001	1	100	0	0.004
Oranges	VKM	1	100	0	0.002	1	0	0.003	0.003	1	100	0	0.001	1	100	0	0.004
Strawberries	VKM	1	100	0	0.002	1	100	0	0.001	1	100	0	0.001	1	100	0	0.004
Strawberry jam	VKM	1	0	0.003	0.003	1	0	0.014	0.014	1	0	0.004	0.004	1	100	0	0.0006
Meat																	
Bovine meat ^a	VKM	1	0	0.030	0.031	1	0	0.006	0.009	1	0	0.011	0.011	1		0	0.001
Edible offal, farmed animals	EFSA	495	80.4	0.87	1.18	542	94.4	0.092	0.36	285	83.5	0.087	0.316	170	99.2	0.014	0.52
Game birds	EFSA	9	100	0	0.38	9	100	0	0.37	1	100	0	0.126	1	100	0	0.088
Game mammals	EFSA	574	71.4	0.94	1.59	572	91.4	0.39	1.23	33	100	0	0.70	28	96.4	0.015	0.68
Lamb/sheep meat	VKM	1	100	0	0.002	1	100	0	0.005	1	100	0	0.005	1	100	0	0.002
Livestock meat	EFSA	461	92.8	0.028	0.17	459	95.5	0.028	0.17	348	98.9	0.0004	0.14	222	99.7	0.0002	0.087
Meat and meat products (including edible offal)	EFSA	23	91.3	0.046	0.17	23	95.7	0.021	0.16	23	95.7	0.0011	0.13	23	95.7	0.0002	0.088
Pastes, pâtés and terrines	EFSA	15	100	0	0.051	15	92.9	0.009	0.069	15	100	0	0.062	15	100	0	0.041
Poultry	EFSA	169	98.8	0.009	0.13	185	97.9	0.002	0.15	170	100	0	0.14	130	100	0	0.11
Poultry meat ^a	VKM	1	0	0.011	0.012	1	0	0.0026	0.029	1	0	0.003	0.006	1	100	0	0.002
Pork meat ^a	VKM	1	0	0.016	0.016	1	0	0.011	0.011	1	0	0.003	0.005	1	0	0.0006	0.002
Sausages ^d	VKM	2	100	0	0.015	2	100	0	0.18	na	na	na	na	2	100	0	0.006
Sausages	EFSA	43	93.1	0.066	0.14	43	100	0	0.10	36	100	0	0.067	36	100	0	0.061
Milk and milk products																	
Cheese	EFSA	115	99.5	0.003	0.13	115	98.7	0.007	0.13	53	98.2	0.0004	0.098	53	100	0	0.071
Cheese	VKM	1	0	0.006	0.007	1	0	0.007	0.009	1	0	0.008	0.011	1	100	0	0.001
Concentrated milk	EFSA	2	100	0	0.12	2	100	0.001	0.13	1	100	0	0.10	1	100	0	0.081
Cream and cream products	EFSA	13	100	0	0.099	13	100	0	0.11	12	100	0	0.11	12	100	0	0.063
Fermented milk products	EFSA	66	100	0	0.075	65	100	0	0.083	64	100	0	0.092	63	100	0	0.050
Fresh whole cow milk	VKM	1	100	0	0.001	1	100	0	0.002	1	100	0	0.002	1	100	0	0.001

		PFOS				PFOA				PFNA				PFHxS			
Liquid milk	EFSA	235	96.4	0.0008	0.14	236	100	0	0.15	111	99.9	<0.0001	0.11	126	100	0	0.095
Milk and dairy products	EFSA	13	84.6	0.0008	0.12	13	84.6	0.001	0.13	13	92.3	<0.0001	0.10	13	92.3	<0.0001	0.081
Grain																	
Bread	VKM	1	0	0.017	0.017	1	0	0.051	0.051	1	0	0.010	0.010	1	0	0.002	0.002
Corn	VKM	1	100	0	0.002	1	100	0	0.001	1	100	0	0.001	1	100	0	0.004
Grains and grain-based products	EFSA	93	100	0	0.13	86	98.8	0.0001	0.10	87	100	0	0.094	80	100	0.000	0.079
Oat ^a	VKM	1	100	0	0.003	1	0	0.015	0.025	1	100	0	0.011	1	100	0	0.007
Rice ^a	VKM	1	100	0	0.002	1	100	0	0.001	1	100	0	0.001	1	100	0	0.004
Rye flour	VKM	1	100	0	0.004	1	100	0	0.02	1	100	0	0.02	1	100	0	0.01
Wheat (white)	VKM	1	100	0	0.004	1	100	0	0.02	1	100	0	0.02	1	100	0	0.01
Other food groups																	
Composite food (including frozen products)	EFSA	48	100	0	0.078	48	96.1	0.0003	0.076	40	100	0	0.065	40	100	0	0.059
Chocolate	VKM	1	100	0	0.093	1	100	0	0.097	1	100	0	0.19	1	100	0	0.184
Herbs, spices and condiments	EFSA	9	100	0	0.051	8	100	0	0.017	9	100	0	0.024	8	100	0	0.033
Honey	VKM	1	100	0	0.002	1	100	0	0.001	1	100	0	0.001	1	100	0	0.004
Legumes, nuts and oilseeds	EFSA	15	100	0	0.11	14	88.5	0.003	0.13	15	100	0	0.11	15	100	0	0.099
Snacks, desserts, and other foods	EFSA	46	100	0	0.59	46	100	0	0.58	46	100	0	0.58	46	100	0	0.572
Sugar and confectionary	EFSA	47	93.7	0.001	0.05	47	87.3	0.002	0.052	10	100	0	0.069	10	100	0	0.055
Vegetables																	
Asparagus	VKM	1	100	0	0.009	1	0	0.012	0.012	1	100	0	0.009	1	100	0	0.006
Beans	VKM	1	100	0	0.009	1	0	0.009	0.009	1	100	0	0.009	1	100	0	0.006
Cabbages	VKM	1	100	0	0.009	1	0	0.004	0.004	1	100	0	0.009	1	100	0	0.006
Carrots ^a	VKM	1	0	0.0003	0.005	1	0	0.006	0.006	1	100	0	0.006	1	100	0	0.003
Cauliflowers	VKM	1	100	0	0.009	1	100	0	0.003	1	100	0	0.009	1	100	0	0.006
Chicory	VKM	1	100	0	0.009	1	0	0.088	0.088	1	0	0.022	0.022	1	100	0	0.006

		PFOS				PFOA				PFNA				PFHxS			
Cucumbers	VKM	1	100	0	0.009	1	100	0	0.003	1	100	0	0.009	1	100	0	0.006
Cultivated mushrooms	VKM	1	100	0	0.009	1	100	0	0.003	1	100	0	0.009	1	100	0	0.006
Lettuce (or salad) ^a	VKM	1	0	0.00009	0.005	1	0	0.005	0.005	1	100	0	0.005	1	100	0	0.003
Peppers	VKM	1	100	0	0.009	1	0	0.012	0.011	1	100	0	0.009	1	100	0	0.006
Peas	VKM	1	100	0	0.009	1	0	0.083	0.083	1	100	0	0.009	1	100	0	0.006
Potatoes ^a	VKM	1	0	0.0005	0.005	1	0	0.018	0.018	1	100	0	0.007	1	100	0	0.003
Spinaches	VKM	1	100	0	0.002	1	0	0.011	0.011	1	100	0	0.005	1	100	0	0.01
Starchy roots and tubers	EFSA	289	99.7	0.004	0.61	289	96.6	0.004	0.61	139	98.3	0.0002	0.63	130	100	0	0.66
Tomatoes	VKM	1	100	0	0.009	1	0	0.003	0.003	1	100	0	0.009	1	100	0	0.006
Vegetables and vegetable products (including fungi)	EFSA	477	94.8	0.003	0.15	489	85.6	0.006	0.16	275	95.8	0.0005	0.12	274	98	0.0001	0.10

*Database: VKM refers to database that contains concentrations in fish and other seafood based on data from the Institute of Marine Research and data on other food from PERFOOD (supplementary data, Papadopoulou et al. (2017)). EFSA refers to concentrations in Annex A, Table A4 in EFSA (2020).

^an: number of samples

^bThe mean value was used for both Lower bound and Upper bound.

^cna: not available

7.2.3 Methyl mercury

The occurrence data for total mercury in wild fish were provided by NFSA in 2018 for the VKM opinion "Scenario calculations of mercury exposure from fish and overview of species with high mercury concentrations" (VKM, 2019). Observations prior to 2010 were not included, as we wanted to use the most recent data as basis for exposure. Only observations from fish caught in open sea (Norskehavet, Skagerrak, Nordsjøen, Barentshavet, Nordøstatlanteren, Barentshavet – vest, and Norskehavet – sør) were included in the dataset for the assessment of mercury in fish in 2019. Analytical results from suspect sampling or from area with particular pollution were not included in the VKM database. EFSA did not include samples resulting from suspect sampling in the database. The occurrence data for farmed salmon and trout were provided by the Institute of Marine Research in 2021 and cover the years 2019 and 2020. The concentration of mercury in farmed fish is affected by fish meal and is therefore dependent on the inclusion rate of fish meal in the fish feed. The relative amount of fish meal in the fish feed has been relatively constant during the recent years (Yttrestøyl et al., 2015; Aas et al., 2016).

Occurrence data for other seafood were obtained from the Institute of Marine Research, 2021 (<https://sjomatdata.hi.no>) and cover the period 2010 to 2020.

The number of samples with concentrations <LOQ is very low for total mercury in fish and other seafood (<https://sjomatdata.hi.no/>) and a possible small difference between LB and UB concentrations is therefore not further addressed.

The mean and 95-percentile concentrations of total mercury in fish and other seafood are presented in table 7.2.3-1. The total number of samples is high, although variable for different species. The mean concentration is highest in the fatty species, Atlantic halibut followed by wolffish and rose fish. The concentration in the commonly consumed lean species, such as Atlantic cod, is somewhat higher than in haddock and the fatty species, herring and mackerel. The lowest concentration is found in farmed Atlantic salmon and farmed trout.

Table 7.2.3-1 Occurrence of total mercury (mg/kg wet weight) in fish and other seafood used for exposure assessment.

	Sampling year	n	Mean	P95 (or max value)
Lean fish (≤5% fat)				
Atlantic cod	2010-2018	2007	0.08	0.2
Haddock	2013-2018	531	0.06	0.14
Plaice	2014-2017	226	0.06	0.15
Rose fish	2014-2017	87	0.12	0.26
Saithe	2011-2017	619	0.06	0.15
Tuna, canned	2015	50	0.08	0.28
Wolffish	2013-2014	46	0.12	0.29

	Sampling year	n	Mean	P95 (or max value)
Fatty fish (>5% fat)				
Atlantic halibut	2013-2016	66	0.19	0.4
Atlantic salmon, farmed	2019-2020	135	0.02	0.04
Herring	2011-2017	50	0.05	0.09
Mackerel	2012-2016	375	0.06	0.16
Trout, farmed	2019-2020	16	0.02	na ¹
Cod liver				
Cod liver	2010-2019	2585	0.05	1.6 ^a
Other seafood				
Blue mussel, farmed	2010-2020	413	0.01	0.03 ^a
Crab, brown and white meat	2010-2015	589	0.07	0.35 ^a
Great scallop muscle and roe	2011-2020	24	0.01	0.03 ^a
Norway lobster white meat	2011-2014	145	0.35	0.62 ^a
Shrimp, peeled	2010-2021	74	0.07	0.28 ^a

¹na – not available due to low number of samples.

^aMaximum value, not P95.

For exposure calculations, total mercury was assumed to be methyl mercury. This is a conservative approach, as 80-100% of total mercury is methyl mercury in fish, and a lower proportion (50-80%) is methyl mercury in crustaceans (EFSA 2012).

7.3 Dietary surveys

7.3.1 Description of food consumption surveys

The four most recent national dietary surveys, conducted among children, adolescents and adults, were used in the present benefit and risk assessment. These four surveys are the basis for the calculations of intake of fish and nutrients, and exposure to contaminants in the Norwegian population. The dietary survey in adults (Norkost 3) includes too few pregnant and lactating women to assess diet in these groups specifically. An overview of the included surveys is given in Table 7.3.1-1.

Table 7.3.1-1 The Norwegian national food consumption surveys used for the exposure estimations.

Study	Year of data collection	Age groups (years)	Participants (number)	Participation rate (%)	Method used
Spedkost 3	2019	1	1966 girls and boys	66	Web-based or paper-based (optional) food frequency questionnaire

Study	Year of data collection	Age groups (years)	Participants (number)	Participation rate (%)	Method used
Småbarnskost 3	2019	2	1413 girls and boys	47	Web-based or paper-based (optional) food frequency questionnaire
Ungkost 3	2015/2016	4	399 (girls: 195; boys: 204)	20	Web-based food diary
		8-9	636 (girls: 341; boys: 295)	55	
		12-13	687(girls: 355; boys: 332)	53	
Norkost 3	2010-2011	18-70	1787 (women*: 925; men: 862)	37	Two 24-hour recalls by telephone
			1453	30	Food propensity questionnaire

*Women of childbearing age, 18-45 years: 466.

In addition, we present the intake estimates of fish from the seventh survey of the Tromsø Study (Tromsø7) in 2015-2016.

7.3.1.1 1- and 2-year-olds

The national dietary survey Spedkost 3 (1-year-olds) was conducted by the University of Oslo in collaboration with the Norwegian Institute of Public Health (Paulsen et al., 2020). The data collection period was March to May 2019. Three thousand children, one year of age, were randomly selected from the National Population Register. The FFQ was based on a validated paper based FFQ (Andersen et al., 2003). A total of 1957 1-year-olds participated, which gave a participation rate of 66%.

The national dietary survey Småbarnskost 3 (2-year-olds) was conducted by the University of Oslo in collaboration with the Norwegian Institute of Public Health (Astrup et al., 2020). The data collection period was February to April 2019. Three thousand children, two years of age, were randomly selected from the National Population Register. A total of 1413 2-year-olds participated. Thus, the participation rate was 47%.

In both Spedkost 3 and Småbarnskost 3, a letter was sent to the children's caregivers with an invitation to take part in the dietary survey and a link to the web-based semi-quantitative food frequency questionnaire (FFQ), which could be filled-in either online or on paper. The caregivers were asked to have the last two weeks of food intake in mind, when filling in the questionnaire. If after 1.5 weeks the FFQ was not filled in, and the participants had not actively declined the invitation, study staff would call the participant and give them a gentle reminder. The questionnaires had approximately 180 food frequency questions, of which eleven asked about fish intake. Food portion sizes were estimated using photographs and predefined household units. Questions about seasonal variation in fish intake were not included. Questions about dietary supplements were included in the FFQ, both as a list of

common supplement products and, additionally, as an open text field for the caregiver to add information about any other supplement products.

The semi-quantitative FFQ is a dietary assessment method used mostly in surveys and epidemiological studies, with a predefined list of questions, to assess habitual diet over a predefined time range. It is crucial that the food list in a FFQ is prepared specifically, and thus is representative for, the study population in question. FFQs give a crude overall estimate of habitual diet and results are often biased (Thompson and Subar, 2017). FFQs are, however, generally evaluated as a good tool for assessing diet at the group level and for ranking individuals by intake (Lovegrove et al., 2015).

7.3.1.2 Children and adolescents

The national dietary survey Ungkost 3 (4-, 9-, and 13-year-olds) was conducted by the University of Oslo, in collaboration with the Norwegian Directorate of Health and the Norwegian Institute of Public Health (Hansen et al., 2015, Hansen et al., 2016). The data was collected during autumn 2015 for 9- and 13-year-olds and spring 2016 for 4-year-olds using a validated web-based four-day food record (Medin et al., 2015, Medin et al., 2016, Medin et al., 2017). Two thousand 4-year-olds were randomly selected from the National Population Register. Between February and May 2016, their caregivers received letters of invitation and consent forms. If after one week the caregivers did not respond to the invitation, study staff made gentle reminders over the phone or via SMS. For the 9- and 13-year-olds, 108 schools in 50 municipalities were randomly selected and invited, including 1164 9-year-olds and 1290 13-year-olds. Study staff visited the included school classes to inform about the survey and demonstrate how to use the web-based food record.

The dietary registrations were conducted over four consecutive days and started on either a Wednesday or a Sunday. Participants with at least three days of completed dietary registrations are included in the analyses of the present assessment. The web-based food diary used a food database of 570 of the most commonly consumed foods and beverages in Norway, of which 14 foods were fish and fish products. The web-based food diary was structured around meals, with photographs to estimate portion sizes, and with the possibility to enter food items not found in the food lists in an open text field. Questions about seasonal variation in fish intake were not included. Questions about dietary supplements were included in the web food record and the participants could choose supplement(s) from the provided food list or add information about dietary supplement use in an open text field. A total of 399 4-year-olds, 636 9-year-olds, and 687 13-year-olds participated in the survey. The participation rates were 20%, 55%, and 53%, respectively.

A food record/diary is an open dietary assessment method that allow for more detail in the registrations compared to an FFQ. With regard to the variety and detail in food items registered, the method is limited by the number of food items in the associated food database and the potential food entries made in the open text fields. It is generally evaluated as a good tool for assessing diet at the group level (Lovegrove et al., 2015).

7.3.1.3 *Adults*

The national dietary survey Norkost 3 was conducted by the University of Oslo in collaboration with the Norwegian Directorate of Health. Norkost 3 assessed diet using two 24-hour-recall interviews conducted over the telephone at least one month apart. Food amounts were estimated using a printed leaflet, showing portion sizes in household measures or photographs (Totland et al., 2012). The study was conducted from January 2010 to August 2011, thus covering all seasons. Five thousand men and women, aged 18-70 years, randomly selected from the National Population Register, were invited and 1787 participated with two days of recordings (participation rate of 37%). Questions about dietary supplements were included in the 24-hour recall interviews. A total of 97 different fish and fish-containing foods were reported in the two 24-hour recalls. The participants were asked to fill in a food propensity questionnaire after having completed the two 24-hour recalls. The propensity questionnaire asked about the habitual intake of 216 different food items, including food items seldomly eaten, drinks, dishes, and supplements. Twenty-one questions asked about fish intake. A total of 1453 participants filled in the questionnaire.

Two 24-hour recall is evaluated to be a good method to assess habitual diet and the estimates of intake of frequently eaten food items, at group level (Lovegrove et al., 2015). However, the estimates are less suitable for food items that tend to have an irregular or low frequency of intake, e.g., fish. Therefore, some results from the propensity questionnaire in the Norkost 3 survey are also included in the description of the fish intake in Chapter 8.

7.3.1.4 *The Tromsø Study*

In addition to the national dietary surveys, we also present dietary data from The seventh survey of the Tromsø Study (Tromsø7) in 2015-2016. This survey conducted dietary assessments using a semi-quantitative FFQ (Lundblad et al., 2019). The participants were 40 to 99 years of age and dietary data from 11425 participants were eligible for analysis. Data on intake of fish for dinner, fish spread, and fish products are presented in the present assessment for additional information about the fish intake in the Norwegian population.

7.3.2 Methodological challenges in dietary food surveys

Tools based on self-reporting are frequently used in nutrition research. All methods used to either assess long-term or short-term diet, prospectively or retrospectively, have associated measurement errors. For example, dietary assessment methods for long-term retrospective intake challenge participants' memory and their ability to take into account the variability of intake by day or season. Many participants also find it challenging to estimate portion sizes and frequencies of intake. Some of these inherent methodological challenges are more prominent in the 24-hour recall method, while others are more prominent when using FFQs.

Direct comparison of diet or dietary components, assessed in different age groups and with different dietary assessment methods, is challenging without validation and calibration studies for the population, food group or the substance in question. In this assessment FFQ

is used for the 1-year-olds and 2-year-olds, and 24-hours recall and food diary for the other age groups. Generally, there is a tendency for FFQ surveys to overestimate energy intake, and for recording methods such as 24-hours recall and food diary to underestimate energy intake.

In Norkost 3, having only two days of data per respondent increases the risk that these days are unrepresentative for the habitual individual intake of food items eaten seldom or infrequently. Due to the low number of sampled days in the dietary surveys that used 24-hours recall and food diary (2 days in Norkost 3 and 3-4 days in Ungkost 3), intake distribution estimates based on observed individual means (OIMs, presented in Chapter 7.5), for the age groups from 4-year-olds and above, tends to give a wider distribution in intake than the true intake. These assessment methods are particularly prone to overestimation of the tails of an intake distribution (for further details, see Chapter 7.5).

Estimated energy intake can be used to evaluate potential under- or overreporting of food intake in the dietary surveys (Black et al., 2000). Under- and overreporting in the dietary surveys used in this assessment is described in Chapter 11 Uncertainties (subchapter 11.2.3). It should be noted that under- and overreporting of energy is not corrected for by either the OIM or the mixed model approach.

7.4 Fish intake estimated from composite dishes

The estimates for intake of fish in adults are used in the quantitative modelling of health outcomes in Chapter 9.2, and in the estimates for exposures to nutrients and contaminants in all age groups.

Fish is eaten in many forms: raw, cooked, baked, and grilled, as a fillet or as an ingredient in composite fish dishes and products. An important share of the fish intake in Norway comes from composite fish dishes. To estimate fish intake, the amounts of fish in composite fish dishes and fish products were converted into raw fish-fillet equivalents and then into prepared fish fillet equivalents.

First, the amount of fish in composite fish products/dishes was converted into raw fish-fillet equivalents. For those composite products that had a recipe in the food composition database, the amount of fish was calculated based on the ingredient list and the nutrient content of the product and its ingredients. If a composite product contained fish, but lacked a recipe, having the nutrient values established through direct analyses or from the product declaration, fish content was estimated. These estimates were based on the best match between the nutrient values of the product and the nutrient values of the ingredients (i.e., fish content was estimated based on the nutrient content in the composite dish and the nutrient content of the fish fillet in question). The ingredient lists, if not specifically known, were based on generic recipes and expert judgement. Nutrient intake from all other ingredients in composite fish products and dishes, like flour, eggs, or tomato sauce, were included in the nutrient estimates for the total diet.

Second, as the recommendation for fish intake is given in grams of prepared (ready to eat) fish fillet per week, amounts of raw fish fillet were converted into amounts of prepared fish fillet using the conversion factor of 0.85. The recalculation from raw into prepared fish fillet adjusted for the loss of water from processing. However, it did not apply retention factors for the vitamins or the fatty acids, because these were not available in the nutrient calculation system. The concentrations of nutrients and contaminants in fish are often analysed only in raw fish fillet. Thus, the concentrations of vitamins and fatty acids may be overestimated in the present analyses.

The most common fish species used as ingredients in composite fish dishes were identified and used in the recipes and recalculations. According to the dietary surveys, several fish dishes were frequently eaten and therefore merited particular attention: fish cakes, fish pudding, fish balls, and mackerel in tomato sauce (the latter used as a bread spread). For fish cakes, fish pudding and fish balls, the main fish ingredients are Alaska pollock and smelt (Aakre et al., 2019). As VKM did not have nutrient content information for Alaska pollock and smelt, nutrient content values for a similar fish species had to be used instead. The nutritional values for haddock were used for both Alaska pollock and smelt. Intake of mackerel in tomato sauce was converted to raw mackerel fillet. The content of fat in mackerel varies during the year. Based on information from the manufacturers of mackerel in tomato sauce, autumn mackerel is the main type of mackerel used in this product. Therefore, the nutrient values from autumn mackerel were used in the present assessment.

In summary, the estimates of the fish intake are given as the sum of fish fillet equivalents from all types of fish dishes and products consumed.

7.5 Approaches to estimate habitual intake of fish and nutrients and chronic exposure to contaminants

In this benefit and risk assessment, both statistical estimation and simulation were used to characterise the habitual intake of fish and nutrients and chronic exposure to contaminants in individuals, and to increase population representativity.

In assessing habitual intake or chronic exposure to the compounds of interest (fish, nutrients, and contaminants) at the population level in Norway, two main challenges were faced: (i) estimation of habitual dietary intake, in particular of fish, when based on short-term dietary assessment methods, and (ii) generalisation from survey respondents to the Norwegian population due to varying response rates in the national surveys (Table 7.3.1-1).

The first challenge was addressed by adopting the observed-individual-means (OIM) approach (Chapter 7.5.1) or a mixed model approach (Chapter 7.5.4) to reduce the effects of day-to-day variability. A universal approach could not be applied because different dietary assessment methods were used in different age groups, and distributional assumptions of the mixed model were met to varying degrees for the different compounds. To address the second challenge, the survey responses were weighted for respondents' demographic

characteristics (gender, education, age, and geographical region) and calendar representativity of consumption (weekday and month), as described in Chapter 7.5.2.

The remainder of Chapter 7.5 presents the different methodological approaches used in more detail and different adaptations made for the various estimates.

7.5.1 Observed individual means (OIMs)

OIMs are the arithmetic mean over the dietary survey days, often used as estimates of individual habitual intake, or chronic exposure. OIMs can be calculated from dietary survey data that include multiple days of registration (e.g., dietary records, or repeated 24-hour recalls). The number of survey days included in the mean varies by the length of the dietary survey (as presented in Chapter 7.3) and the standard error (a measure of precision) of OIMs is inversely related to the number of registration days. Averaging over the survey days produces narrower distribution estimates, but few registration days may lead to an overestimation of the variation between individuals because the variability in the OIMs is overestimated. This results in distributions that are too wide in the tails. Second, for distributions that are skewed with long right tails, upper intake percentiles may be overestimated.

In the following cases, only weighted or unweighted OIMs were used:

- For 1- and 2-year-olds the dietary assessment method (FFQ) queries the habitual intake directly in the form of two-week averages reported by the caregiver. These values are just one observation per individual and not calculated OIMs, but they are treated and presented as OIMs as the FFQ-method averages out the day-to-day variation by default. OIMs weighted by demographic characteristics (see Chapter 7.5.3) were considered to give the best estimate of the habitual intake in these age groups at the population level.
- It was more feasible to calculate the contribution of different food groups to the total intake or exposure based on OIMs, rather than a mixed model as this would require a more complex, multivariate model with higher dimensionality than the main model used.
- For intake of fish and exposure to methyl mercury (which has fish as the only source) only OIMs were used because the distributional assumptions of the mixed model could not be met. The mixed model approach is sensitive to reported intakes with many zeros, as was the case for fish intake.

Please note: OIM data that are not explicitly stated to be weighted, are unweighted.

7.5.2 Survey weighting by demographic factors

The food consumption surveys used in the current assessment had varying participation rates (Table 7.3.1-1). Consequently, some survey respondents may be overrepresented or underrepresented compared with the demographic characteristics of the target population. If all survey responses are treated as equally representative, the estimated distributions describe the variation across survey respondents and not necessarily variations in the whole population.

increase population representativity of estimated intakes, VKM applied weights to the survey respondents based on their demographic characteristics. This was performed adopting the procedure called “raking” (otherwise known as iterative proportional fitting and sample-balancing). Gender, education, age, and geographic regions were chosen as the demographic characteristics used for weighting of surveys, which is in line with the standard choice of characteristics used for survey weighting (Pew Research Center et al., 2018).

The data used in estimation of weights for individual survey respondents were obtained from microdata.no. Microdata.no combines several national registries including the Norwegian Tax Administration (*Skatteetaten*) and the Norwegian State Educational Loan Fund (*Lånekassen*). The database covers all individuals registered in Norway in the past.

The information from microdata.no was collected as of January 1, 2018, and included the following variables:

- Gender
- Education level: The education levels were set to 1 for respondents with a college degree and 0 otherwise. For Ungkost 3, Småbarnskost 3, and Spedkost 3 information on parental education was used, setting the variable to 1 if at least one parent had a college degree.
- Age group (Norkost 3 only): Age was categorized into five age groups: 18–29, 30–39, 40–49, 50–59, 60+. For Ungkost 3, Småbarnskost 3, and Spedkost 3 each age cohort was analysed separately, and therefore there was no need to weight by age.
- County of registration: Counties were used to assign individuals geographically to regions. There were 7 regions (*landsdele*, as defined by Statistics Norway) in Norway as of January 1, 2018.

This approach resulted in 140 groups for adults ($2 * 2 * 5 * 7$): two genders, two education levels, five age groups, and seven regions. For 1-, 2-, 4-, 9-, and 13-year-olds, there were 28 groups ($2 * 2 * 7$): two genders, two education levels, and seven regions.

The sizes of the corresponding 140 groups for adults and the 28 groups for 1-, 2-, 4-, 9-, and 13-year-olds in the population were collected from microdata.no and used to compute relative group sizes or, equivalently, group frequencies. Population group frequencies were

compared to the corresponding dietary survey group frequencies, and weights were calculated in such a way that the weighted survey frequencies were equal to the population frequencies.

7.5.3 Weighted OIMs

By applying the weights described in Chapter 7.5.2, distributions of person-day observations were made representative for the demographic characteristics. The raw person-day observations were used directly together with the corresponding weights to compute weighted OIM distributions, means, standard deviations and percentiles, for 1- and 2-year-olds (FFQ data) as described in Appendix VI, Chapter 19.

7.5.4 Intake and exposure modelling using mixed models

To address the limitations of the OIM method described previously (Chapter 7.5.1), a mixed model based on Bayesian estimation, was used to estimate distributions more representative of the habitual intake/chronic exposure (for technical details, see Appendix VI, Chapter 19).

The mixed model is a statistical model containing fixed and random effects. Mixed models allow estimation of day-to-day variation (treated as random effects) in the modelled exposure for each survey participant and of clustered variation between survey participants, and simulation of long-term chronic exposure. The model is used to correct for day-to-day variation in the modelled exposure for each survey participant, and for variation between survey participants. Thus, distributions estimated by mixed models will be narrower than OIM distributions. The mixed model approach is considered to give more precise estimates of long-term intake/chronic exposure than OIMs, when distributional assumptions of the model are met. This was not the case for fish. Thus, the mixed model approach was only used for nutrients and contaminants, except methyl mercury for which fish was the only source.

The person-day observations together with the corresponding weights described in Chapter 7.5.2 were also used for constructing distributions based on the mixed model estimates, as described in Appendix VI, Chapter 19.

The mixed model approach was used in the semi-quantitative benefit and risk assessment of nutrients and contaminants to:

- Quantify day-to-day variation within individuals
- Simulate long-term averages for habitual intake and exposure levels
- Transform the habitual intake and exposure levels to distributions representative of the general population within a given age group by applying the demographic weights described in Chapter 7.5.2
- Estimate confidence intervals around the average exposure distributions

The mixed-model approach was considered to give the most representative results for adults, and children age 4-, 9-, and 13-years because the dietary survey method was based on multiple days of registration, but it could not be applied to fish or methyl mercury which had fish as the only source of exposure. A general description of the mixed model used is presented below. For more technical details, please see Appendix VI, Chapter 19.

7.5.4.1 *Model specification*

Model specification: Fixed effects

Habitual intake of nutrients and exposure to contaminants included contributions from both food and dietary supplements. For two nutrients, vitamin D and LC n-3 FAs, high doses of supplements resulted in very skewed distributions, which required some adaptations to improve the fit of the main model.

The main model specified below was used to estimate the intake or exposure to compounds that were only moderately affected by supplementation. Iodine, selenium, vitamin B₁₂, as well as PCDD/Fs, DL-PCBs and PFASs in adults were modelled this way:

$$f(\text{Compound}) \sim \text{Const} + \text{Sex} + \text{Age} + \text{Education.High} + \text{Region} + \text{Weekday} + \text{Month},$$

- $f()$ is the Box-Cox transformation of the compound $(\text{Compound}_i^\lambda - 1)/\lambda$ using the optimal value for the exponent, lambda (λ)
- *Const* is the constant term (or intercept)
- *Sex*, *Education.High*, *Region*, *Weekday*, and *Month* are treated as factor (or dummy) variables
- *Age* is treated as a continuous variable, measured in years

The Box-Cox transformation was applied to all compounds as it was found to improve model fit (evaluated by the persistence of the estimated individual daily intakes). See Appendix VI, Chapter 19 for further technical details of finding the optimal λ . All explanatory variables were included as fixed effects.

Survey responses were also corrected for calendar representativity of consumption. Non-uniform distributions of the data availability across the week and the year were computed. Norkost 3 covers all calendar months, but not uniformly. Similarly, all calendar days are represented, but not equally. The relative weighting can correct for this, making all days and seasons equally “likely”.

For Ungkost 3, surveys of the age cohorts were conducted over only a few months. Thus, seasonal representativity was not achievable. For Ungkost 3, only weighting to make days of the week representative was feasible.

For 4-, 9-, and 13-year-olds, the variables *Month* and *Age* were excluded from the model, as the dietary surveys for these age groups (Ungkost 3) cover only a short period within a calendar year, and each age cohort is estimated in a separate regression.

For vitamin D and LC n-3 FAs one more control variable was added to the main model, a dummy variable for supplementation by the respondent. The variable was set to one for a respondent that took a supplement containing the given compound on at least one of the surveyed days (and zero otherwise). The same model was used for adults and 4-, 9-, and 13-year-olds, but again, *Month* and *Age* were excluded from the models for these age groups. Although controlling for supplementation greatly improved the model fit for vitamin D and LC n-3 FAs, the fit was still poorer than for other compounds.

Model specification: Random effects

An important advantage of mixed models is their flexibility when it comes to specifying the structure of random effects. Generally, two components (random and residual) are included. The random component allows to model variability by group (here by subject). The residual component captures remaining variability of each observation (see Appendix VI, Chapter 19 for further technical details). In the adopted models, both variability between subjects and variability between days within subjects were clustered by sex.

7.5.4.2 Simulation of habitual intakes and chronic exposure distributions

The main objective of utilizing the mixed model approach was to simulate distributions of habitual intakes (nutrients) or chronic exposures (contaminants) based on the outcomes of the fitted model(s). The simulated distributions were weighted by demographic characteristics to increase national representativeness.

The steps were as follows:

- A model is fit: the coefficients for the fixed effects, and the elements of the variance-covariance matrices for the random effects are estimated.
- For each survey participant, 365*100 daily observations are simulated (equivalent to 100 years of daily data). The transformation of the modelled compound is reversed to arrive at the original unit of measurement.
- Averages of each 365 daily observations are taken. The result is the daily consumption averaged over a year. For each participant, there are 100 simulated results. The effect of the within person variation will be reduced compared to the OIM approach because the variation is averaged over many more days.
- For the 100 simulated distributions across the survey participants, 100 weighted population distributions are computed. When weighting personal annual averages, one no longer needs to control for weekdays and/or month, but demographic characteristics are controlled for, as described above (see Chapter 7.5.2).
- Based on these 100 distribution results, both the average distribution and its confidence intervals are computed. The average distributions form the background

data for the mixed model approach presented in this benefit and risk assessment (semi-quantitative assessment of nutrients and the contaminants, PCDDFs and DL-PCBs and PFASs).

7.6 Overview of data presented in the benefit and risk assessment

The data presented in the benefit and risk assessment chapters (Chapter 9, 10 and 12) are either weighted or unweighted OIMs, or mixed models see Table 7.6-1. In Chapter 8, we additionally present OIM data for exposure to nutrients and contaminants from either fish or food supplements. OIM data that are not explicitly stated to be weighted are unweighted. All estimates of fish intakes are based on recalculated fish intakes from composite dishes and products into fish fillet.

Table 7.6-1 Overview of the data presented in chapter 9, 10 and 12 in the benefit and risk assessment

Survey	Fish intake	Total nutrient intake	Total PCDDF/DL + PFASs exposure	Total methyl mercury exposure	Contribution of food groups to nutrient intake or contaminant exposure
Adults (Norkost 3 survey)	OIMs, unweighted	Mixed models	Mixed models	OIMs, unweighted	OIMs, unweighted
Children aged 4, 9, 13 yrs (Ungkost 3 survey)	OIMs, unweighted	Mixed models	Mixed models	OIMs, unweighted	OIMs, unweighted
Children aged 1 yr (Spedkost survey) and 2 yrs (Småkost survey)	OIMs, unweighted	OIMs, weighted by demographical factors	OIMs, weighted by demographical factors	OIMs, weighted by demographical factors	OIMs, unweighted

7.7 References

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8 Intake of fish and nutrients and exposure to contaminants

8.1 Introduction

This chapter presents the intake of fish and estimates of nutrient intake and contaminant exposures from fish. We present the fish intakes from the national dietary surveys by age groups and gender (Chapter 8.2), followed by nutrient intake from total diet (including food supplements), from fish alone and from supplements alone (Chapter 8.3). We present the exposures to contaminants from total diet, fish alone, and from food supplements containing marine oils alone (Chapter 8.4).

Intake of fish and nutrients and exposure to contaminants, is presented separately for women of childbearing age (18-45 years) as exposure during pregnancy is of particular relevance for several of the included health outcomes. It should be noted that the same women also are included in the conclusions for women in general (18-70 years).

8.2 Estimates of fish intake from the Norwegian dietary surveys

Table 8.2-1 gives an overview of fish intake (OIMs), presented in grams of prepared fish fillet per week and per day, by gender and for different age groups. The data presented are from the national dietary surveys Norkost 3, Ungkost 3, Spedkost 3 and Småbarnskost 3. Descriptions of the dietary surveys is presented in Chapter 7.3 and the recalculation of fish products into prepared and raw fish fillet are given in Chapter 7.4.

The intakes of fish are skewed in all age groups, with many participants reporting no or a very low intake, and a smaller share of the participants reporting a high intake. To illustrate the skewed nature of the intake data, we present the mean and percentiles of total fish intake.

The fish intake varied among age groups. Adult women aged 18-70 years consumed on average 238 g prepared fish per week, whilst women aged between 18 and 45 years consumed on average 182 g per week. Men of 18 to 70 years consumed 350 g per week. The younger age groups had an average fish intake from 90 g per week for 1-year-olds to 136 g per week for 13-year-old boys. In children and adolescents, the fish intake did not increase with age. The 4-year-old children had the highest mean fish intake, and the 13-year-old girls had the lowest.

Table 8.2-1 Estimated intake of prepared fish fillet in the national dietary surveys, observed individual means (OIMs) g/day and g/week (rounded estimates).

	Total fish intake						Lean fish						Fatty fish					
	g/day					g/week	g/day					g/week	g/day					g/week
	Mean	SD	P50	P25	P75	Mean	Mean	SD	P50	P25	P75	Mean	Mean	SD	P50	P25	P75	Mean
Norkost 3																		
Women, 18-70 (n=925)	34	50	9	0	56	238	18	38	0	0	19	128	15	32	0	0	15	110
Women, 18-45 (n=466)	26	42	0	0	37	182	13	31	0	0	5	93	13	27	0	0	12	92
Men, 18-70 (n=862)	50	72	17	0	79	350	29	59	0	0	31	206	20	43	0	0	18	134
Ungkost 3																		
Girls, 13-year-olds (n=355)	14	23	3	0	20	97	6	15	0	0	7	42	8	18	0	0	4	54
Boys, 13-year-olds (n=332)	20	33	0	0	26	136	9	18	0	0	9	61	10	27	0	0	0	73
Girls, 9-year-olds (n=341)	15	21	6	0	22	101	7	12	0	0	9	47	8	17	0	0	3	53
Boys, 9-year-olds (n=295)	17	25	8	0	25	122	9	17	0	0	13	63	8	18	0	0	7	57
Girls, 4-year-olds (n=195)	20	19	16	6	28	133	10	15	5	0	15	65	9	12	3	0	15	62
Boys, 4-year-olds (n=204)	18	19	13	2	26	129	9	13	3	0	15	69	8	14	0	0	10	52
Sped- and Småbarnskost 3																		
2-year-olds (n=1413)	15	10	14	9	21	108	9	7	8	5	12	66	6	7	3	0	8	42
1-year-olds (n=1957)	13	9	10	5	17	90	7	6	5	3	9	48	5	6	3	0	7	36

Table 8.2-2 shows the percentages of participants in Norkost 3 who reported intake of fish for dinner or lunch or as bread spread, rarely, 1-2 times/week and ≥ 3 times/week. These general questions were asked as part of the 24-hour recall interview. Sixty percent reported to have fish for dinner or lunch 1-2 times per week and 26% to have it 3 times per week or more. The choice of frequencies in the background questionnaire does not allow to differentiate between at least 2 and at least 3 meals per week. One answer option covers 1-2 meals per week, which does not fulfil the recommendation of at least 2 fish meals. The next answer option is 3-4 meals per week, which fulfils both 2 and 3 meals per week.

Table 8.2-2 Frequency of fish intake in percentage of participants in Norkost 3^a (age group 18-70 years).

	Frequency of fish intake, % of survey participants		
Norkost 3, n=1453	Rarely	1-2 times /week	≥ 3 times / week
Fish for dinner or lunch	14	60	26
Fish as bread spread	30	58	13

^aNorkost 3, Totland et al., 2012.

The participants in Norkost 3 were also asked to fill in a food propensity questionnaire in addition to the two 24-hour recalls. Based on the individual frequency questions on fish consumption from the propensity questionnaire, the share of participants that eat at least 2 (3) fish meals per week was estimated to be 62% (38%) for women and 58% (34%) for men (Table 8.2-3). The share of women of 18-45 years old with 2 (3) fish meals per week was 55% (30%).

The proportion having 2 (3) meals or more per week based on the food propensity questionnaire may be overestimated, since the proportion is estimated from the aggregated frequencies of 13 different questions about fish intake.

Table 8.2-3 Share of adults following the fish-meal recommendations.

	≥ 2 fish meals/ week	≥ 3 fish meals/ week
Women, 18-70 years	62%	38%
Women, 18-45 years	55%	30%
Men, 18-70 years	58%	34%

The contribution of lean and fatty fish (mean percentage) to total fish intake varied to some degree among age groups and genders. Men showed a tendency towards higher percentage of lean fish intake compared to women. However, the differences were not large. The lean fish intake ranged from 50% in women of childbearing age to 53% in all women and 60% in men (Figure 8.2-1).

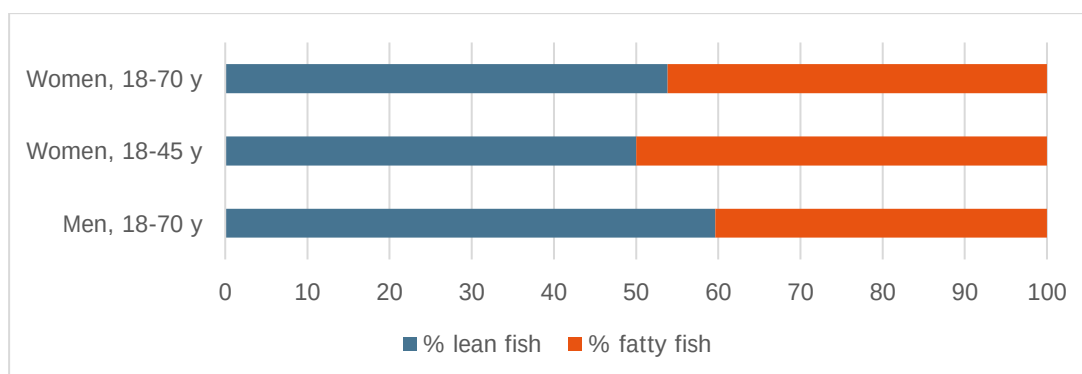


Figure 8.2-1 Percentage of lean and fatty fish, of total fish consumption, in women and men, 18-70 years, in the Norkost 3 survey.

The percentage contributions of lean and fatty fish in children, by gender and age groups for 4-, 9- and 13-year-olds is presented in figure 8.2-2. The percentage of fatty fish increased in adolescents compared to younger children. Both 1-year-olds and 2-year-olds showed a tendency towards a higher contribution of lean fish than fatty fish (see Figure 8.2-3).

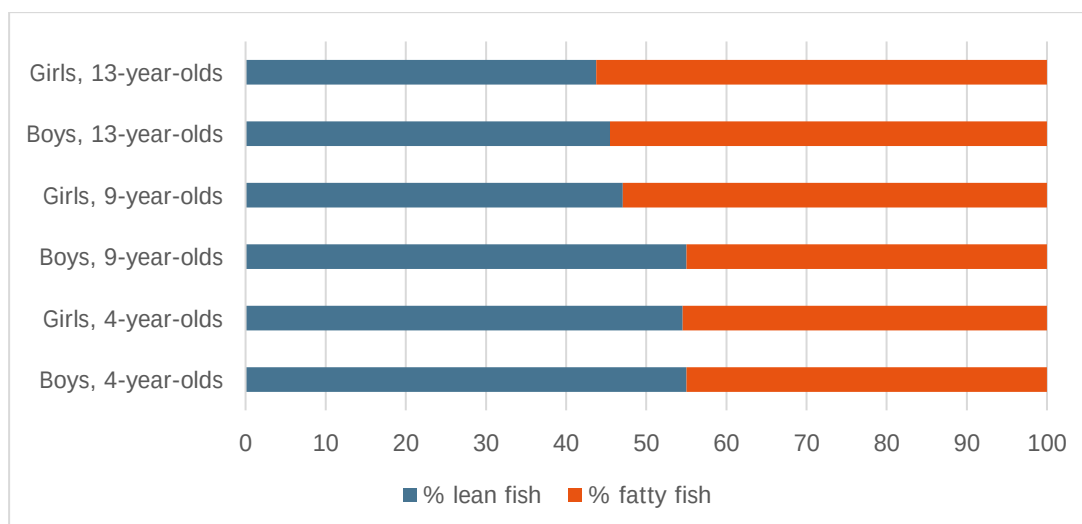


Figure 8.2-2 Percentage of lean and fatty fish, of total fish consumption, in children and adolescents in the Ungkost 3 survey.

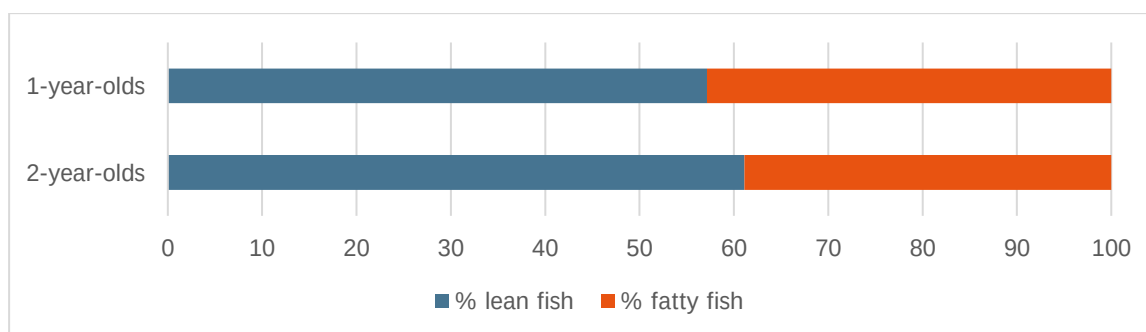


Figure 8.2-3 Percentage of lean and fatty fish, of total fish consumption, in 1-year-olds and 2-year-olds.

The intake of lean and fatty fish species also differed by age group (Figure 8.2-4). Cod, haddock, mackerel, and salmon were the most consumed fish species in all age groups. For adults, cod, haddock, saithe, and tuna constituted the majority of lean fish intake. Fatty fish intake in adults consisted mostly of salmon, mackerel, and herring. In 1-year-olds and 2-year-olds, the intake of lean fish consisted of cod and haddock, while mackerel and salmon constituted the majority of the intake of fatty fish in this age group. In 4- and 9-year-old children and in adolescents, salmon and mackerel constituted the majority of the fatty fish intake, while cod and haddock constituted the majority of the lean fish intake.

As described in Chapter 7.4, the total intake of fish, the intake of lean and fatty fish, and the different fish species are based on the content of fish fillet in the composite fish products and dishes. See Chapter 7.4 for further description of these recalculations.

Due to the open dietary assessment method used in Norkost 3, a greater variety of fish species were registered consumed in this survey. In the surveys using dietary assessment methods like food diaries and FFQ (Ungkost 3 and Småkost3/Spedkost 3), fewer fish species were registered, probably due to the predefined options in these assessment methods. Figure 8.2-4 illustrates the distribution of intake of various fish species in all age groups, for lean and fatty fish.

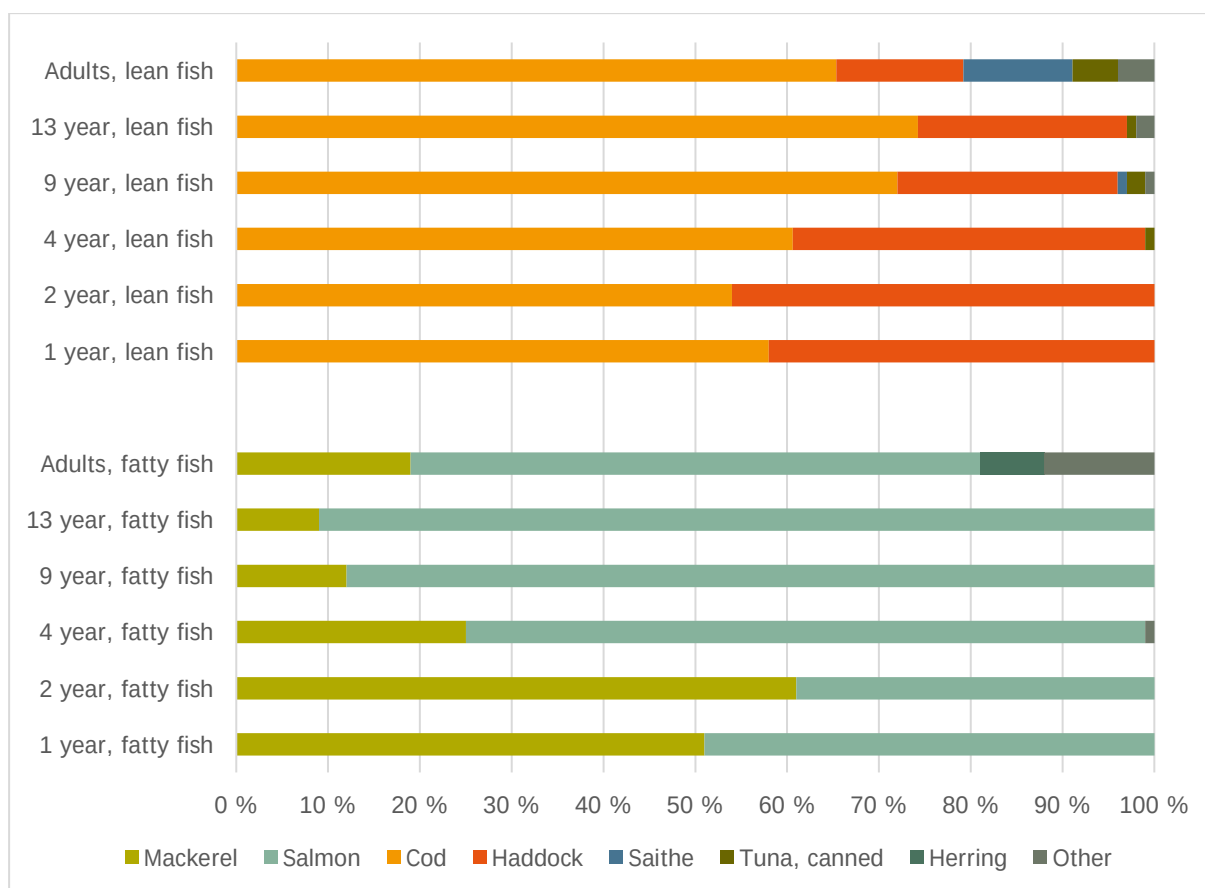


Figure 8.2-4 Percentage contributions of intake of different fish species divided in lean fish and fatty fish, in all age groups.

Mackerel in tomato sauce is a common bread spread product in Norway. All intake of mackerel in 1- , 2- and 4-years-olds was from this bread spread. Table 8.2-4 presents the intake of the mackerel in tomato sauce bread spread (the complete composite product, not only the fish fillet), in 1-, 2-, and 4-year-olds. Intake estimates are presented for both all survey participants and the consumers of mackerel in tomato sauce only.

Table 8.2-4 Intake of mackerel in tomato sauce bread spread, in 1- , 2- and 4-year-old children.

Dietary survey	All participants, mean intake, g/day	Consumers, %	Consumers only, mean intake, g/day
Ungkost 3			
Girls, 4-year-olds (n=195)	3	35	10
Boys, 4-year-olds (n=204)	3	26	12
Spedkost and Småbarnskost			
2-year-olds (n=1413)	5	47	11
1-year-olds (n=1957)	4	40	10

Percentage of consumers of the mackerel in tomato sauce bread spread was higher among 1- and 2-year-olds compared to 4-year-olds. However, the average amount in g/day consumed was approximately the same for all three age groups.

'Kaviar' is another popular bread spread. It contains 30-88% cod roe, depending on brand. In 4-year-old boys 35% consumed 'Kaviar' and the mean intake among consumers was 8 g per day. In 4-year-old girls 29% consumed 'Kaviar' and the mean intake among consumers was 7 g/day (data not shown in table).

In 1-year-olds, 31% consumed Kaviar, with the mean intake among consumers of 3 g/day. In 2-year-olds, 45% consumed Kaviar, with the mean intake among consumers of 5 g/day (data not shown in table).

The Tromsø Study: Tromsø7

The Tromsø7 included participants aged 40 to 99 years. The mean intakes of fish for dinner, both fatty and lean, composite fish dishes and bread spread, are shown in Figure 8.2-5 for all participants and for women and men. Men had higher absolute intakes of all fish categories in Tromsø7 compared to women.

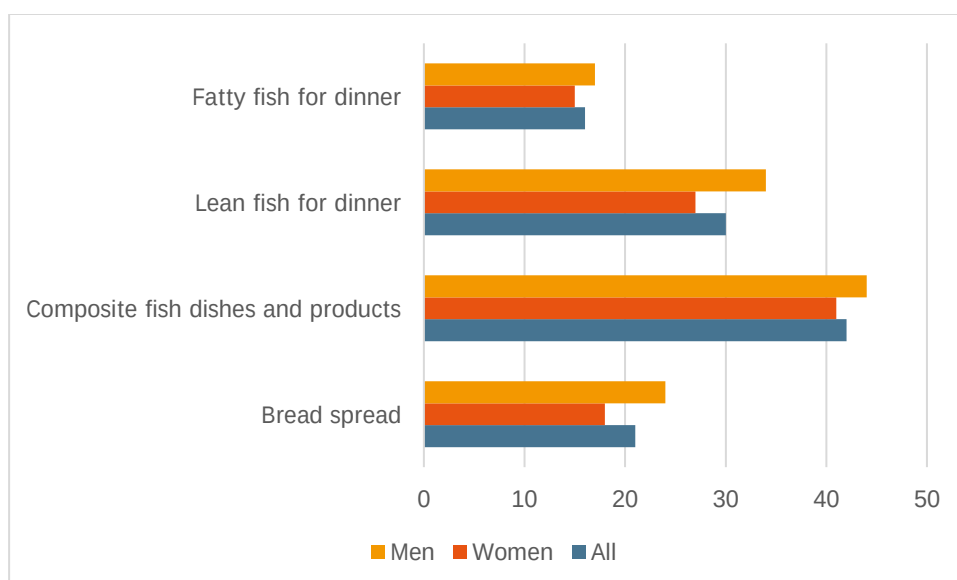


Figure 8.2-5 Intake of fish and fish products in all participants, women and men (aged 40-99 years), g/day, in the Tromsø Study: Tromsø7.

Please note that the fish intake from the Tromsø7 survey is only used to give additional information about the fish intake in Norway. The composite fish dishes are not spilt into ingredients. The dietary data from the Tromsø7 thus presents intake of whole dishes. This is in contrast to the estimates from the national dietary surveys presented earlier in this chapter. In addition, due to the geographical area of The Tromsø Study, the traditional diet pattern along the coast of Norway and the age group included, the general habitual fish intake in the Tromsø region is expected to be higher than the intakes estimated from Norkost 3.

8.3 Estimates of nutrient intakes

In this chapter, we present the intakes of the nutrients, LC n-3 FA, vitamin D, vitamin B₁₂, iodine and selenium. The estimates are presented as observed individual means (OIM) and include intakes from the total diet (including food supplements), from fish alone, and from food supplements alone. Additionally, we also present habitual intake from mixed model results for the intakes estimated from the total diet.

8.3.1 LC n-3 FA

LC n-3 FAs are commonly known as the fatty acids, eicosapentanoic acid (EPA), docosapentanoic acid (DPA) and docosahexanoic acid (DHA). There are few natural sources of the LC n-3 FA in the diet. In adults, fish and seafood contributed approximately 65% of the LC n-3 FA in the diet. In addition, food supplements contributed approximately 19% of total LC n-3 FA intake, including 17% from marine oil supplements.

In women, 18-70 years, 65% of total LC n-3 FA intake came from fish, while supplements contributed with 20%. In women 18-45 years, fish and seafood contributed with 64%, fish only with 63% of total LC n-3 FA intake, while supplements contributed 21%, including 16% from marine oil supplements.

In men, fish contributed 65% of the LC n-3 FA intake and supplements contributed 18%.

Table 8.3.1-1 Intake of LC n-3 FA from total diet, fish and supplements in adults, mg/day, OIM¹.

	Sum EPA, DPA and DHA, mg/day				
	Mean	SD	P50	P25	P75
Women, 18-70 (n=925)					
Intake from total diet	958	1188	511	185	1238
Intake from fish	618	1072	61	0	776
Intake from supplements	196	465	0	0	279
Women, 18-45 (n=466)					
Intake from total diet	832	1170	370	144	986
Intake from fish	524	1001	8	0	593
Intake from supplements	172	560	0	0	206
Men, 18-70 (n=862)					
Intake from total diet	1217	1539	611	230	1613
Intake from fish	787	1432	118	0	931
Intake from supplements	220	503	0	0	279

¹Total diet includes food supplements. OIM, observed individual mean.

In adolescents, fish and seafood contributed 62% of the total LC n-3 FA intake, while supplements contributed 13%, including 12% from marine oil supplements. In 13-year-old

girls, 60% and 14% came from fish and supplements, respectively. In boys, fish contributed with 64% of total LC n-3 FA intake, while 12% came from supplements.

In 9-year-old children, 61% of total LC n-3 FA intake came from fish and seafood, while 16% came from supplements, of which 15% were from marine oil supplements. In girls this age group, fish contributed 61% and supplements 15% of total LC n-3 FA intake. In 9-year-old boys 60% came from fish and 16% came from supplements.

Table 8.3.1-2 Intake of LC n-3 FA from total diet, fish and supplements in children and adolescents, mg/day, OIM¹.

	Sum EPA, DPA and DHA, mg/day				
	Mean	SD	P50	P25	P75
Girls, 13-year-olds (n=355)					
Intake from total diet	459	612	204	109	548
Intake from fish	277	597	4	0	194
Intake from supplements	64	127	0	0	76
Boys, 13-year-olds (n=332)					
Intake from total diet	587	937	248	125	551
Intake from fish	373	928	0	0	144
Intake from supplements	71	138	0	0	90
Girls, 9-year-olds (n=341)					
Intake from total diet	439	606	191	101	460
Intake from fish	269	579	9	0	145
Intake from supplements	67	125	0	0	112
Boys, 9-year-olds (n=295)					
Intake from total diet	499	631	242	132	587
Intake from fish	299	606	25	0	306
Intake from supplements	79	144	0	0	112
Girls, 4-year-olds (n=195)					
Intake from total diet	504	449	412	151	726
Intake from fish	323	383	174	30	497
Intake from supplements	98	200	0	0	113
Boys, 4-year-olds (n=204)					
Intake from total diet	481	507	293	128	616
Intake from fish	284	461	75	6	342
Intake from supplements	108	187	0	0	224

¹OIM, observed individual mean. Total diet includes food supplements.

In 4-year-old children, fish and seafood contributed 62% of total LC n-3 FA intake, supplements contributed 21% of which all came from marine oil supplements. In 4-year-old girls, fish and supplements contributed to total LC n-3 FA intake 64% and 19%, respectively, while in boys, the corresponding percentages were 59% and 22%, respectively.

In 2-year-olds, fish and seafood contributed 58% of total LC n-3 FA intake, and supplements contributed 29%, including 23% from marine oil supplements. In 1-year-olds, the corresponding percentages were 59% from fish and seafood, 28% from supplements, including 26% from marine oil supplements.

Table 8.3.1-3 Intake of LC n-3 FA from total diet, fish and supplements in 1- and 2-year-olds, mg/day, OIM¹.

	Sum EPA, DPA and DHA, mg/day				
	Mean	SD	P50	P25	P75
2-year-olds (n=1413)					
Intake from total diet	505	429	410	186	695
Intake from fish	292	354	183	62	392
Intake from supplements	147	230	0	0	295
1-year-olds (n=1957)					
Intake from total diet	422	394	308	133	586
Intake from fish	251	318	152	54	346
Intake from supplements	117	201	0	0	189

¹Total diet includes food supplements. OIM, observed individual mean, in mg/day.

Table 8.3.1-4 shows the LC n-3 FA intake estimates for all groups with habitual intakes based on mixed modelling for Norkost 3 and Ungkost 3, and weighted OIMs for 1- and 2-year-olds. These current (habitual) intakes are the basis for the benefit characterisation in Chapter 9.3. The lowest mean intake was seen in 9-year-olds girls, while adult men reported the highest intake of LC n-3 FA.

Table 8.3.1-4 LC n-3 FA intake (mg/day, including intake from food supplements) in all age groups, current (habitual) intake based on mixed modelling for Norkost 3 and Ungkost 3 and weighted OIM¹ for Spedbarnskost 3 and Småbarnskost 3.

	Sum EPA, DPA and DHA, mg/day						
	Mean	SD	P05	P25	P50	P75	P95
Norkost 3							
Women, 18-70 (n=925)	1008	784	237	466	782	1316	2538
Women, 18-45 (n=466)	783	607	204	374	605	1000	1978
Men, 18-70 (n=862)	1230	990	293	570	932	1577	3170
Ungkost 3							
Girls, 13-year-olds (n=355)	412	372	104	185	291	500	1155
Boys, 13-year-olds (n=332)	489	382	153	241	351	613	1277
Girls, 9-year-olds (n=341)	378	323	107	176	264	462	1047
Boys, 9-year-olds (n=295)	445	362	139	217	315	539	1210
Girls, 4-year-olds (n=195)	531	400	163	255	386	709	1342
Boys, 4-year-olds (n=204)	440	347	126	208	319	575	1125
Spedbarnskost 3 and Småbarnskost 3							
2-year-olds (n=1413)	497	429	68	178	399	691	1310

	Sum EPA, DPA and DHA, mg/day						
1-year-olds (n=1957)	418	383	45	133	309	585	1102

¹OIM, observed individual mean.

8.3.2 Vitamin D

There are few natural rich sources of vitamin D in food. In adults, 23% of total dietary vitamin D intake came from fish and seafood. In addition, 44% came from supplements, including 29% from marine oil supplements.

Fish and seafood contributed 22% of total dietary vitamin D intake in women, while 48% came from supplements. In men, fish and seafood contributed 23% of total dietary vitamin D intake and 40% came from supplements. In women 18 to 45 years, 21% of the total dietary vitamin D intake came from fish, while 46% came from supplements, including 25% from marine oil supplements.

Table 8.3.2-1 Intake of vitamin D from total diet, fish and supplements in adults, µg/day, OIM¹.

	Vitamin D, µg/day				
	Mean	SD	P50	P25	P75
Women, 18-70 (n=925)					
Intake from total diet	10.2	19.4	6.5	3.3	12.7
Intake from fish	2.2	4.0	0.4	0	2.7
Intake from supplements	4.9	18.7	0	0	5.0
Women, 18-45 (n=466)					
Intake from total diet	8.6	10.6	5.4	2.7	10.8
Intake from fish	1.7	3.5	0.1	0	1.8
Intake from supplements	4.0	9.7	0	0	5.0
Men, 18-70 (n=862)					
Intake from total diet	12.6	12.4	8.4	4.8	16.4
Intake from fish	2.8	4.8	0.8	0	3.4
Intake from supplements	5.1	10.9	0	0	5.0

¹OIM, observed individual mean. Intake from total diet includes supplements.

In adolescents (13-years-olds), fish and seafood contributed 18% of total dietary intake of vitamin D, and supplements contributed 45%, including 22% from marine oil supplements. In 9-year-olds, fish and seafood contributed 16% of total dietary vitamin D intake, while supplements contributed 46%, including 24% from marine oil supplements. Further, in 4-year-olds, 15% came from fish and seafood, 57% came from supplements, including 27% from marine oil supplements.

In 13-year-old girls, 16% of total dietary vitamin D intake came from fish, while 46% came from supplements. In 13-year-old boys, the corresponding contributions were 19% and 45%, respectively.

In 9-year-old girls, 15% of total dietary vitamin D intake came from fish, and 48% came from supplements. For 9-year-old boys, the corresponding contributions were 16% and 44%, respectively.

In 4-year-old girls, 16% of total dietary vitamin D intake came from fish, and 55% came from supplements. For boys of the same age group the corresponding contributions were 14% and 58%, respectively.

Table 8.3.2-2 Intake of vitamin D from total diet fish and supplements in children and adolescents, µg/day, OIM¹.

	Vitamin D, µg/day				
	Mean	SD	P50	P25	P75
Girls, 13-year-olds (n=355)					
Intake from total diet	6.3	5.4	4.5	2.2	9.0
Intake from fish	1.0	2.1	0	0	0.8
Intake from supplements	2.9	4.7	0	0	5.0
Boys, 13-year-olds (n=332)					
Intake from total diet	7.4	6.8	4.6	2.3	11.0
Intake from fish	1.4	3.3	0	0	0.8
Intake from supplements	3.3	5.4	0	0	5.2
Girls, 9-year-olds (n=341)					
Intake from total diet	6.6	5.2	5.1	2.6	9.4
Intake from fish	1.0	2.0	0.1	0	0.8
Intake from supplements	3.2	4.3	1.2	0	5.0
Boys, 9-year-olds (n=295)					
Intake from total diet	7.0	5.5	5.2	2.9	9.3
Intake from fish	1.1	2.1	0.2	0	1.2
Intake from supplements	3.1	4.7	0	0	5.0
Girls, 4-year-olds (n=195)					
Intake from total diet	7.6	5.3	6.5	3.4	10.7
Intake from fish	1.2	1.3	0.7	0.2	1.8
Intake from supplements	4.2	4.8	2.5	0	7.5
Boys, 4-year-olds (n=204)					
Intake from total diet	7.8	5.1	6.8	3.7	10.8
Intake from fish	1.1	1.6	0.6	0.1	1.7
Intake from supplements	4.5	4.7	3.8	0	7.5

¹OIM, observed individual mean. Total diet includes food supplement.

Fish and seafood contributed 8% of total dietary vitamin D intake in 2-year-olds, and supplements contributed 54%, including 26% from marine oil supplements.

In 1-year-olds, fish and seafood contributed 4% of total dietary vitamin D intake, and supplements contributed 47%, including 16% from marine oil supplements.

Table 8.3.2-3 Intake of vitamin D from total diet fish and supplements in 1-year-olds and 2-year-olds, µg /day, OIM¹.

	Vitamin D, µg /day				
	Mean	SD	P50	P25	P75
2-year-olds (n=1413)					
Intake from total diet	9.9	7.0	7.9	4.2	14.0
Intake from fish	0.8	0.7	0.6	0.3	1.0
Intake from supplements	5.4	6.5	3.0	0	10.0
1-year-olds (n=1957)					
Intake from total diet	14.9	7.5	14.6	9.7	18.9
Intake from fish	0.7	0.7	0.5	0.2	0.9
Intake from supplements	7.0	5.8	7.2	0	10.0

¹OIM, observed individual mean. Total diet includes food supplements.

Table 8.3.2-4 shows the vitamin D intake for all groups with current (habitual) intake based on mixed modelling for Norkost 3 and Ungkost 3, and weighted OIM for 1- and 2-year-olds. These current (habitual) intakes are the basis for the benefit characterisation in Chapter 9.3. The rationale for reporting results with weighted OIM and using estimates based on mixed model approach are presented in Chapter 7.5.4. The mean intakes range from 6.4 µg/day in 9-year-old girls to 15.1 µg/day in 1-year-olds.

Table 8.3.2-4 Vitamin D intake (µg/day, including intake from food supplements) in all age groups, current (habitual) intake based on mixed modelling for Norkost 3 and Ungkost 3 and weighted OIM¹ for Spedbarnskost 3 and Småbarnskost 3.

	Vitamin D, µg /day						
	Mean	SD	P05	P25	P50	P75	P95
Norkost 3							
Women, 18-70 (n=925)	9.8	6.5	2.6	4.7	7.9	13.9	22.3
Women, 18-45 (n=466)	8.1	5.6	2.3	3.9	6.2	11.3	19.2
Men, 18-70 (n=862)	12.3	7.9	3.7	6.3	9.7	17.0	27.9
Ungkost 3							
Girls, 13-year-olds (n=355)	6.5	5.0	1.3	2.7	4.8	9.3	16.4
Boys, 13-year-olds (n=332)	7.3	5.8	1.4	3.0	5.5	10.3	18.8
Girls, 9-year-olds (n=341)	6.4	4.2	1.5	3.0	5.4	9.1	14.4
Boys, 9-year-olds (n=295)	6.6	4.5	1.7	3.2	5.3	9.3	15.4
Girls, 4-year-olds (n=195)	7.7	4.3	2.1	3.8	7.6	10.8	15.0
Boys, 4-year-olds (n=204)	7.9	4.3	2.1	4.1	7.8	11.0	15.3
Spedbarnskost 3 and Småbarnskost 3							
2-year-olds (n=1413)	9.8	7.1	2.2	4.1	7.6	13.9	23.0
1-year-olds (n=1957)	15.1	7.6	4.1	9.8	14.5	19.1	29.1

¹OIM, observed individual mean.

8.3.3 Iodine

Fish and seafood contributed 41% of total iodine intake in adults and, 7% came from supplements. In women, 36% of total iodine intake came from fish and 9% came from supplements. In men, the corresponding contributions were 42% and 5%, respectively. In women 18-45 years, 29% of total iodine intake came from fish and seafood (28% from fish only), and 10% came from supplements. Marine oils contain little or no iodine. In Norway, the use of iodized salt is not implemented as a measure to increase the general intake of iodine, and thus iodized salt is not an important source of iodine.

Table 8.3.3-1 Intake of iodine from total diet, fish and supplements in adults, µg/day, OIM¹.

	Iodine, µg/day				
	Mean	SD	P50	P25	P75
Women, 18-70 (n=925)					
Intake from total diet	173	142	126	78	221
Intake from fish	63	125	3	0	66
Intake from supplements	15	43	0	0	0
Women, 18-45 (n=466)					
Intake from total diet	154	116	114	77	198
Intake from fish	44	95	1	0	22
Intake from supplements	16	47	0	0	0
Men, 18-70 (n=862)					
Intake from total diet	238	212	169	101	299
Intake from fish	100	189	6	0	111
Intake from supplements	12	72	0	0	0

¹OIM, observed individual mean. Intake from total diet includes supplements.

In adolescents, fish and seafood contributed 23% of total iodine intake, 2% came from supplements. In 13-year-old girls, 21% of total iodine intake came from fish, while 3% came from supplements. In boys of the same age group, corresponding contributions were 25% and 1%, respectively.

In 9-year-old children, fish and seafood contributed 24% of total iodine intake, and supplements contributed 2%. In 9-year-old girls, 23% of total iodine intake came from fish, while 2% came from supplements. In boys, of the same age group, the corresponding contributions were 25% and 2%, respectively.

In 4-year-old children, fish and seafood contributed 31% of total iodine intake, while 3% came from supplements. In girls, of the same age group, 32% of total iodine intake came from fish, while 3% came from supplements. In boys, the corresponding contributions were 31% and 3%, respectively.

Table 8.3.3-2 Intake of iodine from total diet, fish and supplements in children and adolescents, µg/day, OIM¹.

	Iodine, µg /day				
	Mean	SD	P50	P25	P75
Girls, 13-year-olds (n=355)					
Intake from total diet	104	69	86	60	130
Intake from fish	22	49	1	0	29
Intake from supplements	3	16	0	0	0
Boys, 13-year-olds (n=332)					
Intake from total diet	130	86	108	71	168
Intake from fish	32	63	0	0	45
Intake from supplements	1	9	0	0	0
Girls, 9-year-olds (n=341)					
Intake from total diet	109	64	96	64	137
Intake from fish	25	42	2	0	36
Intake from supplements	2	13	0	0	0
Boys, 9-year-olds (n=295)					
Intake from total diet	130	70	117	82	164
Intake from fish	33	57	3	0	47
Intake from supplements	2	12	0	0	0
Girls, 4-year-olds (n=195)					
Intake from total diet	120	59	107	77	152
Intake from fish	38	49	19	1	58
Intake from supplements	3	17	0	0	0
Boys, 4-year-olds (n=204)					
Intake from total diet	129	69	117	83	160
Intake from fish	40	55	20	1	61
Intake from supplements	4	17	0	0	0

¹OIM, observed individual mean. Intake from total diet includes supplements.

In 2-year-olds, fish and seafood contributed 24% of total iodine intake, while supplements contributed with 7%. In addition, in 1-year-olds, 21% came from fish and seafood, while 6% came from supplements.

Table 8.3.3-3 Intake of iodine from total diet, fish and supplements in 1- and 2-year-olds, µg/day, OIM¹.

	Iodine, µg /day				
	Mean	SD	P50	P25	P75
2-year-olds (n=1413)					
Intake from total diet	156	67	146	110	188
Intake from fish	38	26	34	20	50
Intake from supplements	9	32	0	0	0
1-year-olds (n=1957)					
Intake from total diet	129	72	116	81	159
Intake from fish	27	23	22	12	37
Intake from supplements	8	31	0	0	0

¹OIM, observed individual mean. Intake from total diet includes supplements.

Table 8.3.3-4 shows the iodine intake for all groups with current (habitual) intake based on mixed modelling for Norkost 3 and Ungkost 3, and weighted OIM for 1- and 2-year-olds. These current (habitual) intakes are the basis for the benefit characterisation in Chapter 9.3. The rationale for reporting results with weighted OIM and mixed model are presented in Chapter 7.5.4. The mean intakes range from 100 µg/day in 13-year-old girls to 229 µg/day in adult men.

Table 8.3.3-4 Iodine intake (µg/day, including intake from food supplements) in all age groups, current (habitual) intake based on mixed modelling for Norkost 3 and Ungkost 3, and weighted OIM¹ for Spedbarnskost 3 and Småbarnskost 3.

	Iodine, µg/day						
	Mean	SD	P05	P25	P50	P75	P95
Norkost 3							
Women, 18-70 (n=925)	168	73	81	119	155	202	307
Women, 18-45 (n=466)	152	64	74	109	142	183	275
Men, 18-70 (n=862)	229	126	90	145	203	283	569
Ungkost 3							
Girls, 13-year-olds (n=355)	100	46	41	65	89	124	185
Boys, 13-year-olds (n=332)	123	60	50	81	114	161	236
Girls, 9-year-olds (n=341)	102	46	45	70	94	128	188
Boys, 9-year-olds (n=295)	121	38	68	91	114	144	189
Girls, 4-year-olds (n=195)	121	44	61	87	113	143	200
Boys, 4-year-olds (n=204)	127	46	66	95	119	153	214
Spedbarnskost 3 and Småbarnskost 3							
2-year-olds (n=1413)	156	67	69	109	148	190	285
1-year-olds (n=1957)	133	74	48	83	118	163	276

¹OIM, observed individual mean.

8.3.4 Selenium

In adults, fish and seafood contributed 30% of total selenium intake, while 9% came from supplements. Marine oils contain little or no selenium. In women, 25% of total selenium intake came from fish, while 11% came from supplements. In men, the corresponding contribution were 29% and 7%, respectively. In women 18-45 years, 25% of total selenium intake came from fish and seafood (24% from fish only), and 12% came from supplements.

Table 8.2.4-1 Intake of selenium from total diet, fish and supplements in adults, µg/day, OIM¹.

	Selenium, µg/day				
	Mean	SD	P50	P25	P75
Women, 18-70 (n=925)					
Intake from total diet	56	35	49	34	67

	Selenium, µg/day				
Intake from fish	14	21	5	0	21
Intake from supplements	6	18	0	0	0
Women, 18-45 (n=466)					
Intake from total diet	51	31	45	31	62
Intake from fish	12	22	0	0	17
Intake from supplements	6	17	0	0	0
Men, 18-70 (n=862)					
Intake from total diet	73	46	63	47	87
Intake from fish	21	34	7	0	30
Intake from supplements	5	25	0	0	0

¹OIM, observed individual mean. Intake from total diet includes supplements.

In adolescents, fish and seafood contributed 16% of total selenium intake, while supplements contributed 1.6%. In 13-year-old girls, 15% of total selenium intake came from fish, and 3% from supplements. In boys of the same age group, fish also contributed 15%, with no contribution from supplements.

In 9-year-old children, fish and seafood contributed 16% of total selenium intake, while 1.5% came from supplements. Both girls and boys had a 16% intake of selenium from fish, and very low and no intake of selenium from supplements, respectively.

In 4-year-old children, 21% of total selenium intake came from fish and seafood and only 2.5% from supplements. Fish contributed 23% and 19% of total selenium intake, in girls and boys, respectively.

Table 8.2.4-2 Intake of selenium from total diet, fish and supplements in children and adolescents, µg/day, OIM¹.

	Selenium, µg/day				
	Mean	SD	P50	P25	P75
Girls, 13-year-olds (n=355)					
Intake from total diet	33	14	30	23	39
Intake from fish	5	8	1	0	6
Intake from supplements	1	4	0	0	0
Boys, 13-year-olds (n=332)					
Intake from total diet	40	17	35	28	49
Intake from fish	6	11	0	0	9
Intake from supplements	0	2	0	0	0
Girls, 9-year-olds (n=341)					
Intake from total diet	32	12	30	24	38
Intake from fish	5	7	2	0	6
Intake from supplements	1	3	0	0	0
Boys, 9-year-olds (n=295)					
Intake from total diet	38	16	35	28	45

	Selenium, µg/day				
Intake from fish	6	10	3	0	9
Intake from supplements	0	3	0	0	0
Girls, 4-year-olds (n=195)					
Intake from total diet	30	12	28	22	35
Intake from fish	7	6	6	2	9
Intake from supplements	1	4	0	0	0
Boys, 4-year-olds (n=204)					
Intake from total diet	32	11	30	24	39
Intake from fish	6	7	4	1	8
Intake from supplements	1	4	0	0	0

¹OIM, observed individual mean. Intake from total diet includes supplements.

In 2-year-olds, fish and seafood contributed 17% of total selenium intake, while 6% came from supplements. In 1-year-olds, the corresponding contributions were 16% and 6%, respectively.

Table 8.2.4-3 Intake of selenium from total diet, fish and supplements in 1- and 2-year-olds, µg/day, OIM¹.

	Selenium, µg/day				
	Mean	SD	P50	P25	P75
2-year-olds (n=1413)					
Intake from total diet	36	15	33	26	43
Intake from fish	6	5	5	3	8
Intake from supplements	2	8	0	0	0
1-year-olds (n=1957)					
Intake from total diet	31	15	28	21	37
Intake from fish	5	5	3	2	6
Intake from supplements	2	7	0	0	0

¹OIM, observed individual mean. Intake from total diet includes supplements.

Table 8.3.4-4 shows the selenium intake for all groups with current (habitual) intake based on mixed modelling for Norkost 3 and Ungkost 3, and weighted OIM for 1- and 2-year-olds. These current (habitual) intakes are the basis for the benefit characterisation in Chapter 9.3. The rationale for reporting results with weighted OIM and using mixed model are presented in Chapter 7.5.4. The mean intakes range from 28 µg/day in 4-year-old girls to 72 µg/day in adult men.

Table 8.3.4-4 Selenium intake (µg/day, including intake from food supplements) in all age groups, current (habitual) intake based on mixed modelling for Norkost 3 and Ungkost 3, and weighted OIM¹ for Spedbarnskost 3 and Småbarnskost 3.

	Selenium, µg/day						
	Mean	SD	P05	P25	P50	P75	P95

	Selenium, µg/day						
Norkost 3							
Women, 18-70 (n=925)	55	17	29	41	52	65	86
Women, 18-45 (n=466)	52	17	28	39	50	62	83
Men, 18-70 (n=862)	79	25	38	54	69	86	117
Ungkost 3							
Girls, 13-year-olds (n=355)	32	11	18	25	31	38	52
Boys, 13-year-olds (n=332)	39	13	22	30	37	46	63
Girls, 9-year-olds (n=341)	31	9	19	25	30	36	47
Boys, 9-year-olds (n=295)	37	11	22	29	35	43	57
Girls, 4-year-olds (n=195)	28	7	18	23	27	32	41
Boys, 4-year-olds (n=204)	32	9	19	25	31	38	47
Spedbarnskost 3 and Småbarnskost 3							
2-year-olds (n=1413)	36	15	18	27	33	43	67
1-year-olds (n=1957)	31	15	13	22	28	38	61

¹OIM, observed individual mean.

8.3.5 Vitamin B₁₂

In adults, fish and seafood contributed 24% of total vitamin B₁₂ intake, and 21% came from supplements. In women, 17% of total vitamin B₁₂ intake came from fish, while 33% came from supplements. The corresponding contributions in men were 24% and 9%, respectively. In women of 18-45 years, fish and seafood contributed 19% of total vitamin B₁₂ intake (16% from fish only), while 23% came from supplements.

Table 8.2.5-1 Intake of vitamin B₁₂ from total diet, fish and supplements in adults, µg/day, OIM¹.

	Vitamin B ₁₂ , µg/day				
	Mean	SD	P50	P25	P75
Women, 18-70 (n=925)					
Intake from total diet	8.4	28.4	5.1	3.7	7.6
Intake from fish	1.4	2.2	0.5	0	2.1
Intake from supplements	2.8	28.5	0	0	0
Women, 18-45 (n=466)					
Intake from total diet	6.8	16.1	5.0	3.6	7.1
Intake from fish	1.1	1.9	0.2	0	1.6
Intake from supplements	1.6	15.9	0	0	0
Men, 18-70 (n=862)					
Intake from total diet	9.3	14.3	7.4	5.3	10.4
Intake from fish	2.2	3.4	0.9	0	3.0
Intake from supplements	0.8	12.1	0	0	0

¹OIM, observed individual mean. Intake from total diet includes supplements.

In adolescents, fish and seafood contributed 12% of total vitamin B₁₂ intake, while 4% came from supplements. In 13-year-old girls, 10% of total intake came from fish, while 4% came from supplements. In boys of the same age group, the corresponding contributions were 12% and 4%, respectively.

In 9-year-olds, 13% of total vitamin B₁₂ intake came from fish and seafood, 4.2% from supplements. Both genders of this age group got 12% of total vitamin B₁₂ intake from fish only, while the contribution from supplements were 5% and 4%, in girls and boys, respectively.

In 4-year-olds, fish and seafood contributed 19% of total vitamin B₁₂ intake, while 7% came from supplements. Fish only contributed 19% and 18% in girls and boys, respectively, while contributions from supplements were 7% for both sexes.

Table 8.2.5-2 Intake of B₁₂ from total diet, fish and supplements in children and adolescents, µg/day, OIM¹.

	Vitamin B ₁₂ , µg/day				
	Mean	SD	P50	P25	P75
Girls, 13-year-olds (n=355)					
Intake from total diet	4.8	4.3	4.4	3.2	5.6
Intake from fish	0.5	0.9	0.1	0	0.6
Intake from supplements	0.2	0.6	0	0	0
Boys, 13-year-olds (n=332)					
Intake from total diet	5.7	2.7	5.3	3.8	7.0
Intake from fish	0.7	1.3	0	0	0.7
Intake from supplements	0.2	0.6	0	0	0
Girls, 9-year-olds (n=341)					
Intake from total diet	4.3	1.8	4.0	3.0	5.2
Intake from fish	0.5	0.9	0.2	0	0.6
Intake from supplements	0.2	0.4	0	0	0
Boys, 9-year-olds (n=295)					
Intake from total diet	5.0	2.1	4.6	3.5	6.2
Intake from fish	0.6	0.9	0.2	0	1.0
Intake from supplements	0.2	0.4	0	0	0
Girls, 4-year-olds (n=195)					
Intake from total diet	4.2	1.4	4.0	3.2	5.1
Intake from fish	0.8	0.7	0.7	0.3	1.1
Intake from supplements	0.3	0.5	0	0	0.3
Boys, 4-year-olds (n=204)					
Intake from total diet	4.4	1.7	4.1	3.3	5.3
Intake from fish	0.8	0.9	0.5	0.1	1.1
Intake from supplements	0.3	0.6	0	0	0.3

¹OIM, observed individual mean. Intake from total diet includes supplements.

Fish and seafood contributed 16% to total vitamin B₁₂ intake in 2-year-olds, and 17% in 1-year-olds. Supplements contributed approximately 3% of total vitamin B₁₂ intake in both age groups.

Table 8.2.5-3 Intake of B₁₂ from total diet, fish and supplements in 1- and 2-year-olds, µg/day, OIM¹.

	Vitamin B ₁₂ , µg / day				
	Mean	SD	P50	P25	P75
2-year-olds (n=1413)					
Intake from total diet	4.3	1.8	4.0	3.1	5.1
Intake from fish	0.7	0.7	0.5	0.3	0.9
Intake from supplements	0.1	0.7	0	0	0
1-year-olds (n=1957)					
Intake from total diet	3.2	1.5	3.0	2.2	3.9
Intake from fish	0.5	0.6	0.4	0.2	0.7
Intake from supplements	0.1	0.3	0	0	0

¹OIM, observed individual mean. Intake from total diet includes supplements.

Table 8.3.5-4 shows the vitamin B₁₂ intake for all groups with current (habitual) intake based on mixed modelling for Norkost 3 and Ungkost 3, and weighted OIM for 1- and 2-year-olds. These current (habitual) intakes are the basis for the benefit characterisation in Chapter 9.3. The rationale for reporting results with weighted OIM and using mixed model are presented in Chapter 7.5.4. The mean intakes range from 3.3 µg/day in 1-year-olds to 8.9 µg/day in adult men.

Table 8.3.5-4 Vitamin B₁₂ intake (µg/day, including intake from food supplements) in all age groups, current (habitual) intake based on mixed modelling for Norkost 3 and Ungkost 3, and weighted OIM¹ for Spedbarnskost 3 and Småbarnskost 3.

	Vitamin B ₁₂ , µg / day						
	Mean	SD	P05	P25	P50	P75	P95
Norkost 3							
Women, 18-70 (n=925)	6.6	2.6	3.3	4.8	6.2	8.0	11.4
Women, 18-45 (n=466)	6.4	2.5	3.2	4.7	6.1	7.8	11.0
Men, 18-70 (n=862)	8.9	2.5	5.4	7.1	8.6	10.4	13.5
Ungkost 3							
Girls, 13-year-olds (n=355)	4.6	1.5	2.4	3.5	4.4	5.5	7.4
Boys, 13-year-olds (n=332)	5.6	2.1	2.7	4.1	5.3	6.9	9.5
Girls, 9-year-olds (n=341)	4.1	1.4	2.1	3.1	4.0	5.0	6.7
Boys, 9-year-olds (n=295)	4.9	1.6	2.6	3.7	4.7	5.8	7.7
Girls, 4-year-olds (n=195)	4.1	1.1	2.5	3.3	4.1	4.8	6.1

	Vitamin B ₁₂ , µg/day						
Boys, 4-year-olds (n=204)	4.4	1.3	2.5	3.4	4.3	5.3	6.8
Spedbarnskost 3 and Småbarnskost 3							
2-year-olds (n=1413)	4.3	1.8	2.1	3.1	4.0	5.2	7.5
1-year-olds (n=1957)	3.3	1.5	1.4	2.3	3.0	4.0	5.9

¹OIM, observed individual mean. Intake from total diet includes supplements.

8.4 Estimates of contaminant exposure

To calculate the habitual dietary exposure, KBS food consumption and body weight data at the individual level were used. Occurrence data and consumption data were merged for each food reported eaten in the dietary surveys. For each individual in the surveys, the mean lower bound and/or upper bound occurrence values of the different food samples were combined with the daily consumption of the corresponding food items. The resulting exposures per food were summed to obtain the total exposure at the individual level (divided by the respective individual body weight and converted to weekly exposure by multiplying by seven).

8.4.1 PCDD/F and DL-PCB

PCDD/Fs and DL-PCBs are contributed by all food groups, but fish, crustaceans, meat and dairy are the major contributors.

The exposure estimates for PCDD/Fs and DL-PCBs are based on occurrence data in Norwegian food, supplemented with data from EFSA (see Chapter 7.2.1). For this benefit and risk assessment of fish intake, we present UB mixed-model exposure estimates for PCDD/Fs and DL-PCBs in Table 8.3.1-1. Mean exposures ranged from 4.4 to 12.3 pg TEQ/kg bw/week for the sum of PCDD/Fs and DL-PCBs in different age groups. The 95th-percentile exposure estimates were 1.5 to 2 times higher than the mean in different age groups. The UB exposure was 1.7 times (adults) to 2 times (13-years-olds) higher than the corresponding LB exposure (VKM 2022). When considering only the PCDD/Fs, the mean UB intake ranged from 2.6 to 7.1 pg TEQ/kg bw/week in different age groups. For further details about LB data, see the VKM risk assessment of PCDD/Fs and DL-PCBs from the total diet (VKM 2022).

Table 8.4.1-1 PCDD/F and DL-PCB exposure from total diet^a (pg TEQ_{WHO2005}/kg bw/week, upper bound), VKM database, among adults (Norkost 3) and 13-, 9- and 4-year-olds (Ungkost 3), current (habitual) intake based on mixed modelling, and 2- and 1-year-olds (Småbarnskost 3 and Spedkost 3), weighted OIM¹

	PCDD/Fs and DL-PCBs (29 congeners)							PCDD/Fs (17 congeners)						
	Mean	SD	P05	P25	P50	P75	P95	Mean	SD	P05	P25	P50	P75	P95
Adults, 18-70 (n=1787)	4.62	1.74	2.32	3.38	4.35	5.56	7.88	2.61	0.89	1.39	1.97	2.49	3.11	4.26
Women, 18-45 (n=466)	4.40	1.64	2.25	3.22	4.16	5.30	7.47	2.55	0.87	1.38	1.93	2.44	3.05	4.15
13-year-olds (n=687)	4.67	2.06	2.11	3.21	4.28	5.70	8.52	2.98	1.24	1.38	2.10	2.78	3.63	5.28
9-year-olds (n=636)	6.61	2.28	3.49	5.00	6.30	7.92	10.78	4.24	1.38	2.30	3.25	4.08	5.05	6.74
4-year-olds (n=399)	10.91	3.02	6.67	8.78	10.59	12.71	16.33	6.67	1.62	4.32	5.53	6.54	7.68	9.53
2-year-olds (n=1413)	12.34	6.23	5.38	8.25	11.28	14.83	22.43	7.24	3.12	3.49	5.13	6.75	8.66	12.48
1-year-olds (n=1957)	12.24	7.45	4.34	7.76	10.86	14.94	24.23	7.06	3.38	2.82	4.73	6.47	8.66	13.19

^aContribution from fruits, vegetables, and potatoes is not included. ¹Observed individual mean.

Fish was the largest contributor to mean total UB exposure to PCDD/Fs and DL-PCBs in adults, and was also an important source in children, together with milk and other dairy products (Figure 8.4.1-1).

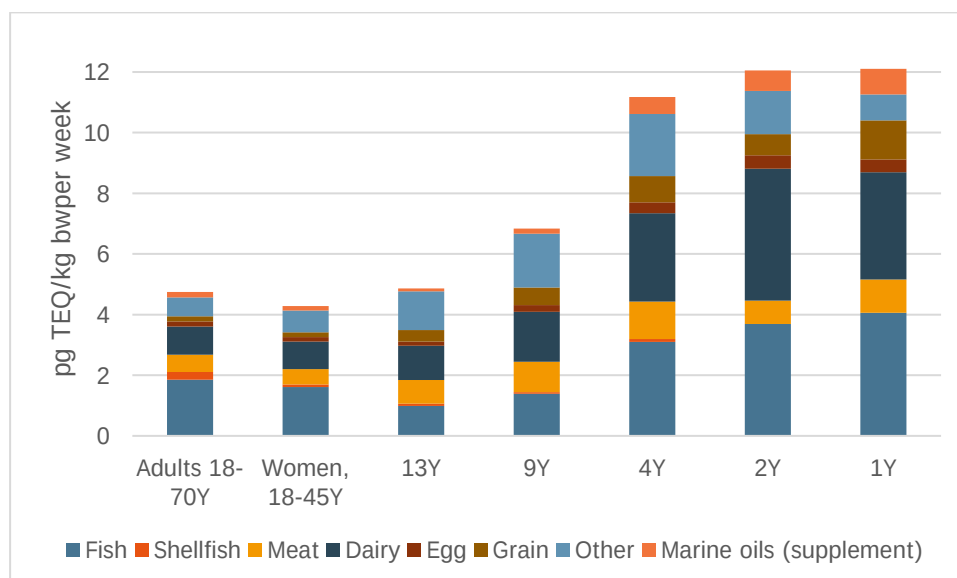


Figure 8.4.1-1 Contribution of food groups to the total dietary exposure of PCDD/Fs and DL-PCBs in adults (Norkost 3), 13- 9-, and 4-year-olds (Ungkost 3), 2- and 1-year-olds (Småbarnskost 3 and Spedkost 3). Intakes are based on mean OIM (upper bound) and concentrations in the VKM dataset. Y: years.

Fish contributed 21-39% to the mean total intake of PCDD/Fs and DL-PCBs for different age groups, whereas dairy contributed 20-35% (Table 8.4.1-2). Fatty fish was the main contributor to the dietary exposure to PCDD/Fs and DL-PCBS from fish (Figure 8.4.1-2).

Table 8.4.1-2 Percent contribution of food groups to the total^a dietary intake of PCDD/Fs and DL-PCBs in adults (Norkost 3), 13- 9-, and 4-year-olds (Ungkost 3), 2- and 1-year-olds (Småbarnskost 3 and Spedkost 3). Intakes are based on mean OIM¹ and concentrations in the VKM dataset (upper bound).

	Adults, 18-70 years (n=1787)	Women, 18-45 years (n=466)	13-year- olds (n=687)	9-year- olds (n=636)	4-year- olds (n=399)	2-year- olds (n=1413)	1-year- olds (n=1957)
Fish	39	38	21	20	28	30	33
Shellfish	5.5	2.1	1.4	0.7	0.8	<0.1	<0.1
Meat	12	12	16	15	11	6.2	9
Dairy	20	21	23	24	26	35	29
Egg	3.5	3.5	2.9	3.2	3.2	3.6	3.5
Grain	3.3	3.8	7.8	8.4	7.8	5.7	11
Other	13	17	26	26	18	12	7
Marine oils (supplement)	3.8	3.5	1.8	2.5	5	7.6	7.3

^aContribution from fruits, vegetables, and potatoes is not included. ¹Observed individual mean.

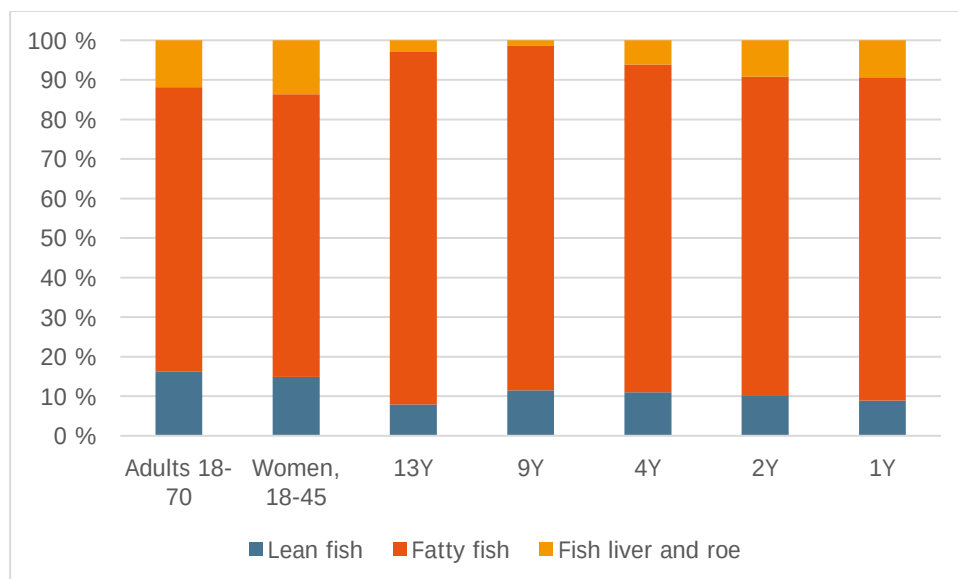


Figure 8.4.1-2 The contribution of categories of fish to the upper bound intake of the sum of PCDD/Fs and DL-PCBs from fish in adults (Norkost 3), 13- 9-, and 4-year-olds (Ungkost 3), 2- and 1-year-olds (Småbarnskost 3 and Spedkost 3). Intakes are based on mean OIM (upper bound) and concentrations in the VKM dataset. Y: years.

8.4.2 PFASs

The LB exposure to PFASs is presented in the main body of the text in this chapter, and UB exposures can be found in Appendix IX, Chapter 22. The LB is selected because of the high proportion of food samples with non-detected concentrations because of low sensitivity in the analytical methods for PFASs, resulting in high LOQs and large uncertainties reflected in large difference between LB and UB exposure estimates. Furthermore, based on toxicokinetic modelling and available serum concentrations EFSA (2020) reported that the true exposure is more likely to be closer to the LB than the UB estimates. The impact of the detection limits on exposure assessments is further discussed in Chapter 11 (Uncertainty).

Exposure estimates obtained with the occurrence database from EFSA 2020 (EFSA dataset) is presented in the main body of the text. This dataset was selected because it is considered by VKM to be more robust and less influenced by single data than the VKM dataset. Compared to the VKM dataset the number of samples is higher in the EFSA dataset and the proportion of samples with levels below the LOQ was lower. Furthermore, the data from PERFOOD were also submitted to EFSA and contributes to the fraction of samples with concentrations above the LOQ. Also, a large proportion of fish samples in the EFSA database was submitted by Norway. Finally, the EFSA dataset results in 1.4 to 1.9 times higher exposures than the VKM dataset (see appendix IX, Chapter 22). Exposures at LB

underestimate the true exposure and the slightly higher exposure estimate based on the EFSA dataset is a more conservative estimate. VKM prefers to take a conservative approach and applies exposure estimates based on the EFSA dataset for the risk characterization in Chapter 9. The LB and UB results obtained with the VKM dataset are shown in appendix IX, chapter 22 in which also a comparison of the findings with the two datasets is presented.

Exposure to the PFASs (PFOS, PFOA, PFNA and PFHxS and the sum of the four PFASs) based on the EFSA dataset is shown in table 8.4.2-1. The mean intakes of the sum of four PFASs ranged from 6.5 to 18 ng/kg bw/week. The corresponding 95th-percentile estimates ranged from 8.8 to 35 ng/kg bw/week. The highest estimated exposure to the sum of the four PFASs was in 1-, 2- and 4-year-olds, reflecting their higher energy requirements compared to adults, who have approximately half the estimated exposure compared to these groups of children. The exposure in 1-year olds was similar to that in 4-years-olds. Of note, exposure in 1-year olds is considered an underestimate, since contribution from 'food for infants and young children', which is a major food group in this age groups in terms of consumption, is missing because there were no reliable concentration data available (explained in Chapter 7.2.2).

Table 8.4.2-1 Mean, median and 95th percentile of PFAS exposure (LB) from total diet (ng/kg bw per week) for adults (Norkost 3), 13- 9-, and 4-year-olds (Ungkost 3), current (habitual) intake based on mixed modelling, and 2- and 1-year-olds (Småbarnskost 3 and Spedkost 3), weighted OIM¹. Intakes are based on the EFSA-dataset.

	PFOS			PFOA			PFNA			PFHxS			Sum of PFASs		
	Mean	P50	P95	Mean	P50	P95	Mean	P50	P95	Mean	P50	P95	Mean	P50	P95
Adults, 18-70 (n=1787)	4.66	4.18	9.08	1.64	1.54	2.87	0.28	0.25	0.58	0.77	0.72	1.40	7.37	6.85	13.07
Women, 18-45 (n=466)	3.76	3.44	7.01	1.60	1.53	2.60	0.26	0.24	0.52	0.82	0.78	1.42	6.47	6.11	10.99
13-year-olds (n=687)	2.95	2.63	5.92	1.10	1.01	2.03	0.15	0.13	0.37	0.32	0.25	0.85	4.51	4.08	8.76
9-year-olds (n=636)	4.21	3.88	7.62	1.50	1.41	2.54	0.26	0.23	0.53	0.52	0.46	1.11	6.47	6.05	11.23
4-year-olds (n=399)	9.50	8.77	17.29	2.69	2.57	4.30	0.73	0.66	1.47	1.39	1.30	2.46	14.33	13.48	24.65
2-year-olds (n=1413)	11.36	10.21	22.78	3.48	3.09	6.86	1.04	0.92	2.18	2.28	2.10	4.60	18.16	16.70	33.86
1-year-olds (n=1957)	10.14	8.74	23.59	3.35	2.79	7.44	0.96	0.82	2.12	1.96	1.67	4.22	16.40	14.45	35.41

¹Observed individual mean.

The highest contribution to the total intake was from PFOS, followed by PFOA, together contributing approximately 80-90% of the sum of the four PFASs (Figure 8.4.2-1).

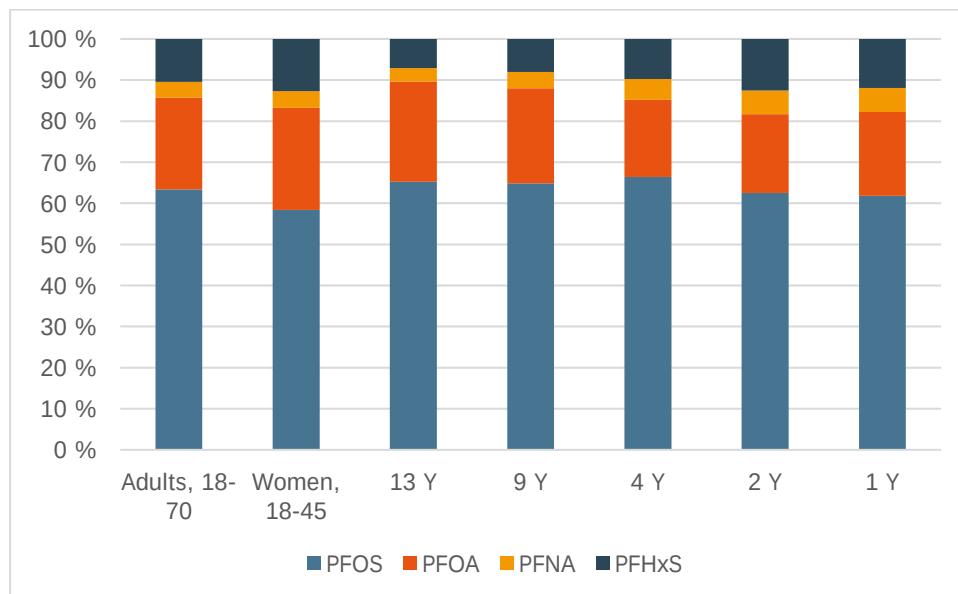


Figure 8.4.2-1 Mean relative contribution of PFOS, PFOA, PFNA and PFHxS (LB) to the sum of 4 PFASs for adults (Norkost 3), 13- 9-, and 4-year-olds (Ungkost 3), habitual intake based on mixed modelling, and 2- and 1-year-olds (Småbarnskost 3 and Spedkost 3), weighted OIM. Intakes are based on concentrations in the EFSA-dataset. Y: years.

Many food groups contribute to the exposure to the sum of 4 PFASs, as illustrated in Figure 8.4.2-2. Fish contributed most (31-42 % for different age groups), followed by fruit/vegetables/potatoes 17-32 %) and eggs (13-19 %) (Table 8.4.2-2).

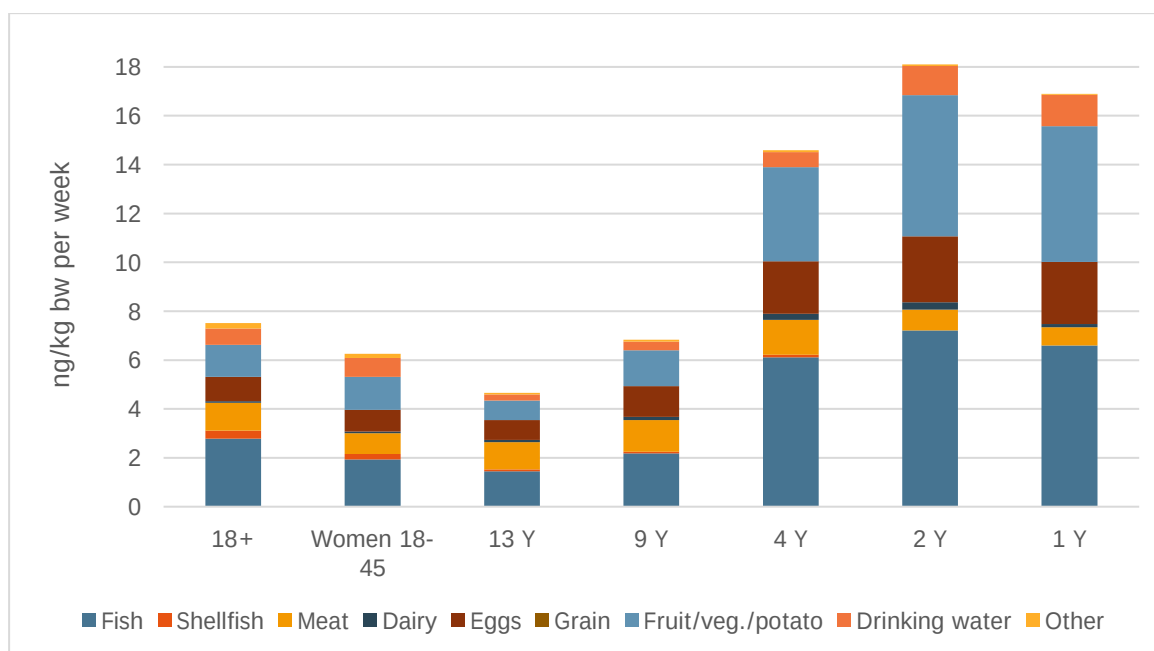


Figure 8.4.2-2 Contribution of food groups to the total dietary exposure of sum of 4 PFASs in adults (Norkost 3), 13- 9-, and 4-year-olds (Ungkost 3), 2- and 1-year-olds (Småbarnskost 3 and Spedkost 3). Intakes are based on mean OIM and concentrations in the EFSA dataset. Y: years.

Table 8.4.2-2 Percent contribution of food groups to the total dietary intake of sum of 4 PFASs in adults (Norkost 3), 13- 9-, and 4-year-olds (Ungkost 3), 2- and 1-year-olds (Småbarnskost 3 and Spedkost 3). Intakes are based on mean OIM¹ (lower bound) and concentrations in the EFSA dataset.

	Adults, 18-70 years (n=1787)	Women, 18-45 years (n=466)	13- year- olds (n=687)	9-year- olds (n=636)	4-year- olds (n=399)	2-year- olds (n=1413)	1-year- olds (n=1957)
Fish	37	31	31	32	42	40	39
Shellfish	4.3	3.5	1.7	1	0.8	0	0
Meat	15	14	24	19	9.7	4.6	4.4
Dairy	0.9	1.1	1.9	1.9	1.7	1.7	0.8
Eggs	13	14	18	19	15	15	15
Grain	0	0	0	0	0	0	0
Fruit/veg./potato	17	22	17	21	26	32	33
Drinking water	8.9	12	5.6	5.3	4.2	6.6	7.6
Other	3.1	2.7	1.3	1	0.5	0.3	0.2

¹Observed individual mean.

Information regarding the contribution of different food groups to the intake of each of the 4 PFASs are shown in appendix IX, Chapter 22 Tables 1-7.

Lean fish contributed approximately equally as fatty fish to the total intake from fish based on the EFSA dataset (figure 8.4.2-3).

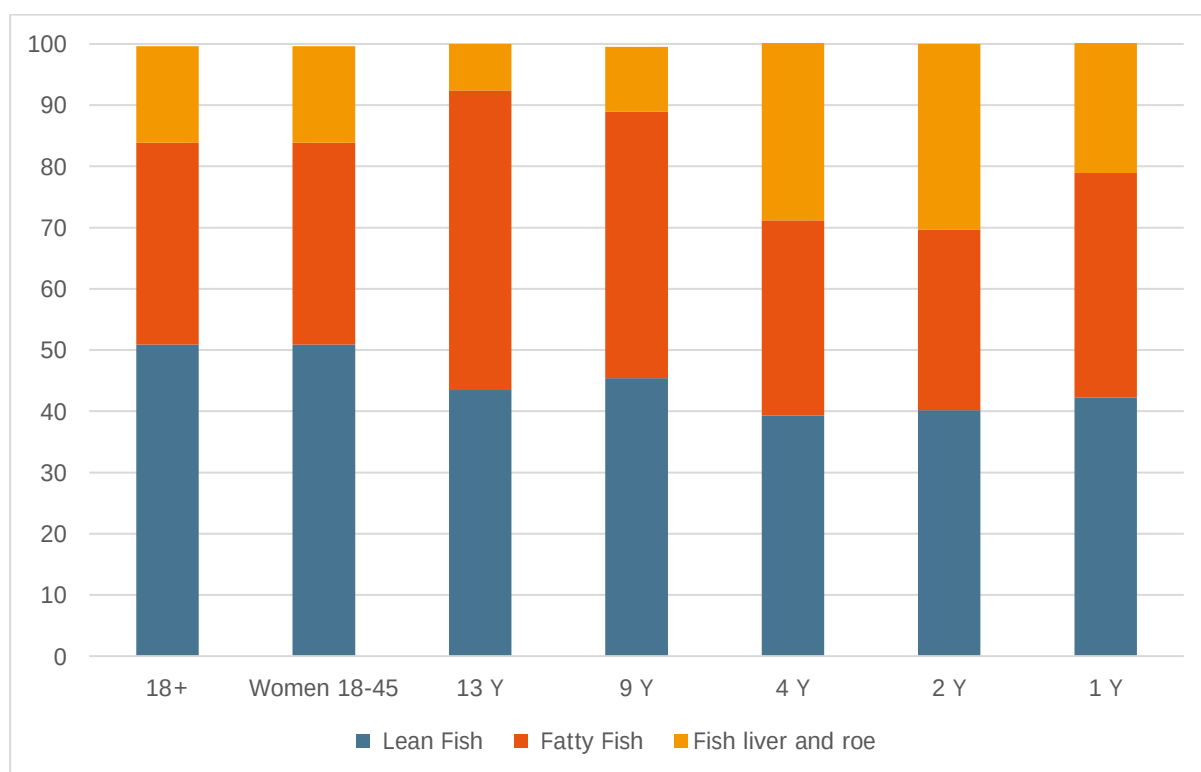


Figure 8.4.2-3 Mean relative contribution of fish categories to the sum 4 PFASs from fish in adults (Norkost 3), 13- 9-, and 4-year-olds (Ungkost 3), 2- and 1-year-olds (Småbarnskost 3 and Spedkost 3). Intakes are based on mean OIM and concentrations in the EFSA dataset. Y: years.

8.4.3 Methyl mercury

The weekly exposure to methyl mercury based on total mercury in fish and other seafood, as described in Chapter 7.2.3 for different age groups, is given in Table 8.4.3-1. The mean exposure from the seafood diet ranged from 0.56 µg/kg bw per week in 1- and 2-year-olds to 0.12 µg/kg bw per week in 13-year-olds. High (95th percentile) exposure was highest in 1-year-olds (1.42 µg/kg bw/week) but was not much higher than the high exposure in adults (1.17 µg/kg bw/week). It should be noted that the 95th -percentile exposure is to be considered an overestimate for Ungkost 3 and Norkost 3, as it is based on OIMs. This is due to the survey methods used (for further details, see chapter 7.5.1). Furthermore, the assumption that all mercury in fish and seafood is methyl mercury is a conservative approach. Methyl mercury in fish liver and roe is not included, as the levels are so low that it will not affect the risk assessment.

Table 8.4.3-1 Methyl mercury exposure from total diet presented as OIM estimates from current intake ($\mu\text{g}/\text{kg}$ bw per week), among adults (Norkost 3) and 13-, 9- and 4-year-olds (Ungkost 3), and 2- and 1-year-olds (Småbarnskost 3 and Spedkost 3), presented as weighted OIM¹

	Methyl mercury						
	Mean	SD	P05	P25	P50	P75	P95
Adults, 18-70 (n=1787)	0.28	0.48	0	0	0.09	0.36	1.17
Women, 18-45 (n=466)	0.20	0.38	0	0	0.04	0.26	0.85
13-year-olds (n=687)	0.12	0.22	0	0	0.01	0.16	0.57
9-year-olds (n=636)	0.19	0.29	0	0	0.08	0.27	0.81
4-year-olds (n=399)	0.44	0.55	0	0.07	0.27	0.61	1.33
2-year-olds (n=1413)	0.56	0.38	0.07	0.29	0.48	0.74	1.23
1-year-olds (n=1957)	0.56	0.47	0.05	0.23	0.45	0.76	1.42

¹Observed individual mean.

Fish contributed 86-100% of the mean intake, depending on the age group. Lean fish was the major source, contributing 54-74% of the total intake depending on age groups. Fatty fish contributed 23-46% depending on age group (Figure 8.4.3-1 and Table 8.4.3-2).

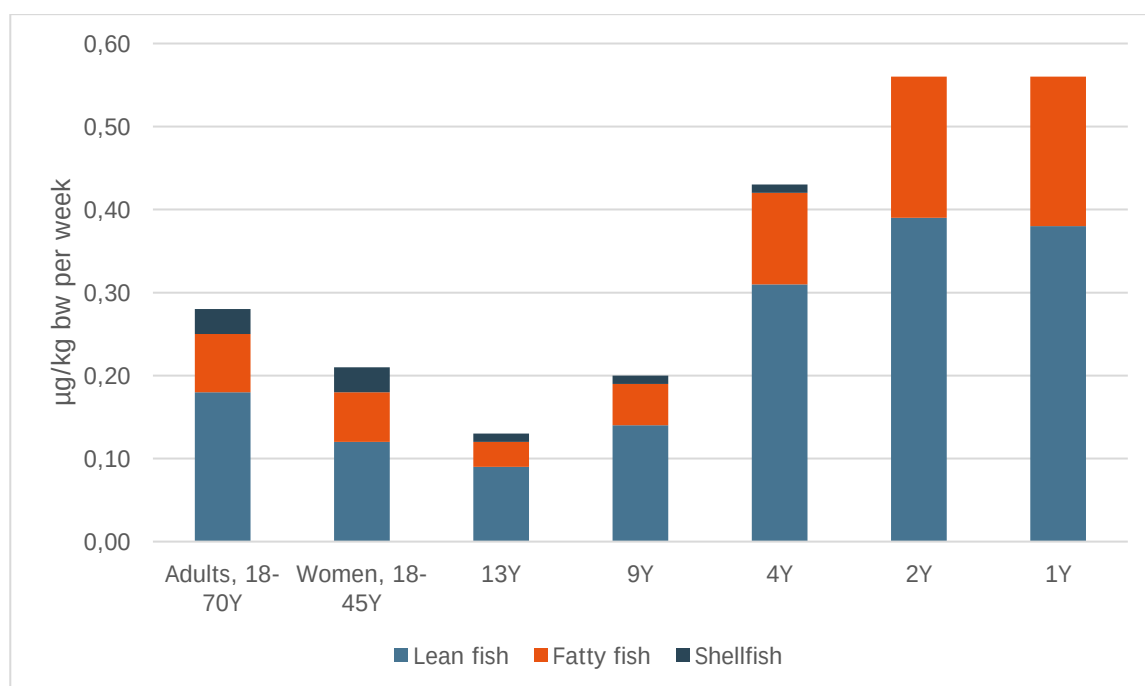


Figure 8.4.3-1 Contribution of food categories to intake of methyl mercury exposure in adults (Norkost 3), 13- 9-, and 4-year-olds (Ungkost 3), 2- and 1-year-olds (Småbarnskost 3 and Spedkost 3). Intakes are based on mean OIM. Y: years.

Table 8.3.3-2 Percent contribution of food groups to the total dietary intake of methyl mercury in adults (Norkost 3), 13- 9-, and 4-year-olds (Ungkost 3), 2- and 1-year-olds (Småbarnskost 3 and Spedkost 3). Intakes are based on mean OIM¹.

	Adults, 18-70 years (n=1787)	Women, 18-45 years (n=466)	13- year- olds (n=687)	9-year- olds (n=636)	4-year- olds (n=399)	2-year- olds (n=1413)	1-year- olds (n=1957)
Lean fish	64	57	69	70	72	70	68
Fatty fish	25	29	23	25	26	30	32
Shellfish	11	14	8	5	2	0	0

¹Observed individual mean.

8.5 References

EFSA (2020) Risk to human health related to the presence of perfluoroalkyl substances in food. EFSA Journal 18:e06223. DOI: <https://doi.org/10.2903/j.efsa.2020.6223>.

VKM (2022) Knutsen H., Amlund H., Beyer J., Bukhvalova B., Engeset D., Lillegaard I.T., Mariussen E., Mathisen G.H., Brantsæter A.L., Bremer S., Samdal I.A., Thomsen C., Eriksen G.S. Risk assessment of dioxins, furans and dioxin-like PCBs in food in Norway. VKM Report 2022; 16.

9 Characterisation of benefits and risks

In this Chapter we present

- 1) the fish intake scenarios
- 2) a quantitative benefit and risk assessment of fish and selected health outcomes based on the weight of evidence conclusions for health outcomes related to fish consumption in Chapter 4 and fish consumption estimates from Chapter 8.
- 3) a semi-quantitative benefit assessment of nutrients in fish based on average requirements described in Chapter 2, nutrient intake estimates from Chapter 8, and the weight of evidence conclusions for health outcomes related to nutrient intakes in Chapter 5.
- 4) a semi-quantitative risk assessment of contaminants in fish based on tolerable weekly intakes described in Chapter 2 and contaminant exposure estimates from Chapter 8, and the adverse effects related to contaminant exposure described in Chapter 6.

The quantitative modelling of fish intake and health outcomes is the major part of this benefit and risk assessment. The nutrients and contaminants in fish could not be included in the quantitative modelling due to lack of available methodology and models as described in the following. There is a lack of consensus for the use of linear no-threshold dose-response model for methyl mercury published in connection to the Global Burden of Foodborne Disease project (also referred to as WHO's Foodborne Disease Burden Epidemiology Reference Group (FERG)), which is not in line with EFSA's TWI for methyl mercury. The dioxin model also from the Global Burden of Foodborne Disease project has not been updated with the new TWI for PCDD/Fs and DL-PCBs set by EFSA in 2018. There is no established existing model for PFASs. Quantitative models exist for intake of LC n-3 FAs and vitamin B₁₂, but not for vitamin D, iodine, or selenium intake. Development of quantitative models for the contaminants and nutrients could not be conducted within the time frame of this benefit and risk assessment but is suggested for further research in Chapter 13 Datagaps. As none of the contaminants could be included in the quantitative modelling, to keep the balance, we decided not to include any nutrients either. Consequently, the benefit and risk assessment of nutrients and contaminants in fish are semi-quantitative.

9.1 Current fish intake and fish intake scenarios used for benefit and risk characterisation

VKM was requested to evaluate health consequences in the Norwegian population from current fish consumption, and from changes in fish consumption in accordance with recommendations from the Norwegian Directorate of Health. The fish recommendations, and how VKM has interpreted and recalculated these for intake of total fish and fatty fish for all age groups, are described in Chapter 2.1, see Table 2.1-1.

In this chapter, we present a description of the fish intake scenarios that have been constructed for this benefit and risk assessment of fish consumption, including the reasons/justifications for the various choices and decisions made to construct these scenarios. The current fish intake estimated from the national dietary surveys, and the fish intake in the scenarios form the basis for the changes in benefits and risks resulting from changes in fish consumption presented in the Chapters 9.2 to 9.4.

Recommendations for fish consumption are presented in Chapter 2.1. Based on the lower and upper range of recommendation for weekly fish consumption for adults, 300-450 g prepared fish fillet per week, VKM constructed two fish scenarios. A 300 g scenario (scenario 2) corresponding to 300 g of prepared fish fillet (200 g fatty fish and 100 g lean fish) per week, and a 450 g scenario (scenario 3) corresponding to 450 g of prepared fish fillet (200 g fatty fish and 250 g lean fish) per week.

The scenarios are structured as follows: all fish consumption is subtracted from the overall food intake, and then the fixed quantities of fish are added to each day. All individuals belonging to the same age group get the same fish consumption added daily. The fish intake scenarios differ only in the amounts of lean and fatty fish, see Table 9.1-1.

It is recommended that at least 200 g of the total fish intake is fatty fish. We decided to keep this constant in both scenario 2 and 3. Accordingly, fatty fish represent 67% and lean fish 33% of the total fish intake in scenario 2, and 44 and 56%, respectively in scenario 3. In current fish intakes in various age groups, the proportion of fatty fish ranges from 39-57%. Scenario 3 could in principle contain a larger proportion of fatty fish. However, 44% fatty fish in scenario 3 is in line with the average proportions of fatty fish in current fish intake among adults which is 41%.

To have the possibility to consider health effects of a fish intake lower than the recommendations, we additionally decided to include a lower fish intake scenario with 150 g total fish per week (scenario 1). As this scenario is not according to the recommendations for fish intake, it was not part of the terms of reference from the Norwegian Food Safety Authorities. Scenario 1 was based on the mid-point in the fish recommendations, i.e., 375 g, of which 200 g fatty fish, i.e., 53% fatty fish. The scenarios are numbered based on increasing amount of fish.

To construct the fish scenarios in the younger age groups, we used the same method as when extrapolating the recommendations for younger age groups in Chapter 2.1; the weekly amount of fish intake was adjusted based on reference values for energy requirements (NNR, 2012). The energy adjusting factors range from 0.96 for 13-year-olds to 0.34 for 1-year-olds. The energy adjusting factors are derived from the mean estimated energy requirement in girls and boys with an average physical activity level at the given age (NNR 2012, Table 8.6). For 1-year-olds, the average daily energy requirements were given in kJ/kg body weight (NNR 2012, Table

8.5). The mean body weight in Spedkost 3 was 10 kg, and the mean energy requirement was estimated to be 3.4 MJ per day.

The weekly and current fish intake and fish intake in the three scenarios (total, fatty and lean fish) that have been used for the quantitative and semi-quantitative benefit and risk characterisations in the Chapters 9.2 to 9.4 are given in Table 9.1-1.

Table 9.1-1 Mean estimated current intake of fish and fish intake in the three scenarios used for the quantitative and semi-quantitative benefit and risk characterization. The intakes are presented as prepared fish fillet, OIMs, g/week (rounded estimates).

Age group	Current fish intake**			Fish intake in scenario 1**			Fish intake in scenario 2**			Fish intake in scenario 3**		
	Total	Fatty	Lean	Total	Fatty	Lean	Total	Fatty	Lean	Total	Fatty	Lean
Adults*, 18-70	294	126	168	150	80	70	300	200	100	450	200	250
Women, 18-70	238	110	128									
Women, 18-45	182	92	93									
Men, 18-70	350	134	206									
13-year-olds*	114	66	54	144	77	67	288	192	96	432	192	240
Girls	97	54	42									
Boys	136	73	61									
9-year-olds*	114	54	54	111	59	52	222	148	74	333	148	185
Girls	101	53	47									
Boys	122	57	63									
4-year-olds*	132	54	66	86	46	40	171	114	57	257	114	143
Girls	133	62	65									
Boys	129	52	69									
2-year-olds*	108	42	66	45	34	30	129	86	43	194	86	108
1-year-olds*	90	36	48	51	27	24	102	68	34	153	68	85

*Energy adjusting factor based on reference values for energy requirements (Nordic Nutrition Recommendations, 2012) for adults=1, 13-year-olds=0.96, 9-year-olds=0.74, 4-year-olds=0.57, 2-year-olds=0.43, 1-year-olds=0.34.

**Difference between total fish intakes and the sum of fatty and lean fish intakes is fish offal. Fish offal is not included in the scenario calculations. Weekly fish intakes are rounded figures calculated from g/day.

It should be noted that the fish scenarios are simple constructed scenarios, without any substitution or energy adjustment, i.e., reduced fish intake is not replaced by other foods or adjusted for reduced energy intake. Increased fish intake is neither adjusted by subtracting any other foods nor adjusted for increased energy intake. It should be anticipated that the scenario 1, with 150 g fish, is in fact a reduction of energy intake for several participants, and therefore might represent an underestimation of nutrients and contaminants, whereas the scenarios with increased fish, and in particular the scenario 3, might represent an overestimation compared to current fish intake.

For the purpose of estimating exposure to nutrients and contaminants in the scenarios, the scenarios are constructed out of the most common consumed fish species, see explanation in Chapter 9.1.1.

The scenarios are conducted with a simplistic procedure, which reduces variability to a less realistic level. However, as this does not have an impact on the quantitative modelling in Chapter 9.2, which is the major part of this benefit and risk assessment, the procedure for the scenarios was considered “fit for purpose”.

9.1.1 Estimating nutrient intake and contaminant exposure in the scenarios

To be able to also estimate nutrient intake and contaminant exposures from the fish scenarios, we used the combination of fish species that are specific for each age group as reported in the dietary surveys, with accompanying concentrations of nutrients and contaminants. These age-specific fish combinations were based on the relative quantities consumed of various fish species reported by the survey respondents in the various age groups, see Figure 8.2-4.

In the scenarios, there was no need to include fish in the modelling of habitual intake or exposure (Chapter 7.5) because all individuals were assigned to eat the same quantity of fish without variability from day-to-day or between individuals. The distributions of nutrients and contaminant were estimated by first modelling the contribution from all food sources other than fish (identical to what is presented in Chapter 7.5). Then the contribution from fish (nutrient or contaminant) was added to these distributions as fixed amounts, giving three sets of distributions (one for each scenario) that were used in the present assessment.

9.2 Quantitative benefit and risk characterization of fish intake

The overall aim of the quantitative assessment was to estimate the effect on disease incidence and mortality in the Norwegian population as a result of eating fish in other quantities than the currently consumed amount.

In the weight of evidence analyses in Chapter 4, there were no adverse associations between fish consumption and any of the included health outcomes. Consequently, the quantitative modelling only contains beneficial results from fish consumption. As the fish as such contains both nutrients and contaminants, the quantitative modelling is regarded as a benefit and risk characterisation of fish.

9.2.1 Current fish intake and intake scenarios

Based on the national dietary surveys, the current mean total fish intake in the Norwegian adult population (18-70 years) was estimated to 50 g/day (350 g/week) and 34 g/day (238 g/week) for men and women, respectively (see Table 8.2-1). For the health outcome “preterm-birth” the current mean fish fillet intake of women of childbearing age (18-45 years) was used, which was estimated to 26 g/day (182 g/week) (see Table 8.2-1). For the health outcome “CHD incidence”, the mean intake in men and women combined (18-70 years) was used, which was estimated to 42 g/day (294 g/week). This is because the data used for CHD incidence was only available for genders combined. The estimates for total fish intakes were used. This was done,

as epidemiological evidence of an association between intake of sub-types of fish (fatty or lean) and different health outcomes were more limited than for total fish.

The fish scenarios are given in Table 9.1-1. Scenario 1 is 150 g fish per week (equal to 21 g/day), scenario 2 is 300 g fish per week (equal to 43 g/day), and scenario 3 is 450 g fish per week (equal to 64 g/day).

9.2.2 Health effects included

According to the protocol, quantitative assessments were performed for health outcomes for which the evidence for an association with fish intake is graded at least “probable”. Based on the systematic literature review and the weight of evidence conclusions for associations with total fish intake, the following ten health outcomes were included in the quantitative assessment (reference to the relevant chapter that describe the evidence behind each association is given in brackets):

Incidence:

CHD (weight of evidence, see Chapter 4.3)

Stroke (weight of evidence, see Chapter 4.5)

Dementia (weight of evidence, see Chapter 4.14)

Alzheimer`s disease (weight of evidence, see Chapter 4.13)

Preterm birth (weight of evidence, see Chapter 4.23)

Mortality:

CVD (weight of evidence, see Chapter 4.7)

CHD (weight of evidence, see Chapter 4.7)

Myocardial infarction (MI) (weight of evidence, see Chapter 4.7)

Stroke (weight of evidence, see Chapter 4.7)

All-cause (weight of evidence, see Chapter 4.8)

Of note, the evidence is graded “probable” also for low birth weight (LBW), but there was no association when controlling for gestational age and preterm birth (PTB). Because LBW (weight of evidence, see Chapter 4.25) was explained by PTB in studies of fish intake during pregnancy, only PTB was included in the quantitative assessment.

The evidence is graded “probable” for stroke mortality and MI mortality, but no dose-response meta-analysis was found that included studies of stroke- or MI mortality only. Thus, MI mortality and stroke mortality were only included in the quantitative assessments as nested within other outcomes. MI mortality was nested within CHD mortality and stroke mortality within CVD mortality. A high proportion of CHD deaths will be MI deaths. The difference between CVD mortality and CHD mortality will give an indication of the impact on stroke mortality.

9.2.3 Modelling approach

Data to derive dose-response functions describing the relation between fish intake and the relative risk (RR) of each of the included health outcomes were identified in the literature (Table 9.2.4-1). The dose-response functions were derived by assuming a RR of 1 at zero consumption and a log-linear association (Barendregt & Veerman, 2010):

$$\ln(RR) = \beta x \quad (1),$$

where x is the intake amount in g/day and β can be estimated from a RR for a given x derived from the scientific literature. The dose-response parameter, β , is used to estimate the RR at any intake level x from the following exponential function:

$$RR(x) = e^{\beta x} \quad (2)$$

In the following, current incidence refers to the currently observed annual number of cases or deaths of the included health outcomes in the Norwegian population; the current intake refers to the intakes reported in the national dietary surveys in Norway. Alternative incidence and intake refer to the calculated incidence when intake is equal to either of the three alternative fish intake scenarios.

The absolute risk of each health outcome at alternative fish intake levels, is derived by assuming that the current incidence is a reflection of the current fish intake in the population (Hoekstra et al., 2013). The absolute risk of each health outcome, $r_{outcome}$, is then given by:

$$r_{outcome} = I_{current} / RR(x_{current}),$$

where $I_{current}$ is the current annual number of new cases of the given health outcome in the adult population and $RR(x_{current})$ is the relative risk when the intake, x , is equal to the current intake. No age-specific RR or incidences were used.

The incidence at any alternative intake level in the fish intake scenarios, $I_{alternative}$ is estimated by multiplying the current incidence, $I_{current}$ by the ratio of the RR when the intake is equal to any alternative intake level, $RR(x_{alternative})$ and $RR(x_{current})$. The absolute difference in incidence between the current intake level and any alternative intake level in the fish intake scenarios, is then given by:

$$I_{alternative} - I_{current} = (r_{outcome} * RR(x_{alternative})) - I_{current} = I_{current} * (RR(x_{alternative})/RR(x_{current})-1)$$

Aside from the absolute difference in annual new cases or deaths, also the fractional change attributed to the specific change in fish consumption, referred to as the potential impact fraction (PIF), were calculated from the above.

In the following, the data applied to calculate the annual number of incident cases and deaths as well as potential impact fractions in each scenario of fish intake are described.

9.2.4 Data - relative risks

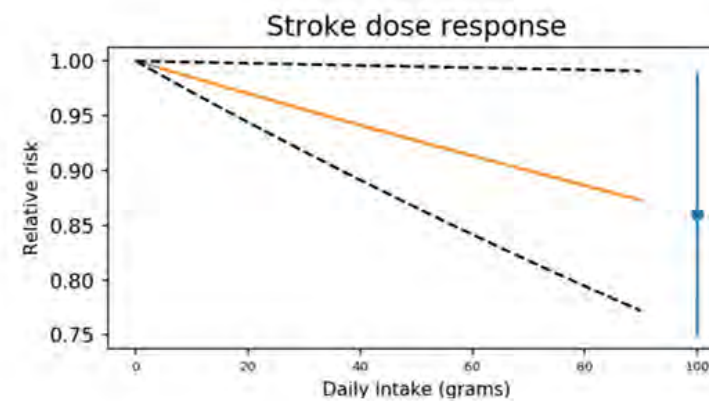
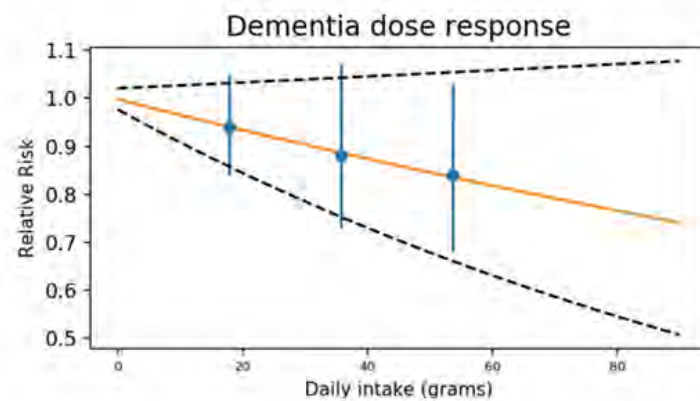
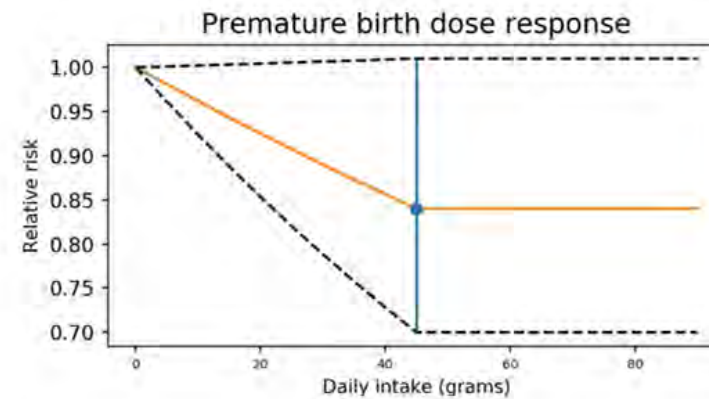
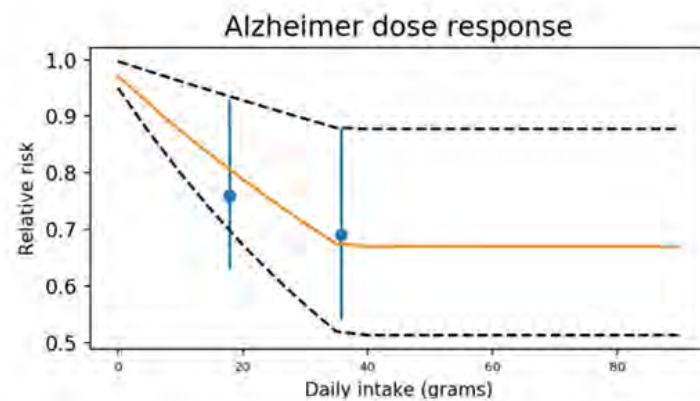
The dose-response parameter (β) was derived using RR dose-response relationships found in meta-analyses identified in VKM's systematic search for systematic reviews and meta-analyses on fish intake and health outcomes. Applied RR dose-response relationships were selected from either the most recent meta-analysis or other meta-analyses with eligibility criteria similar to VKM's criteria. The selection of dose-response studies is described in more detail below.

In Table 9.2.4-1, the RRs for each health outcome identified in the literature are reported along with the dose (g/day), the calculated dose-response parameter, β and associated assumptions. The rationale behind the selection of RRs for each association is given below. In Figure 9.2.4-1, the dose-response relationships for each health outcome as applied in the assessment are illustrated.

Table 9.2.4-1 Relative risks (95% CI) per dose (g/day) and dose-response parameter derived from relative risk and dose per health outcome.

Health outcome	Relative Risk (95% CI)	Dose (g/d)	Dose-response parameter, β	Assumptions	Reference
Dementia	0.94 (0.84,1.05) 0.88 (0.73,1.07) 0.84 (0.68,1.03)	17.9 35.7 53.6	-0.003 (-0.007, 0.001)	No test for non-linearity 125 g/serving Observed intake range 0-600 g/week	Kosti, 2021
Alzheimer`s disease	0.76 (0.63,0.93) 0.69 (0.54, 0.88)	17.9 35.7	-0.01 (-0.02, -0.004)	No test for non-linearity 125 g/serving No further risk reduction for intakes >2 servings/wk Observed intake range 0-350 g/wk	Kosti, 2021
Preterm birth	0.84 (0.70, 1.01)	45	-0.004 (-0.008, 0)	Non-linear 105 g/serving No further risk reduction for intakes >45 g/d Observed intake range 0-80 g/d	Zhao, 2020
Stroke incidence	0.86 (0.75, 0.99)	100	-0.002 (-0.003, 0)	Linear Observed intake range 0-130 g/d	Bechthold, 2019
CHD incidence	0.96 (0.95, 0.97)	20	-0.002 (-0.003, -0.002)	Non-linear, no risk reduction for intake below 40 g/d Observed intake range 0-175 g/d	Zhang, 2020
All-cause mortality	0.93 (0.90, 0.96) 0.90 (0.84, 0.96)	100 200	-0.001 (-0.001,0)	Non-linear 100 g/serving Observed intake range: 0-225 g/d	Schwingshackl, 2017

Health outcome	Relative Risk (95 % CI)	Dose (g / d)	Dose-response parameter, β	Assumptions	Reference
CVD mortality	0.96 (0.94, 0.99)	20	-0.002 (-0.003, -0.001)	Non-linear No further risk reduction for intakes >40 g/d Observed intake range 0-100 g/d	Jiang, 2021
CHD mortality	0.96 (0.95, 0.98)	20	-0.002 (-0.003, -0.001)	Non-linear No further risk reduction for intakes >60 g/d Observed intake range 0-200 g/d	Zhang, 2020



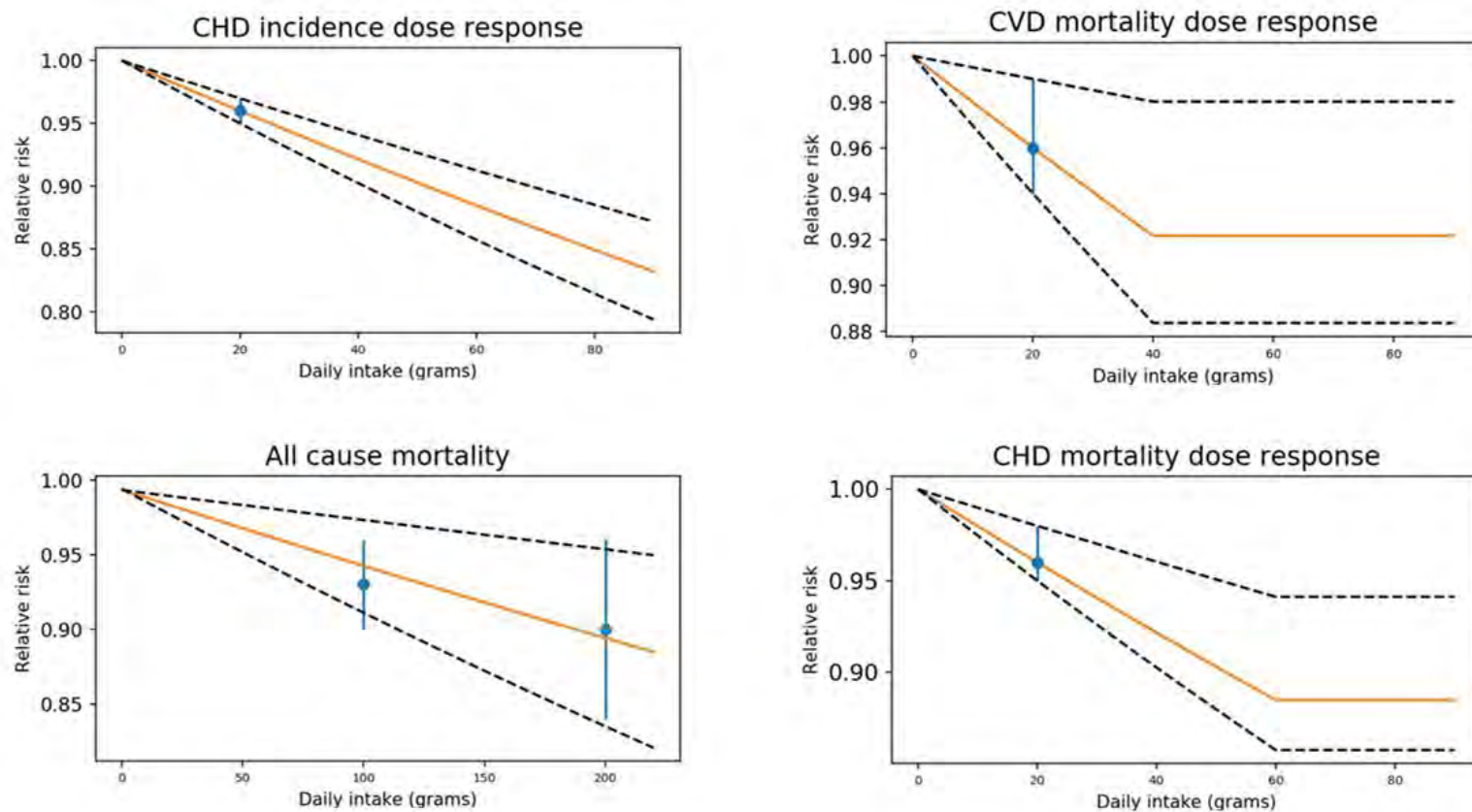


Figure 9.2.4-1 Dose-response relationships between fish intake (g/day) and relative risk for each health outcome as applied in the impact modelling. The blue points with error bars represent the relative risks and their 95% confidence intervals (CI) obtained from the scientific literature, and to which a log-linear function was fitted to obtain the dose-response relationship. The yellow graph represents the dose-response relationship derived when using the mean relative risk. The dashed graphs represent the dose-response relationships derived using the lower and upper limits of 95% CI of the relative risks.

9.2.4.1 Alzheimer's disease and dementia

Kosti et al. (2021) was the most recent meta-analysis and examined the dose-response relationship between fish intake (g/week) and the risk of dementia and Alzheimer's disease. Kosti et al. (2021) was also the meta-analysis with the best overlap with the primary studies included by VKM. The high/low summary RRs calculated by VKM and Kosti et al. (2021) both suggest a significant high/low relationship between higher intake of fish and lower risk of Alzheimer's disease and dementia (see Chapter 4.13).

Even though a statistically significant high/low relationship between fish intake and dementia was identified in the above-mentioned analyses, no significant dose-response relationship was identified. Despite this, it was still chosen to apply the RRs per g fish per week for dementia as presented in Kosti et al. (2021). Although higher fish intake was associated with lower dementia risk, the slope of risk reduction was gradually reduced with intakes higher than approximately 250 g/week. Compared to no fish intake, an intake of 125 grams fish/week was associated with a marginally non-significant 6% lower risk of dementia (RR of 0.94 (95% CI 0.84, 1.05), a further increase to 250 g/week was associated with a non-significant 12% reduction in the RR (RR=0.88 (95% CI 0.73, 1.07)) and an increase to 375 g/week with a non-significant 16% lower risk of dementia (RR=0.84 (95%CI 0.68, 1.03)). No test for non-linearity was performed, but a qualitative description identified that the slope of the risk reduction was gradually reduced with intakes higher than 250 g/week. For Alzheimer's disease, significant RRs were found when comparing any fish intake per week with no intake. Still no test for non-linearity was performed, but a gradual levelling off of the risk reduction as fish intake increased was more evident in the case of Alzheimer's disease than for dementia. Compared with no fish consumption, the risk of Alzheimer's disease decreased by 24% at intakes of 125 g/week (RR = 0.76, (95% CI 0.63, 0.93)) and by 31% at intakes of 250 g/week (RR=0.69 (95% CI: 0.54, 0.88)). Any increase in fish intake beyond this did not seem to be related to additional benefit. In sensitivity analyses, the associations between fish intake and dementia and Alzheimer's disease were attenuated excluding studies with short follow-up.

Rrs from Kosti et al. (2021) (Table 9.2.4-1), were used to describe the dose-response relationship for dementia and Alzheimer's disease (Figure 9.2.4-1), by fitting a loglinear model to the reported RR, and then derive the RR at any other intake level (equation 2).

9.2.4.2 Preterm birth

The dose-response relationship used for total fish intake and preterm birth is from Zhou et al. (2020). Zhou et al. (2020) was the only identified systematic review with a quantitative meta-analysis. The authors performed both a linear (per 45 g/day increase, equal to 315 g/week) and non-linear meta dose-response analysis (see Chapter 4.23). Potential non-linearity of the association was evaluated by calculating restricted cubic splines for each study with ≥ 3 categories of exposure, at 3 fixed knots (10th, 50th, and 90th percentiles) over the intake range

(0- 80 g/day). The deviation from linearity was statistically significant ($P=0.01$) with little or no further risk reduction for intakes above 45 g/day.

The RR of 0.84 per 45 g increase in fish per day reported by Zhou et al. (2020) (Table 9.2.4-1) was used to describe the dose-response relationship applying the assumptions relating to equation 1 and 2. No change in risk for intakes above 45 g/day was assumed resulting in the dose-response function for preterm birth illustrated in Figure 9.2.4-1.

9.2.4.3 Stroke incidence

The dose-response relationship of total fish intake with stroke incidence is based on a meta-analysis by Bechthold et al. (2019). Among 6 identified systematic reviews of fish intake and stroke (Chen et al., 2021; Jayedi et al., 2020; Bechthold et al., 2019; Zhao et al., 2019; Qin et al., 2018; Xun et al., 2012) the eligibility criteria in Bechthold et al. (2019) were most similar to VKMs criteria (studies of stroke incidence, but not mortality) (see Chapter 4.5). Chen et al. (2021) was the most recent, but not the most comprehensive. Jayedi et al. (2020) was limited to patients with type 2 diabetes, and Qin et al. (2018) did not examine total fish (fatty and lean fish only). Zhao et al. (2019) included the largest number of studies, but also studies of stroke mortality. Despite this difference, the relative risks reported in Bechthold et al. (2019) (14% lower risk per 100 g increase per day) was similar to that reported in Zhao (12% lower risk per 700 g increase per week). Bechthold et al. (2019) evaluated potential non-linearity of the association by calculating restricted cubic splines for each study with ≥ 3 categories of fish exposure, using 3 fixed knots at 10%, 50%, and 90% through the total distribution of the reported fish intake (0-130 g/day), and combined them using multivariate meta-analysis. The deviation from linearity was not statistically significant ($P=0.37$).

The RR of 0.86 per 100 g increase in fish per day reported by Bechthold et al. (2019) (Table 9.2.4-1) was used to describe the dose-response relationship applying the assumptions relating to equation 1 and 2. The resulting dose-response function as applied in the assessment is illustrated in Figure 9.2.4-1.

9.2.4.4 CHD incidence

The dose-response relationship of total fish intake with CHD incidence is based on the meta-analysis by Zhang et al. (2020). Among three identified systematic reviews of fish intake and CHD risk (Zhang et al., 2020; Bechthold et al., 2019; Jayedi et al., 2020), Jayedi et al. (2020) was limited to studies in patients with type 2 diabetes, and Zhang et al. (2020) was the most recent review of studies in the general population. The inclusion of primary studies in both Zhang et al. (2020) and Bechthold et al. (2019) differed somewhat from VKM's study inclusion, but the high-low estimate in Zhang et al. (2020) was similar to that found by VKM (see Chapter 4.3). The dose-response relationship in Zhang et al. (2020) was derived by plotting the adjusted RRs for each exposure quantile of fish intake from the primary studies and fitting a restricted

cubic spline model with three knots (25%, 50%, and 75% percentiles) over the intake range (up to 175 g/day, but with limited data for intake above 100 g/day). Each additional daily 20 g of fish was associated with a 4% reduction in the risk of CHD (RR=0.96, 95% CI: 0.95, 0.97). Based on the authors' report of the dose-response curve, the risk of CHD only decreased when fish consumption was above 40 g/day.

The RR of 0.94 per 20 g increase in fish per day reported by Zhang et al. (2020) (Table 9.2.4-1) was used to describe the dose-response relationship applying the assumptions relating to equation 1 and 2. Even though Zhang et al. (2020) describe that no change in risk occurs for intakes below 40 g/day, the log linear association was assumed from zero intake. The resulting dose-response function for CHD incidence is illustrated in Figure 9.2.4-1.

9.2.4.5 *All-cause mortality*

The dose-response relationship of total fish intake with all-cause mortality is based on Schwingshackl et al. (2017) (see Chapter 4.8). Among 5 identified systematic reviews on fish and all-cause mortality (Jayedi et al., 2020; Jayedi et al., 2018; Schwingshackl et al., 2017; Wan et al., 2017; Zhao et al., 2016), the most recent (Jayedi et al., 2020) was limited to patients with type 2 diabetes. Among studies of the general population, Schwingshackl et al. (2017) identified the largest number of primary studies with sufficient dose-response data (19 prospective studies, compared with 10 studies in Jayedi et al. (2018)). Schwingshackl et al. (2017) evaluated potential non-linearity of the association by calculating restricted cubic splines for each study with ≥ 3 categories of fish exposure, using 3 fixed knots at 10%, 50%, and 90% through the total distribution of the reported fish intake (0-250 g/day), and combined them using multivariate meta-analysis. There was a deviation from linearity ($P=0.09$), and relative risks were reported separately for 1 and 2 servings of fish per day, assuming 1 serving equal to 100 g.

The RRs at 1 and 2 servings per day from Schwingshackl et al. (2017) (Table 9.2.4-1), were used to describe the dose-response relationship for all-cause mortality (Figure 9.2.4-1), applying the assumptions relating to equation 1 and 2, in the observed intake range of 0-250 g/day. The resulting dose-response function as applied in the assessment is illustrated in Figure 9.2.4-1.

9.2.4.6 *CVD mortality*

The meta-analysis dose-response relationship of total fish intake with CVD mortality is based on Jiang et al. (2021) (see Chapter 4.7). Two systematic reviews with dose-response meta-analyses were identified; Jiang et al. (2021) and Jayedi et al. (2018). Jiang et al. (2021) was the most recent and comprehensive. Potential non-linearity of the association was evaluated by restricted cubic splines using four knots at 5%, 35%, 65% and 95% over the intake range (0-

100 g/day). The deviation from linearity was statistically significant ($P < 0.001$) with no further risk reduction after 40 g/day.

A RR of 0.96 per 20 g increase in fish per day as reported by Jiang et al. (2021) (Table 9.2.4-1) was used to describe the dose-response relationship applying the assumptions relating to equation 1 and 2, up to an intake of 40 g/day, after which no change in risk occur. The resulting dose-response function as applied in the assessment is illustrated in Figure 9.2.4-1.

9.2.4.7 CHD mortality

The dose-response relationship of total fish intake with CHD mortality is based on Zhang et al. (2020) (see Chapter 4.7). Two systematic reviews with dose-response meta-analyses were identified (Zhang et al., 2020; Zheng et al., 2012). Zhang et al. (2020) was the most recent and most comprehensive. The inclusion of primary studies differed somewhat from VKMs selection. However, the results of studies excluded by VKM did not stand out from other primary studies in the high-low forest plot in Zhang et al. (2020). Potential non-linearity of the association was evaluated by restricted cubic splines using three knots at 25%, 50%, and 75% over the intake range (0-200 g/day, but with limited data for intake above 125 g/day). Based on the authors' report of the dose-response curve, the risk reduction levelled out at 60 g/day, with no further decrease in risk.

A RR of 0.96 per 20 g increase in fish per day as reported by Zhang et al. (2020) (Table 9.2.4-1) was used to describe the dose-response relationship applying the assumptions relating to equation 1 and 2, up to an intake of 60 g/day, after which no change in risk occur. The resulting dose-response function as applied in the assessment is illustrated in Figure 9.2.4-1.

9.2.5 Data - current number of incident cases and deaths

To calculate incident cases and/or deaths in each of the fish intake scenarios assessed, measures of occurrence of disease in the Norwegian population were taken from publicly available data sources in the following order of priority: 1) national health registry, 2) official public health report for Norway and 3) research papers based on Norwegian data. The most recent data was applied, if data from several years was available.

In Table 9.2.5-1, estimates of the current incidence applied in the models are reported along with the respective data sources and main assumptions. Below, the measures of occurrence used for each health effect is described in detail.

Table 9.2.5-1 Annual number of incident cases or deaths in Norway of included health outcomes.

Health outcome	Age groups	Annual new cases, men	Annual new cases, women	Assumptions	Reference
Dementia	70-90+ years	4652	5941	Incidence is derived from the prevalence and assumption of duration of dementia. It is assumed that mortality is not associated with dementia and that duration equals life expectancy at onset of disease	GjØra et al., 2020
Alzheimer`s disease	70-90+ years	2605	3445	Assumption that 56% and 58% of dementia is Alzheimer`s disease for men and women, respectively	GjØra et al., 2020
Preterm birth	Fertile age		173 (22-27 weeks) + 2751 (28-36 weeks)	Total births 52,897 in 2020, 0.3% early preterm and 5.2% preterm births	Norwegian Institute of Public Health, 2020
Stroke incidence	0-74 years	2754	1465	Stroke incidence derived by stroke cases admitted to hospital per 1,000. Absolute number of incident stroke cases derived by multiplying with Norwegian population age 0-74	Norwegian Institute of Public Health, 2018
CHD incidence	0-74 years	36 790 (men and women combined)	36 790 (men and women combined)	Annual CHD admissions (hospital or specialist care)	Public Health Report Norway (web publication), 2020
All-cause mortality	20-69 years	3884	2745	Estimated as the mean of the total mortality of all diseases (excluding "external causes" and "lacking death certificate") from year 2016-2020	Norwegian Institute of Public Health, 2020
CVD mortality	20-69 years	942	352	Estimated as the mean of the total mortality of "diseases in the circulation system (cardio-vascular diseases) from year 2016-2020	Norwegian Institute of Public Health, 2020
CHD mortality	20-69 years	498	127	Current incidence is calculated as the mean of the total mortality of CHD=ischemic heart disease from year 2016-2020	Norwegian Institute of Public Health, 2020

9.2.5.1 Dementia and Alzheimer`s disease

The number of incident cases of dementia and Alzheimer`s disease in the population was derived from estimates of prevalence from a publicly available report from 2020 (GjØra et al., 2020) of the prevalence of dementia in Norway.

The reported prevalence of dementia in the age group 70-90+ was converted to an annual incidence per 100 000, assuming that a person diagnosed with dementia will live until the expected age of death at the age of diagnosis. This is a crude assumption, as evidence shows that life expectancy is considerably reduced in patients with dementia (Strand et al., 2018), but included for simplicity. Expected age of death was obtained from Statistics Norway (Forventet gjenstående levetid, Statistics Norway, 2019) and was derived as the mean expected age of death for the age group 70-99 (approximately 8 years for men and 9.3 years for women). Absolute number of incident cases of dementia was derived by multiplying with the population of 70–99-year-olds (Statistics Norway, 2019) and dividing by 100,000. According to the report (GjØra et al., 2020), 56% and 58% of dementia is Alzheimer`s disease for men and women, respectively, in the age group 70-90+. Therefore, to derive the number of annual incident cases of Alzheimer`s disease, the annual incident cases of dementia was multiplied with 0.56 and 0.58 for men and women, respectively.

9.2.5.2 Preterm births

The annual number of preterm births (defined as birth lengths of 22-36 weeks gestation based on ultrasound in most women, else last menstrual period) is available from the Medical Birth Registry of Norway. The registry contains information about all births in Norway.

9.2.5.3 Stroke admissions

The annual number of incident cases of acute stroke (in terms of hospital admissions) was used as a proxy for stroke incidence and obtained from the Norwegian Cardiovascular Disease Registry. Diseases are coded according to the International Statistical Classification of Diseases and Related Health Problems, version 10 (ICD-10). The ICD-10 codes included for stroke were I60-I69 (cerebrovascular diseases). Cases of acute stroke were registered for the age groups 0-44 and 45-74 years. Number of cases was given per 1 000 and was multiplied by the population aged 0-44 and 45-74 (Statistics Norway, 2019) to obtain the absolute number of stroke cases.

9.2.5.4 CHD admissions

The number of patients hospitalized or treated in specialist care for ischemic heart disease in 2020 (all ages) was used as a proxy for an annual number of incident cases of CHD and was available from the Norwegian Public Health Report, chapter on cardiovascular diseases (Ariansen et al., 2014 (updated 2021), Table 9.2.5-1). The number includes both new

patients and patients with a history of CHD, as well as CHD deaths in hospital, but not outside hospital. Diseases are coded according to the International Statistical Classification of Diseases and Related Health Problems, version 10 (ICD-10). Ischemic heart disease was defined as acute myocardial infarction (AMI) and diagnosed angina (ICD-10 codes I20-I25). This definition of ischemic heart disease was not directly available from the open-source version of the Norwegian Cardiovascular Disease Registry.

9.2.5.5 *All-cause, CVD and CHD mortality*

The annual number for deaths from all disease causes (excluding violent deaths and missing causes) and deaths from cardiovascular diseases (CVD) overall and by sub-categories (coronary heart disease, myocardial infarctions, and stroke), was taken from the national Cause of Death Registry for Norway, administered by the Norwegian Institute of Public Health. Diseases are coded according to the International Statistical Classification of Diseases and Related Health Problems, version 10 (ICD-10). The ICD-10 codes included for mortality from all diseases were A00-R99, CVD mortality I00-I99 (circulatory diseases), and CHD mortality I20-I25.

9.2.6 Results and discussion

All health outcomes were estimated for men and women separately, except preterm birth which only is relevant for women, and CHD incidence as numbers of current annual incident cases was obtained for the total population only, and not separately for each gender. In Tables 9.2.6-1 and 9.2.6-2, the mean annual number of incident cases and deaths at current fish intake and each alternative intake scenario of 150, 300 and 450 g/week for each health effect, except CHD incidence, are presented for men and women, respectively. In Table 9.2.6-3, the potential impact fractions are presented. They indicate the percent increase or decrease in annual number of cases or deaths from the current, due to changes in fish intakes, and rely on dose-response parameters obtained from the epidemiological studies (Table 9.2.4-1) as well as the current fish intake and an alternative intake (e.g., 150, 300 and 450 g/week). In Figures 9.2.6-1 and 9.2.6-2, the potential impact fractions are plotted against alternative intake amounts in gram per week, for men and women, respectively. Thus, Figures 9.2.6-1 and 9.2.6-2 shows the percent change in disease occurrence and mortality at any alternative intake, not only the investigated scenarios. An impact fraction above 0 indicates that a change to the alternative intake results in an increase in the number of cases or deaths; an impact fraction below 0 indicates that a change to the alternative intake results in a decrease in the number of cases or deaths. The line for each health outcome intersects the x-axis at the current intake relevant for each health outcome (i.e., 182 g/week for preterm birth, 350 g/week for all health outcomes for men). In Table 9.2.6-4, the results for CHD incidence are presented, both the annual number of cases and potential impact fractions. The estimates presented in brackets in Tables 9.2.6-1 to 9.2.6-4, represent an indication of the uncertainty in the RRs from the original studies (Table 9.2.4-1). The lower and upper limits in the brackets are estimated by applying the lower and upper limits of the 95% confidence interval around RRs (Table 9.2.4-1), respectively.

All identified health outcomes have an inverse association with fish intake, i.e., increase in fish intake decrease the risk of the health outcomes and vice versa. Generally, this trend is reflected in the results, and for each health outcome it is seen that disease cases and deaths are prevented with increasing fish intake. However, for some health outcomes, the increase in number of prevented cases only occurs up to a certain intake (e.g., Alzheimer's disease, preterm birth, CVD mortality and CHD mortality). Likewise, for some diseases, the 95% confidence intervals around the RR includes 1, and thus the results using the lower and upper limits around the RR suggest that it cannot be concluded if and by how much an increase in fish intake will prevent or cause the disease. It should be noted, that for all health outcomes, the assessed intake scenarios (150, 300 and 450 g/week) were within the observed regions in the original studies from which the RRs were derived, and thus no extrapolation outside observed intake regions was performed.

Below, the results for each included health effect is described and discussed.

Table 9.2.6-1 Mean annual number of incident cases and deaths in men at current fish intake and estimated for each alternative scenario of fish intake. Numbers in brackets indicate the estimated number of cases or deaths derived when using the lower and upper limits of the 95% confidence intervals around the relative risks.

Health outcome	Current 350 g /week	Scenario 1 150 g /week	Scenario 2 300 g /week	Scenario 3 450 g /week
Dementia, incidence	4652	5111 (4572;5725)	4763 (4632;4899)	4438 (4193;4692)
Alzheimer's disease, incidence	2605	3021 (2741;3332)	2605 (2605;2605)	2605 (2605;2605)
Stroke, incidence	2754	2875 (2762;2989)	2784 (2755;2811)	2695 (2643;2750)
All-cause mortality	3884	3943 (3906;3982)	3898 (3890;3908)	3855 (3836;3872)
CVD mortality	942	978 (951;997)	942 (942;942)	942 (942;942)
CHD mortality	498	527 (512;535)	505 (501;507)	488 (485; 493)

Table 9.2.6-2 Mean annual number of incident cases in women at current fish intake and estimated number for each alternative scenario of fish intake. Numbers in brackets indicates the estimated number of cases or deaths derived when using the lower and upper limits of the 95% confidence intervals around the relative risk.

Health outcome	Current 238 g /week	Scenario 1 150 g /week	Scenario 2 300 g /week	Scenario 3 450 g /week
Dementia, incidence	5941	6193 (5896;6509)	5770 (5570;5972)	5376 (4767;6050)
Alzheimer's disease, incidence	3445	3926 (3603;4279)	3384 (3344;3423)	3384 (3344;3423)
Preterm birth	2924	2976 (2921;3031)	2739 (2558;2934)	2716 (2515;2936)
Stroke, incidence	1465	1493 (1466;1518)	1445 (1428;1463)	1400 (1342;1460)

Health outcome	Current 238 g/week	Scenario 1 150 g/week	Scenario 2 300 g/week	Scenario 3 450 g/week
All-cause mortality	2745	2763 (2752;2775)	2732 (2723;2740)	2701 (2673;2728)
CVD mortality	352	361 (354;365)	347 (345;351)	347 (345;351)
CHD mortality	127	130 (128;131)	125 (124;126)	120 (118;124)

Table 9.2.6-3 Potential impact fractions represented by percent change in annual number of incident cases or deaths estimated for change in fish intakes from the current intake of 350 g/week for men and 238 g/week women, respectively, to 150, 300 or 450 g/week for each health effect and per gender. The numbers in brackets indicate the estimated impact fractions when using the lower and upper limits of the 95% confidence intervals around the relative risks. A negative sign indicates an expected percentwise decrease in number of cases or deaths due to the change in fish intake.

Health outcome	Men			Women		
	Scenario 1 150 g/week	Scenario 2 300 g/week	Scenario 3 450 g/week	Scenario 1 150 g/week	Scenario 2 300 g/week	Scenario 3 450 g/week
Dementia	9.88% (-1.7;23)	2.38% (-0.4;5.3)	-4.6% (-9.8;0.86)	4.23% (-0.8;9.5)	-2.88% (-6.2;0.5)	-9.51% (-19.7;1.8)
Alzheimer's disease, incidence	16% (5.2;28)	0% (0;0)	0% (0;0)	13.95% (4.6;24.2)	-1.76% (-2.9;-0.6)	-1.76% (-2.9;-0.6)
Preterm birth				1.77% (-0.1;3.69)	-6.32% (-12.5;0.4)	-7.1% (-13.9;0.4)
Stroke, incidence	4.4% (0.3;8.5)	1.08% (0;2)	-2.13% (-4;-0.1)	1.91% (0.1;3.7)	-1.32% (-2.5;0)	-4.46% (-8.3;-0.3)
All-cause mortality	1.52% (0.6;2.5)	0.37% (0.1;0.6)	-0.75% (-1.2;-0.3)	0.66% (0.3;1.1)	-0.46% (-0.8;-0.2)	-1.58% (-2.6;-0.6)
CVD mortality	3.86% (0.9;5.9)	0% (0;0)	0% (0;0)	2.6% (0.6;3.9)	-1.22% (-1.8;-0.3)	-1.22% (-1.8;-0.3)
CHD mortality	6% (2.9;7.6)	1.47% (0.7;1.8)	-2.02% (-2.5;-1.3)	2.6% (1.3;3.2)	-1.79% (-2.2;-0.9)	-5.17% (-6.4;-2.6)

Table 9.2.6-4 Mean annual number of CHD cases and potential impact fractions for CHD incidence represented by percent change in annual number of incident cases or deaths estimated for change in fish intakes from the current intake of total population, respectively, to 150, 300 or 450 g/week. The numbers in brackets indicate the estimated impact fractions when using the lower and upper limits of

the 95% confidence intervals around the relative risks. The negative sign indicates an expected percentwise decrease in number of cases or deaths due to the change in fish intake.

CHD Incidence	Scenario 1 150 g/week	Scenario 2 300 g/week	Scenario 3 450 g/week
Annual incidence (current=36790)	38368 (37960;38783)	36726 (36709;36742)	35154 (34746;35562)
Impact fraction	4.29% (3.2;5.4)	-0.17% (-0.2;-0.1)	-4.45% (-5.6;-3.3)

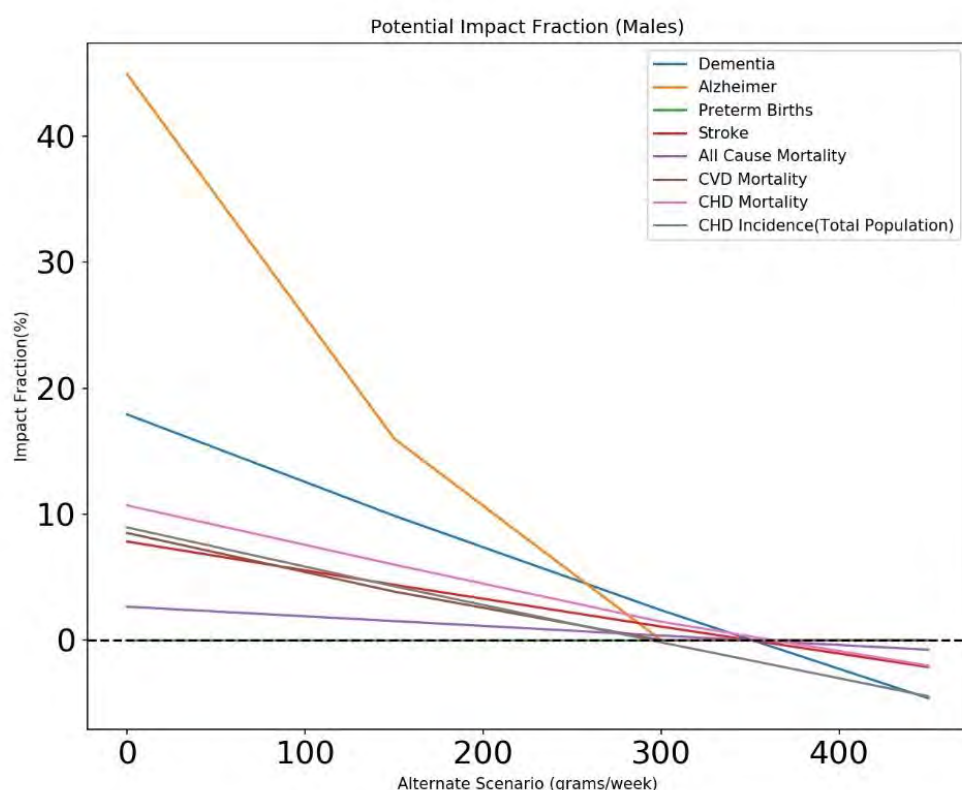


Figure 9.2.6-1 Potential impact fractions for each health outcome for Norwegian adult men per alternative intake amount (in g/week). A positive potential impact fraction indicates the expected percentwise increase in number of cases or deaths when the male population changes intake in fish consumption to a given alternative intake; a negative impact fraction indicates an expected percentwise decrease in number of cases or deaths.

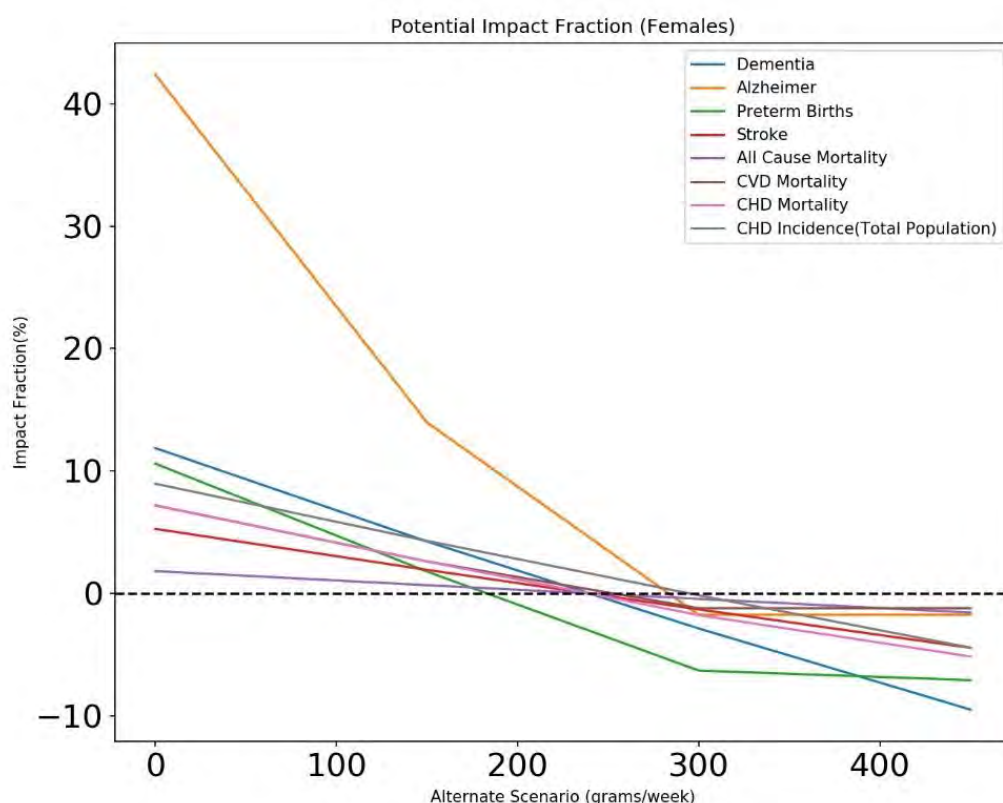


Figure 9.2.6-2 Potential impact fraction for each health outcome for women per alternative intake amount (in g/week). A positive potential impact fraction indicates the expected percentwise increase in number of cases or deaths when the female population changes intake in fish consumption to a given alternative intake; a negative impact fraction indicates an expected percentwise decrease in number of cases or deaths.

9.2.6.1 Dementia

The dose-response RR for dementia reported by Kostis et al. (2021) is not significant with 1 included in the 95% confidence interval. It was estimated that if adult Norwegian men increase the current intake of fish (350 g/week) to 450 g/week, 214 dementia cases will be prevented annually (9.2.6-1) corresponding to approximately a 5% decrease in cases (9.2.6-3). However, when applying the lower and upper limits the 95% CI of the RR, an estimated 459 cases is prevented (approximately 10% decrease in cases) or 40 extra cases caused (approximately 1% increase in cases), respectively. If the intake is decreased to 150 g/week, 459 additional dementia cases was estimated (lower and upper limits estimates: 80 cases prevented (approx. 2% decrease) and 2037 extra cases caused (approx. 23% increase)). The same dose-response relationship was applied for women, thus the same trend estimated: if Norwegian adult women increase the current intake of fish (238 g/week) to within the recommended intake range of 300 to 450 g/week, a decrease in annual cases of 171 (approximately 3% decrease) to 565 (approximately 10% decrease) was estimated.

However, the upper and lower limits of the 95% CI results in ranges of –20 cases to + 247 cases (from current to 300g/week) and –494 cases to + 40 cases (from current to 450 g/week).

Despite a significant summary RR for high versus low intake in Kosti et al. (2021) and our systematic literature review (Chapter 4.13), it can from these quantitative estimates not be concluded by how much changes in fish consumption will impact disease occurrence of dementia.

Besides the non-significant dose-response relationship, it should be noted that the calculated difference in annual number of cases (Tables 9.2.6-1 and 9.2.6-2) was based on an assumption that dementia do not decrease the life expectancy of an individual diagnosed with dementia. As described before, this is not the case and thus likely results in an underestimation of the current annual number of new cases when estimated from the dementia prevalence. Also, it should be noted, that the results in Tables 9.2.6-1, 9.2.6-2 and 9.2.6-3 were derived by assuming that the current recorded fish intake in the age group 18-69 reflect the current annual prevalent cases (converted to annual incident cases) in the age group 70-90+ (see Table 9.2.5-1). This assumption is only valid, if it at the same time is assumed that the individuals currently in the 70-90+ had the same fish consumption patterns as the individuals currently in the 18-69 age group. It is unknown how this assumption affects the quantitative estimates.

9.2.6.2 Alzheimer`s disease

Contrary to dementia, the dose-response relationship for Alzheimer's disease is a significant inverse association between RR and fish intake up to an intake of 250 g/week, after which there is no further risk reduction (see Figure 9.2.4-1). For men, this means that neither an increase of fish intake to 450 g/week or a decrease to 300 g/week was estimated to change the percentage of Alzheimer's disease cases attributed to fish intake (Table 9.2.6-3). A decrease in fish intake to 150 g/week was estimated to cause 416 (upper and lower CI 95% limits: 136; 727) extra cases per year of Alzheimer's disease in men, corresponding to a percent increase of 16% (upper and lower CI 95% limits: 5%; 28%). In women, it was estimated that an increase in fish intake from the current (238 g/week) to all intakes above 250 g/week will prevent 61 annual cases (upper and lower CI 95% limits: 22; 101). A decrease in intake to 150 g/week was estimated to cause 481 (upper and lower CI 95% limits: 158; 843) extra cases per year, corresponding to a percentwise increase in cases of 14% (upper and lower CI 95% limits: 5%; 24%). It should be noted that Alzheimer's disease is a subcategory of dementia, and therefore the impact of changes in fish intake on Alzheimer's disease is integrated in the overall impact on dementia and cannot be added. The same assumptions as in dementia regarding life expectancy and age groups of current intake and prevalence applies to Alzheimer's disease.

9.2.6.3 *Preterm birth*

The dose-response RR for preterm birth reported by Zhou et al. (2020) is not significant with 1 included in the 95% confidence interval. Therefore, it was estimated that the number of annual preterm births using the lower and upper limits dose-response relationships, results in a range of 366 preterm births prevented to an extra 10 births caused when intake of fish is increased from the current (182 g/week) to 300 g/week, which correspond to 13% of cases prevented or an increase in cases of 0.4% (Table 9.2.6-2 and 9.2.6-3). Likewise, if current intake is decreased to 150 g/week, the estimated number of preterm births could either increase by 107 or decrease by 3 annually. Therefore, it can from these quantitative estimates not be concluded by how much changes in fish consumption will impact the number of preterm births per year.

9.2.6.4 *Stroke incidence*

The dose-response relationship between fish intake and stroke incidence is a significant inverse linear association. It was estimated that if men decrease the intake of fish from the current (350 g/week) to 300 g/week or 150 g/week, an increase in number of annual stroke cases of 30 (upper and lower CI 95% limits: 1; 57) and 121 (upper and lower CI 95% limits: 8; 235), respectively, was estimated. This corresponds to a percentwise increase in stroke cases of 1% (300 g/week) and 4% (150 g/week) from the current. If the intake is increased to 450 g/week, the number of stroked cases was estimated to decrease by 59 cases annually (upper and lower CI 95% limits: 4; 111), corresponding to a percentwise decrease of 2%. For women, an increase of 28 stroke cases per year (upper and lower CI 95% limits: 1; 53) was estimated for a decrease in intake from the current (182 g/week) to 150 g/week, corresponding to a percentwise increase of 2% (upper and lower CI 95% limits: 0%; 4%). If women increased the intake to the upper end of the recommended intake, i.e., 450 g/week, 65 (upper and lower CI 95% limits: 5; 123) cases of stroke was estimated to be prevented each year, or a decrease of 4.5% (upper and lower CI 95% limits: 0.3%; 8%).

9.2.6.5 *CHD incidence*

The dose-response relationship for fish intake and CHD incidence is significant inverse association. Zhang et al. (2020) describes a decrease in risk at intakes above 280 g/week. In the quantitative assessment, the decrease in risk was assumed to occur from zero intake (see Figure 9.2.4-1). Changes in annual number of incident cases of CHD due to changes in fish intake were only estimated for the total population (0-74 years of age), not per gender and are shown in Table 9.2.6-4. An increase in fish intake from the current (294 g/week) to 300 and 450 g/week was estimated to decrease the annual incident cases of CHD by 64 cases (upper and lower CI 95% limits: 48; 81) and 1636 cases (upper and lower CI 95% limits: 1228; 2044), respectively. The latter corresponds to a decrease of 4.5% from the current CHD cases. A decrease in the intake from the current to 150 g/week was estimated to cause 1578 (upper and lower CI 95% limits: 1170; 1993) extra CHD cases per year in the Norwegian population (0-74 years).

9.2.6.6 All-cause mortality

The dose-response relationship between fish intake and all-cause mortality is a significant non-linear inverse association. However, from the RRs reported in Schwingshackle et al. (2017) for 1 and 2 servings per day (Table 9.2.4-1), a log-linear association was assumed for the observed intake region (see Figure 9.2.4-1). An increase in annual number of deaths of 14 (upper and lower CI 95% limits: 6; 24) and 59 (upper and lower CI 95% limits: 22; 98) was estimated for decreases in men's fish intake from the current (350 g/week) to 300 and 150 g/week, respectively. This corresponds to percentwise increases of 0.4% (upper and lower CI 95% limits: 0.1%; 0.6%) and 1.5% (upper and lower CI 95% limits: 0.6%; 2.5%) for 300 and 150 g/week, respectively. For increase in intake to 450 g/week, an estimated 44 deaths per year (upper and lower CI 95% limits: 17; 72) were prevented. For women, an increase in annual number of deaths of 18 (upper and lower CI 95% limits: 7; 30) was estimated for a decrease in intake from the current (182 g/week) to 150 g/week, corresponding to 0.7% increase (upper and lower CI 95% limits: 0.3%; 1.1%) from the current annual deaths. With increase in fish intake, annual number of prevented deaths were estimated to 13 (upper and lower CI 95% limits: 5; 22) and 44 (upper and lower CI 95% limits: 17; 72) for 300 and 450/g week intake scenarios, respectively, corresponding to a 1.6% (upper and lower CI 95% limits: 0.6%; 2.6%) decrease in number of deaths from the current at the 450 g/week scenario.

9.2.6.7 CVD mortality

The dose-response relationship for CVD mortality is a significant inverse association between RR and fish intake up to an intake of 280 g/week, after which there is no further risk reduction (see Figure 9.2.4-1). For men, this means that neither an increase of fish intake to 450 g/week or a decrease to 300 g/week changes the percentage of CVD deaths attributed to fish intake (Table 9.2.6-3). A decrease in fish intake to 150 g/week was estimated to cause 36 (upper and lower CI 95% limits: 9; 55) extra deaths per year due to CVD in men, corresponding to a percentwise increase of approximately 4% (upper and lower CI 95% limits: 1%; 6%). In women, it was estimated that an increase in fish intake from the current (238 g/week) to all intakes above 250 g/week will prevent 5 annual deaths (upper and lower CI 95% limits: 1; 7) due to CVD. A decrease in intake to 150 g/week was estimated to cause 9 (upper and lower CI 95% limits: 2; 13) extra CVD deaths per year, corresponding to a percentwise increase in cases of approximately 3% (upper and lower CI 95% limitss: 0.6%; 4%). It should be noted that CVD mortality is a subcategory of all-cause mortality, and therefore the impact of changes in fish intake on CVD mortality is integrated in the overall impact of all-cause mortality and cannot be added.

9.2.6.8 CHD mortality

The dose-response relationship for CHD mortality is a significant inverse association between RR and fish intake up to an intake of 420 g/week, after which there is no further risk reduction (see Figure 9.2.4-1). For men, it was estimated that an increase in fish intake from the current (350 g/week) to all intakes above 420 g/week prevent 10 CHD deaths per year

(upper and lower CI 95% limits: 5; 13), corresponding to a decrease of 2.6% (upper and lower CI 95% limits: 1.3%; 1.3%) from the current CHD deaths. A decrease in intake to 150 g/week was estimated to cause an extra 29 (upper and lower CI 95% limits: 14; 37) CHD deaths per year in men. For women, it was estimated that increase in fish intake from the current (238 g/week) to 300 and 450 g/week will prevent 2 (upper and lower CI 95% limits: 1; 3) and 7 (upper and lower CI 95% limits: 3; 9) CHD deaths per year, respectively, where the latter corresponds to approximately 5% decrease in CHD deaths. On the contrary, a decrease from current intake to 150 g/week was estimated to cause an extra 3 (upper and lower CI 95% limits: 1; 4) CHD deaths per year, corresponding to an increase of 2.6% (upper and lower CI 95% limits: 1.3%; 3.2%) from the current CHD deaths.

9.2.7 Assumptions and limitations

In the following, the main assumptions and limitations of the quantitative assessment of the effect of fish intake scenarios on disease incidence and mortality are listed and briefly discussed.

9.2.7.1 Model assumptions and uncertainty

In the assessment, a change in fish intake was modelled by shifting the population mean of current intake to any of the alternative intake scenarios represented as point estimates. By doing so, the variation in intakes in the population is disregarded, and this assumption is a source of unquantified uncertainty around the size of the estimated effect. This assumption might introduce bias because the difference between true intake and population mean differ for different age-groups or sex.

The mean intake is a simple estimate of the population intake which is an ideal scenario that should be closest to/best representation of the individual intakes. The limitation lies in the fact that different age groups among the population have significantly different intakes. For instance, the mean is "pushed up" due to very high fish consumption in the senior age groups as compared to the younger age groups where the consumption is lower and the RR (relative risk) for most of the diseases have an inverse proportionality with the consumption (as can be seen from the dose-response curves). Due to this, the estimated RR of the "young cohorts" is higher than what it should be and for the "senior cohorts" are lower than what it should be for current consumption and that in turn affects the impact fraction calculations. Considering the scope of the project, the time constraints, and the data constraints some of these limitations are left for future scope. Other sources of uncertainty pertaining to the assessment of fish intake are discussed elsewhere (see exposure assessment Chapter 11).

Estimation of the expected number of new cases and deaths in each alternative intake scenario was done under the assumption that the current intake of fish is reflected in the current disease incidence and mortality. Under this assumption, the lag-time between exposure and onset of disease is ignored (i.e., it is disregarded that lifestyle diseases develop over time due to exposure to modifiable risk factors), as well as changes in fish consumption

over time are ignored (e.g., a 30-year-old today is assumed to have the same consumption pattern as a 30-year-old 10 years ago).

Overall, the quantitative assessment of the effect of changes in fish intake on disease incidence and mortality is performed under the assumption that the distribution of all other risk factors is constant.

9.2.7.2 Parameter assumptions and uncertainty

A considerable source of uncertainty in the assessment is the assumption on the structure and parameters linking relative risks to fish intake. As an indication of the parameter uncertainty of the relative risks, the annual number of new cases and deaths and potential impact fractions in each alternative intake scenario were estimated by applying the lower and upper limits of the 95% confidence interval around the relative risks (Tables 9.2.6-1, 9.2.6-2 and 9.2.6-3). However, additional unquantified uncertainty originating from the relative risks is likely to affect the quantitative estimates due to, for example, residual confounding, extrapolation of relative risks between populations with different distribution of risk factors, mortality rates. Additionally, the structure of the assumed relationship between intake and risk (mostly log-linear, Table 9.2.4-1) as well as the overall assumption of fish intake as the sole changing factor will potentially affect outcome estimates both in magnitude and direction.

Due to lack of epidemiological evidence on the association between lean and fatty fish or even specific fish species and the risk of the different health outcomes, the current assessment used total fish intake only, disregarding proportion of lean and fatty fish or specific fish species consumed in the Norwegian population.

Introduction to semi-quantitative characterization of nutrients and contaminants

In Chapters 9.3 and 9.4 we present semi-quantitative characterizations of nutrients and contaminants in fish. To characterize the intakes of nutrients and contaminants we use dietary reference values and health-based guidance values, i.e., average requirement (AR) for the nutrients and tolerable weekly intake (TWI) for contaminants.

The AR defines the level of a nutrient intake that is sufficient to cover the requirement for half of a defined group of individuals, provided that there is a normal distribution of the requirement (NNR, 2012). Indirectly, it can be deducted that the established AR may not cover the requirements for half of the population, and consequently, even if 100% in the population have intakes above AR, they will not all have covered their nutritional needs.

For the contaminants, the groups having an intake above TWI is defined as the proportion at risk of too high intakes. When exposure is above the TWI the risk of adverse effects is assumed to increase by increasing exceedance, but the increased risk cannot be quantified. A TWI should be interpreted as a safe upper level of intake, and when chronic intake of a contaminant is below a TWI there is no appreciable risk for adverse health effects.

ARs that are used as comparison values for nutrients in Chapter 9.3 must be considered as far less conservative values than the TWIs used as comparison values for contaminants in Chapter 9.4. These are not comparable parameters.

In the semi-quantitative characterization of nutrients and contaminants, we have used mixed model approach to calculate estimates for all compounds for adults and young children and adolescents (data from Norkost 3 and Ungkost 3) except for methyl mercury. For the 1- and 2-year-olds data are based on FFQ-surveys, and we present weighted observed individual means (OIMs). See Chapter 7.5 for description of method for mixed model exposure assessment.

9.3 Semi-quantitative benefit characterization of nutrients

In this benefit and risk assessment, the proportion of the population below the average requirement (AR) for nutrients is defined as having a relatively high probability of inadequate intake.

This chapter presents semi-quantitative benefit assessment of nutrients in fish based on average requirements described in Chapter 2 (overview in Table 2.2.7-1), nutrient intake estimates from Chapter 8, and the weight of evidence conclusions for nutrients and health outcomes in Chapter 5.

VKM have decided to use the dietary reference value average requirement (AR) as comparison value to identify probability of inadequate intake (see Chapter 2), and this is in line with the proposal for harmonised dietary reference values from WHO, FAO, and NASEM

(Allen et al., 2019). For simplicity, we have termed the estimated average requirements (EAR) from IOM used for children as AR in the present chapter. For LC n-3 FA, no AR was available. However, an adequate intake (AI) set by EFSA has been used as comparison value. VKM apply the EAR/AR cut-point method (Carriquiry et al., 1999; Murphy et al., 2002; NNR, 2012). To apply this method, certain conditions need to be met, e.g., that the dietary data reflect habitual intake (NNR, 2012), which is the case for both Sped- and Småbarnskost 3, and also for Ungkost 3 and Norkost 3 since we have adjusted for within- and between variation.

For the nutrients, the proportion of the groups being at risk for having inadequate intake increases the lower the intake is compared to the AR. Therefore, we present the 5th, 25th, 50th, 75th and 95th percentiles for the nutrient intake to facilitate the interpretation of the probability of inadequate intake.

From the intake estimates, we have presented mixed model data for adults and 13-, 9-, and 4-year-olds, and weighted OIM for 1-, and 2-year-olds. The methods for the mixed model and the weighting are described in Chapter 7. The current fish intake, and the fish scenarios that are used for calculation of the estimates for the nutrients given below are presented in Chapter 9.1, see especially Table 9.1-1.

9.3.1 Long-chain n-3 fatty acids

The contributions to LC n-3 FA from various food groups in adults are fish and seafood 66%, food supplements 19%, meat 6%, dairy 3%, eggs 4%, and other food groups 2%, and the contributions to LC n-3 FA from various food groups in 13-year-olds are fish and seafood 62%, food supplements 13%, meat 12%, dairy 4%, eggs 4%, and other food groups 5%.

In Tables 9.3.1-1-9.3.1-3 we have presented how LC n-3 FA intake, including intake from food supplements, is distributed in the different age groups, both at current dietary intake and the three fish scenarios (the fish scenarios are presented in Table 9.1-1). This is also illustrated in Figure 9.3.1-1.

Humans and animals can synthesize most of the fatty acids they need, except for linoleic acid (LA, 18:2 n-6) and α -linolenic acid (ALA, 18:3 n-3). As a result, these two fatty acids are called essential and must be supplied through the diet. EPA, DPA and DHA can be synthesized from the α -linolenic acid (ALA), and ALA is therefore considered the only essential n-3 fatty acid. The synthesis of EPA, DPA and DHA from ALA is limited by various reasons. In a typical Western diet with high intake of linoleic acid, the conversion of ALA to EPA, DPA and DHA is reduced. No AR has been established for LC n-3 FAs, but we have estimated the proportion of the adult population below the adequate intake at 250 mg/day for EPA plus DHA set by EFSA (EFSA, 2010).

Table 9.3.1-1 Total LC n-3 FA intake (mg/day, including intake from food supplements) and proportion below the adequate intake (AI) in adult women and men (Norkost 3) based on mixed model estimates of current (habitual) intake, and fish scenario 1, 2, and 3.

	Mean	SD	P05	P25	P50	P75	P95	<AI *
Women, 18-70 years (n=925)								
Current	1008	784	237	466	782	1316	2538	11%
Scenario 1	764	214	528	581	688	927	1151	0%
Scenario 2	1377	214	1140	1194	1301	1540	1764	0%
Scenario 3	1433	214	1196	1249	1356	1595	1819	0%
Women, 18-45 years (n=466)								
Current	783	607	204	374	605	1000	1978	18%
Scenario 1	717	197	521	566	626	862	1099	0%
Scenario 2	1330	197	1134	1179	1239	1475	1712	0%
Scenario 3	1386	197	1190	1234	1294	1531	1767	0%
Men, 18-70 years (n=862)								
Current	1230	990	293	570	932	1577	3170	9%
Scenario 1	841	269	564	630	722	1038	1354	0%
Scenario 2	1454	269	1177	1243	1335	1651	1967	0%
Scenario 3	1509	269	1232	1298	1391	1706	2023	0%

Current fish intake, and fish intake in the scenarios are presented in Table 9.1-1.

*These estimates do not include contribution from docosapentaenoic acid (DPA) as the AI set by EFSA only includes EPA + DHA. The other estimates in this table include EPA, DPA and DHA.

Table 9.3.1-2 Total LC n-3 FA intake (mg/day, including intake from food supplements) in 4-, 9- and 13-year-old boys and girls (Ungkost 3) based on mixed model estimates of current (habitual) intake, and fish scenario 1, 2, and 3.

	Mean	SD	P05	P25	P50	P75	P95
Girls, 13-year-olds (n=355)							
Current	412	372	104	185	291	500	1155
Scenario 1	574	129	450	487	528	622	848
Scenario 2	1137	129	1013	1050	1091	1184	1411
Scenario 3	1187	129	1063	1100	1141	1235	1461
Boys, 13-year-olds (n=332)							
Current	489	382	153	241	351	613	1277
Scenario 1	612	144	470	510	557	681	910
Scenario 2	1174	144	1033	1073	1120	1244	1472
Scenario 3	1225	144	1083	1124	1170	1294	1523
Girls, 9-year-olds (n=341)							
Current	378	323	107	176	264	462	1047
Scenario 1	472	113	365	394	426	532	708
Scenario 2	905	113	798	827	859	965	1141
Scenario 3	944	113	837	866	898	1004	1180
Boys, 9-year-olds (n=295)							
Current	445	362	139	217	315	539	1210
Scenario 1	494	125	375	409	444	545	759
Scenario 2	927	125	808	842	877	978	1192
Scenario 3	966	125	847	881	916	1017	1231

	Mean	SD	P05	P25	P50	P75	P95
Girls, 4-year-olds (n=195)							
Current	531	400	163	255	386	709	1342
Scenario 1	408	142	276	306	351	487	699
Scenario 2	740	142	608	638	682	818	1030
Scenario 3	765	142	632	662	707	843	1055
Boys, 4-year-olds (n=204)							
Current	440	347	126	208	319	575	1125
Scenario 1	417	136	287	317	356	513	685
Scenario 2	749	136	619	648	687	844	1016
Scenario 3	774	136	644	673	712	869	1041

Current fish intake, and fish intake in the scenarios are presented in Table 9.1-1.

Table 9.3.1-3 LC n-3 FA intake (mg/day, including intake from food supplements) in 1- and 2-year-olds (Spedkost 3 and Småbarnskost 3) based on weighted observed individual mean (OIM) estimates of current (habitual) intake, and fish scenario 1, 2, and 3.

	Mean	SD	P05	P25	P50	P75	P95
2-year-olds, girls and boys (n=1413)							
Current	497	429	68	178	399	691	1310
Scenario 1	444	236	268	291	317	575	916
Scenario 2	787	236	612	635	660	918	1259
Scenario 3	806	236	630	653	679	936	1278
1-year-olds, girls and boys (n=1957)							
Current	418	383	45	133	309	585	1102
Scenario 1	348	202	199	218	244	429	718
Scenario 2	608	202	459	478	504	689	979
Scenario 3	624	202	474	494	520	705	994

Current fish intake, and fish intake in the scenarios are presented in Table 9.1-1.

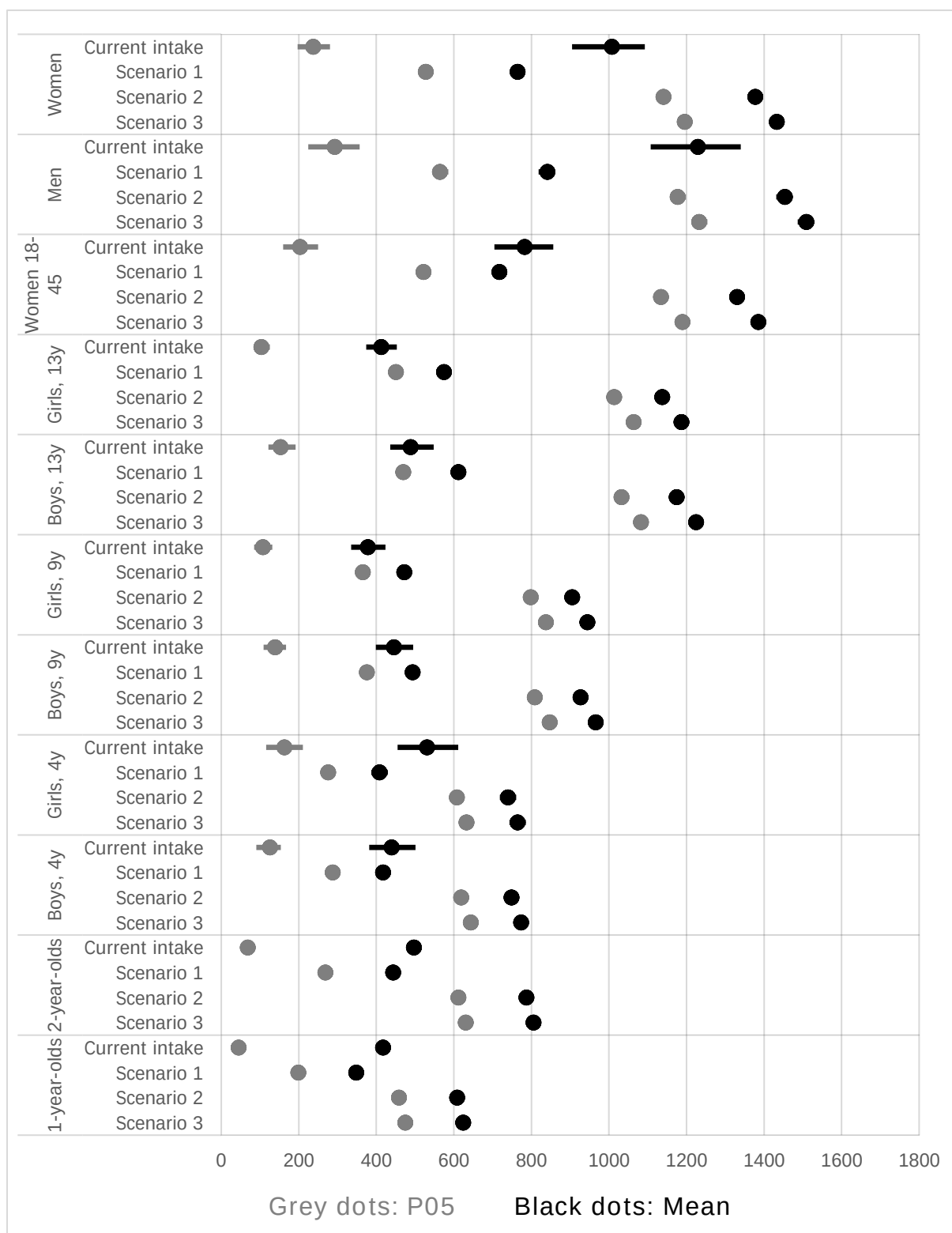


Figure 9.3.1-1 Distribution of the estimated intake of LC n-3 FA (sum EPA+DPA+DHA) in the different age groups, at the current level of fish intake and in the three fish scenarios. Black dots show mean intakes, and grey dots show the 5th percentile, both in mg/day. The horizontal lines around the dots show confidence intervals (5% and 95%). The results are mixed model based, except for 1- and 2-year-olds, for whom weighted OIMs are reported, and, thus, no confidence intervals are available.

No semi-quantitative evaluation of proportion having an intake below average requirement could be presented for LC n-3 FA as it is for the other nutrients in fish. The reason for this is that there is no established AR for LC n-3 FAs. In 2010, EFSA suggested an adequate intake (AI) for EPA + DHA at 250 mg/day.

9.3.1.1 *Benefit characterisation at current intakes*

Estimates from Norkost 3 show that at current intake levels, about 10% of adult women and men receive less than 250 mg of EPA plus DHA per day. Women of childbearing age (18-45 years) have the lowest estimated intakes among adults, and 18% have intakes below the AI.

Groups at risk of low LC n-3 FA intakes

As fatty fish and fish oil supplements are the main sources of marine LC n-3 FA, individuals with low intakes of fatty fish and fish oil supplements will consequently have low intakes. As presented above, intake of ALA may partly compensate for low intakes of EPA and DHA in people with adequate conversion rate from ALA to EPA and DHA.

9.3.1.2 *Benefit characterisation at altered fish intake in the fish scenarios*

In all the fish intake scenarios, all adults have an intake of EPA plus DHA above the adequate intake.

9.3.1.3 *Evidence for health benefits related to intake of LC n-3 FA*

In Chapter 5.2, we evaluated health benefits (and risks) related to LC n-3 FA intake. In the revision of NNR (2012), Schwab et al. (2014) conducted an evidence-based systematic literature review of LC n-3 FA and associated health effects. The review by Schwab et al. (2014) covered cardiovascular diseases (CVD), type 2 diabetes, body weight, and cancer. The evidence for an association between LC n-3 FA and the health outcomes were not judged to be “probable” or “convincing”.

VKM conducted an updated review on the association between LC n-3 FA and all health outcomes relevant for fish consumption except for immune outcomes, i.e., CVD and mortality, neurodevelopmental outcomes in children and adults, including cognition, cognitive decline and mental health disorders, birth outcomes, type 2 diabetes, overweight and obesity, rheumatoid arthritis, multiple sclerosis, and male fertility. The evidence for protective associations between LC n-3 FA intake and the health outcomes CVD mortality, CHD mortality, CHD incidence and MI incidence, was judged to be “probable”. The evidence that LC n-3 FA increases birth weight was also judged to be “probable”. Moreover, the evidence for associations between LC n-3 FA intake and the health outcomes CVD incidence was judged to be “limited, suggestive” for doses <1 g/day LC n-3 FA, but “probable” for higher doses from supplements. The evidence for an association between LC n-3 FA and depression and birth weight (maternal exposure during pregnancy) was judged to be “limited, suggestive”. For the other health outcomes included in the systematic review (all-

cause mortality, stroke incidence, atrial fibrillation, type 2 diabetes, child neurodevelopment (maternal exposure during pregnancy), cognition and cognitive decline in adults, and preterm birth (maternal exposure during pregnancy) the evidence was judged to be "limited, no conclusion".

All the outcomes where it was judged that the evidence for an association between the LC n-3 FA supplementation and the outcome were "probable" are included in the quantitative benefit and risk analysis for fish consumption except from CVD and MI incidence. For CVD incidence, this association was, however, only judged to be "probable" in doses of LC n-3 FA > 1 g/day which is not relevant for LC n-3 FA intake from fish consumption. The overlap between the conclusions on "probable" evidence for the associations for fish and LC n-3 FA and the same outcome may strengthen the assumption that beneficial effects from fish and these outcomes are mediated through the LC n-3 FAs.

9.3.1.4 Summary of benefit characterisation of LC n-3 FA

In summary, there is evidence ("probable") that LC n-3 FA intake is associated with reduced risk of CVD mortality, CHD mortality, CHD incidence, MI incidence and increased birth weight. Of these health outcomes, CHD incidence, CVD mortality and CHD mortality are included in the quantitative modelling of benefits and risks associated with fish consumption in Chapter 9.2. The evidence that LC n-3 FA intake is associated with CVD incidence, depression and low birth weight was judged as "limited, suggestive".

As shown above, at current intake, 18% of women of childbearing age, have intakes below an adequate intake set by EFSA (2010). In women and men 18-70 years, about 10% have intakes below the adequate intake. Fish, and especially fatty fish, is one of very few natural sources to LC n-3 FA, and consequently, increasing intake of fish will have high impact on the total intake of LC n-3 FA. In the fish scenarios, in which all participants in the food dietary surveys are assigned a fixed daily intake of fish, all adults have estimated intakes of EPA plus DHA above the adequate intake.

9.3.2 Vitamin D

The recommended daily intake of vitamin D for children, adolescents, and adults, including pregnant and lactating women, is 10 µg/day, and 20 µg/day in elderly ≥75 years (NNR, 2012). AR for adults, children and adolescents is 7.5 µg/day (NNR, 2012), and for pregnant and lactating women 10 µg/day (NNR, 2012). The critical endpoint for setting an AR for vitamin D is bone health.

Vitamin D is produced by UVB-exposure of the skin or ingested orally by eating foods naturally rich in vitamin D, fortified foods or by taking supplements. Vitamin D is hydroxylated in the liver to 25-hydroxyvitamin D (25(OH)D), which is the main storage form of vitamin D with a half-life of several weeks reflecting the total contribution from diet, supplements and sun.

Vitamin D production in the skin peaks during summer, is limited during spring and autumn, and absent during the darkest part of the year, as all the UVB-rays are absorbed in the atmosphere even on clear, sunny days. As a result, a substantial proportion of the Norwegian population have vitamin D-insufficiency during winter (serum concentration of 25(OH)D under 50 nmol/L), but relatively few have vitamin D-deficiency (<30 nmol/L) (Nasjonalt råd for ernæring, 2018). However, in certain population groups including immigrants from Asia and Africa, the prevalence of vitamin D-deficiency is much higher.

The contributions to vitamin D from various food groups in adults are fish and seafood 23%, food supplements 44%, meat 3%, dairy 8%, eggs 6%, and other food groups 16%, and the contributions to vitamin D from various food groups in 13-year-olds are fish and seafood 18%, food supplements 45%, meat 4%, dairy 11%, eggs 6%, and other food groups 16%.

In Tables 9.3.2-1-9.3.2-3 we have presented how vitamin D intake, including intake from vitamin supplements, is distributed in the different age groups, both at current level, and the three scenarios. This is also illustrated in Figure 9.3.2-1.

Table 9.3.2-1 Total vitamin D intake (µg/day, including intake from food supplements) and proportion below the average requirement (AR) in adult women and men (Norkost 3) based on mixed model estimates of current (habitual) intake, and fish scenario 1, 2, and 3.

	Mean	SD	P05	P25	P50	P75	P95	<AR
Women, 18-70 years (n=925)								
Current	9.8	6.5	2.6	4.7	7.9	13.9	22.3	48%
Scenario 1	8.9	5.2	3.3	4.5	6.8	12.9	18.5	53%
Scenario 2	10.8	5.2	5.1	6.4	8.6	14.7	20.3	41%
Scenario 3	11.2	5.2	5.5	6.8	9.0	15.2	20.8	36%
Women, 18-45 years (n=466)								
Current	8.1	5.6	2.3	3.9	6.2	11.3	19.2	59%
Scenario 1	7.7	4.7	3.1	4.2	5.6	11.1	16.8	62%
Scenario 2	9.6	4.7	5.0	6.0	7.4	13.0	18.7	51%
Scenario 3	10.0	4.7	5.4	6.4	7.9	13.4	19.1	46%
Men, 18-70 years (n=862)								
Current	12.3	7.9	3.7	6.3	9.7	17.0	27.9	35%
Scenario 1	11.0	7.0	3.8	5.7	8.3	15.3	24.8	44%
Scenario 2	12.8	7.0	5.6	7.5	10.2	17.2	26.6	25%
Scenario 3	13.2	7.0	6.1	7.9	10.6	17.6	27.0	20%

Current fish intake, and fish intake in the scenarios are presented in Table 9.1-1.

Table 9.3.2-2 Total vitamin D intake ($\mu\text{g}/\text{day}$, including intake from food supplements) and proportion below the average requirement (AR) among 4-, 9- and 13-year-old girls and boys, (Ungkost 3) based on mixed model estimates of current (habitual) intake, and fish scenario 1, 2, and 3.

	Mean	SD	P05	P25	P50	P75	P95	<AR
Girls, 13-year-olds (n=355)								
Current	6.5	5.0	1.3	2.7	4.8	9.3	16.4	67%
Scenario 1	6.8	4.4	2.3	3.5	5.2	9.3	15.5	66%
Scenario 2	8.7	4.4	4.3	5.4	7.1	11.3	17.4	53%
Scenario 3	9.1	4.4	4.7	5.8	7.5	11.7	17.8	50%
Boys, 13-year-olds (n=332)								
Current	7.3	5.8	1.4	3.0	5.5	10.3	18.8	62%
Scenario 1	7.5	5.0	2.3	3.7	5.8	10.2	17.4	62%
Scenario 2	9.4	5.0	4.3	5.6	7.8	12.1	19.4	48%
Scenario 3	9.8	5.0	4.7	6.0	8.2	12.5	19.8	44%
Girls, 9-year-olds (n=341)								
Current	6.4	4.2	1.5	3.0	5.4	9.1	14.4	65%
Scenario 1	6.6	3.8	2.2	3.5	5.6	9.1	13.6	64%
Scenario 2	8.0	3.8	3.7	4.9	7.1	10.5	15.1	53%
Scenario 3	8.3	3.8	4.0	5.2	7.4	10.8	15.4	51%
Boys, 9-year-olds (n=295)								
Current	6.6	4.5	1.7	3.2	5.3	9.3	15.4	66%
Scenario 1	6.6	4.1	2.2	3.4	5.3	9.0	14.6	66%
Scenario 2	8.1	4.1	3.7	4.9	6.8	10.4	16.1	56%
Scenario 3	8.4	4.1	4.0	5.2	7.1	10.7	16.4	54%
Girls, 4-year-olds (n=195)								
Current	7.7	4.3	2.1	3.8	7.6	10.8	15.0	49%
Scenario 1	7.4	4.1	2.2	3.8	7.2	10.3	14.7	53%
Scenario 2	8.5	4.1	3.3	4.8	8.2	11.4	15.8	46%
Scenario 3	8.7	4.1	3.5	5.0	8.4	11.6	16.0	44%
Boys, 4-year-olds (n=204)								
Current	7.9	4.3	2.1	4.1	7.8	11.0	15.3	49%
Scenario 1	7.4	4.0	2.2	3.8	7.2	10.3	14.4	53%
Scenario 2	8.5	4.0	3.3	4.9	8.3	11.4	15.4	45%
Scenario 3	8.7	4.0	3.5	5.1	8.5	11.6	15.7	43%

Current fish intake, and fish intake in the scenarios are presented in Table 9.1-1.

Table 9.3.2-3 Total vitamin D intake ($\mu\text{g}/\text{day}$, including intake from food supplements) and proportion below the average requirement (AR) in 1- and 2-year-olds (Spedkost 3 and Småbarnskost 3) based on weighted observed individual mean (OIM) estimates of current (habitual) intake, and fish scenario 1, 2, and 3.

	Mean	SD	P05	P25	P50	P75	P95	<AR
2-year-olds, girls and boys (n=1413)								
Current	9.8	7.1	2.2	4.1	7.6	13.9	23.0	50%
Scenario 1	9.5	7.0	2.2	3.9	7.3	13.6	22.9	51%
Scenario 2	10.1	7.0	2.9	4.6	7.9	14.3	23.5	48%
Scenario 3	10.3	7.0	3.0	4.7	8.1	14.4	23.7	48%
1-year-olds, girls and boys (n=1957)								
Current	15.1	7.6	4.1	9.8	14.5	19.1	29.1	16%
Scenario 1	14.8	7.6	3.9	9.5	14.4	18.7	28.6	17%
Scenario 2	15.3	7.6	4.4	10.1	14.9	19.3	29.2	15%
Scenario 3	15.5	7.6	4.6	10.2	15.0	19.4	29.3	14%

Current fish intake, and fish intake in the scenarios are presented in Table 9.1-1.

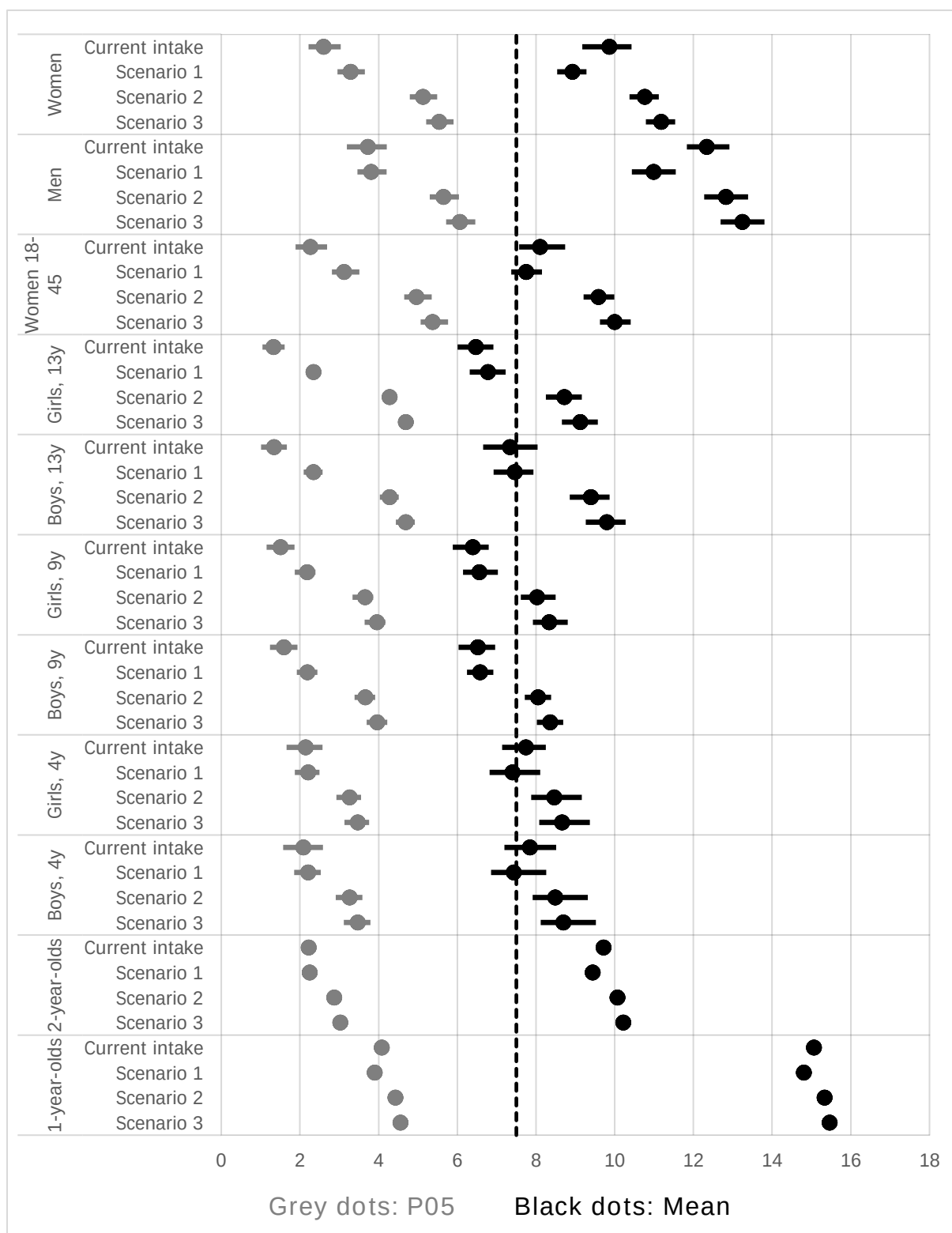


Figure 9.3.2-1 Distribution of the estimated habitual vitamin D intake in the different age groups, at the current level of fish intake and in the three fish scenarios. Black dots show mean intakes, and grey dots show the 5th percentile, both in µg/day. The horizontal lines around the dots show confidence intervals (5% and 95%). The results are mixed model based, except for 1- and 2-year-olds, for whom weighted OIMs are reported, and, thus, no confidence intervals are available. The dashed vertical line is AR used in this assessment.

9.3.2.1 *Benefit characterisation at current intakes*

At current intake levels, all age groups have a relatively high proportion of individuals with an intake of Vitamin D below AR. Women of childbearing age (18-45 years) have the lowest estimated intakes among adults, and 59% have intakes below the AR at 7.5 µg/day. The mean (median) vitamin D intake is 8.1 (6.2) µg/day, and the estimated intake in the 5th percentile is 2.3 µg/day. Among children, the 13- and 9-year-old girls have the lowest estimated vitamin D intake, and 67% and 65%, respectively, of these have intakes below the AR at 7.5 µg/day. The mean (median) estimated vitamin D intake in 9-year-old girls is 6.4 (5.4) µg/day and 13-year-old girls is 6.5 (4.8) µg/day, and the estimated intake in the 5th percentile is 1.5 and 1.3 µg/day, respectively. The age groups with the lowest proportion below AR within current intake of vitamin D were 1-year-olds and adult men, where 16% and 35% respectively had an intake below AR.

Groups at risk of low vitamin D intakes

At risk groups for poor vitamin D-status are those with little sun exposure, little dietary intake of vitamin D and/or not taking supplements containing vitamin D, including cod liver oil. This includes certain immigrant groups and frail elderly. In a Norwegian study, it has also been shown that a significant proportion of teenagers have a poor vitamin D-status (Nasjonalt råd for ernæring, 2018).

Dietary intake of vitamin D and vitamin D supplementation is of major importance during winter in the general population and throughout the year in at risk groups with little or no sun exposure during the summer half of the year. As can be seen from the tables and text above, all age groups have a relatively high proportion with intakes of vitamin D from the diet (including food supplements) below AR.

9.3.2.2 *Benefit characterisation at altered fish intake in the fish scenarios*

For scenario 2, the vitamin D intake among women in childbearing age was increased to on average mean (median) 9.6 (7.4) µg/day, and the estimated intake in the 5th percentile is 5.0 µg/day. The proportion having an intake below the AR was reduced from 59% with current intake of fish to 51%. For scenario 3, the vitamin D intake was increased to mean (median) 10.0 (7.9) µg/day, and the estimated intake in the 5th percentile is 5.4 µg/day. The proportion having an intake below the AR was reduced to 46%.

Among the 13-year-old girls, the intake of vitamin D in scenario 2 was increased to on average mean (median) 8.7 (7.1) µg/day, and the estimated intake in the 5th percentile was increased to 4.3 µg/day and the proportion having an intake below the AR was reduced from 67% with current intake of fish to 53%. In scenario 3, the intake of vitamin D was increased to on average mean (median) 9.1 (7.5) µg/day, and the estimated intake in the 5th percentile was increased to 4.7 µg/day and the proportion having an intake below the AR was reduced to 50%.

In scenario 1, the mean vitamin D intakes are slightly decreased in most age groups except for in the 13- and 9-year-olds, and the proportion below AR is either increased or the same as with the current fish intake in all age groups. The intakes in the 5th percentile are, however, slightly increased in all age groups except for the youngest age groups. This is probably because this scenario represents a small increase in fish/fatty fish in those age groups with the current lowest fish intake, whereas it represents a small decrease in fish intake in other age groups.

9.3.2.3 *Evidence for health benefits related to intake of vitamin D*

Bone health is the selected endpoint to form the basis for reference values (recommended intake and AR) for vitamin D intake in both NNR (2012) and IOM (2010). The selection of bone health as indicator is based on a thorough evidence-based systematic review for all potential health endpoints for vitamin D (IOM, 2010; Lamberg-Allardt, 2013).

In Chapter 5.3, we evaluated health risk related to vitamin D intake. In the revision of NNR (2012), Lamberg-Allardt et al. (2013) conducted an evidence-based systematic literature review of vitamin D and associated health effects. The review by Lamberg-Allardt et al. (2013) covered the following health outcomes; pregnancy outcomes and growth, bone health (all fractures, hip fractures, vertebral fractures, bone mineral density/osteoporosis, bone mass, bone quality, rickets, osteomalacia, dental health), muscle strength, falls; all cancers, breast cancer, colorectal cancer, prostate cancer, diabetes type I, diabetes type II, multiple sclerosis, obesity, total mortality, hypertension/blood pressure, cardiovascular disease (CVD) clinical outcomes, and infections.

The evidence for associations between vitamin D and bone health (including falls) and mortality were concluded to be “probable” by Lamberg-Allardt et al. (2013). It should be noted that bone health is not included in the quantitative modelling of associations between health outcomes and fish consumption. None of the other health outcomes related to vitamin D and relevant for this benefit and risk assessment was judged to be “probable” or “convincing” by Lamberg-Allardt et al. (2013).

We did an updated review on birth weight and judged that the evidence that vitamin D supplementation in pregnancy reduce the risk of LBW was “limited, suggestive” and the evidence that vitamin D supplementation in pregnancy increase birth weight was “limited, suggestive”. Moreover, an update by SACN (2020) on the association between vitamin D intake and respiratory tract infections showed “limited, suggestive” evidence for the notion that vitamin D lowers the risk of respiratory tract infections.

9.3.2.4 *Summary of benefit characterisation of vitamin D*

In summary, there is evidence (“probable”) that vitamin D intake is associated with a beneficial effect on bone health (including falls) and reduced risk of mortality. The evidence that vitamin D is associated with low birth weight (<2500 g), birth weight (as continuous

variable) and respiratory tract infection were judged as “limited suggestive” in our updated reviews.

As shown above, women of childbearing age and young girls have the lowest vitamin D intakes, and at current intake 59% of women of childbearing age and 49-67% of children and adolescents in Ungkost 3 have intakes below AR.

The scenario estimations indicate that increasing intake of fish from the current intake to the recommended intake would lead to a moderate increase in vitamin D-intake at the population level and may be of special importance for those with a very low dietary intake of vitamin D, where even a small increase may be of substantial importance. For example, in 13-year-old girls and boys, the 5th percentile increased from about 1.4 µg with current fish intake to 4.7 µg/day in scenario 3.

Fish, and especially fatty fish is one of very few natural sources for vitamin D intake.

9.3.3 Iodine

The recommended daily iodine intake for adults is 150 µg/day, for pregnant women 175 µg/day and for lactating women 200 µg/day (NNR, 2012). AR is 100 µg/day for adults (NNR, 2012), and higher during pregnancy (160 µg/day), and during lactation (209 µg/day) (IOM, 2001). ARs for children and adolescents 1 to 18 years are in the range 17-45 µg/day (IOM, 2000). In the Nordic Nutrition Recommendations (NNR) (2012), an AR was not set for children <10 years and adolescents. The US Institute of Medicine (IOM, 2001) set the AR for children and adolescents (1 to 18 years) to between 65 to 95 µg/day. The thyroid iodine accumulation and turnover (to prevent goiter) were used to set the AR.

Henjum et al. (2019) concluded that iodine intake is inadequate in Norway among women of childbearing age, also pregnant and breastfeeding women. In particular, the authors of the review expressed concern regarding iodine status among women of childbearing age, as all included studies published after 2016 showed a median urinary iodine concentration (UIC) lower than the cut-off for adequate group median set by the WHO (100–199 µg/L) for this group.

The contributions to iodine from various food groups in adults are fish and seafood 41%, food supplements 7%, meat 1%, dairy 35%, eggs 5%, and other food groups 11%, and the contributions to iodine from various food groups in 13-year-olds are fish and seafood 23%, food supplements 2%, meat 2%, dairy 55%, eggs 5%, and other food groups 13%.

In Tables 9.3.3-1-9.3.3-3, we have presented how iodine is distributed in the different age groups, including intake from food supplements, both at current level and the three scenarios. This is also illustrated in Figure 9.3.3-1. For the age group 18 to 70 years, we have used ARs for adults, but not included the higher ARs during pregnancy and lactation.

Table 9.3.3-1 Total iodine intake ($\mu\text{g/day}$, including intake from food supplements) and proportion below the average requirement (AR) among adult women and men (Norkost 3) based on mixed model estimates of current (habitual) intake, and fish scenario 1, 2, and 3.

	Mean	SD	P05	P25	P50	P75	P95	<AR
Women, 18-70 years (n=925)								
Current	168	73	81	119	155	202	307	14%
Scenario 1	141	44	84	111	135	165	223	15%
Scenario 2	156	44	100	127	151	181	239	5%
Scenario 3	225	44	168	195	221	250	307	0%
Women, 18-45 years (n=466)								
Current	152	64	74	109	142	183	275	20%
Scenario 1	143	44	85	113	138	167	225	15%
Scenario 2	158	44	100	128	153	183	241	5%
Scenario 3	227	44	169	197	224	253	309	0%
Men, 18-70 years (n=862)								
Current	229	126	90	145	203	283	569	8%
Scenario 1	169	60	92	127	159	200	284	8%
Scenario 2	185	60	108	144	176	216	299	3%
Scenario 3	253	60	177	212	246	286	367	0%

Current fish intake, and fish intake in the scenarios are presented in Table 9.1-1.

Table 9.3.3-2 Total iodine intake ($\mu\text{g/day}$, including intake from food supplements) and proportion below the average requirement (AR) among 4-, 9- and 13-year-old girls and boys (Ungkost 3) based on mixed model estimates of current (habitual) intake, and fish scenario 1, 2, and 3.

	Mean	SD	P05	P25	P50	P75	P95	<AR
Girls, 13-year-olds (n=355)								
Current	100	46	41	65	89	124	185	33%
Scenario 1	113	35	67	89	109	133	177	9%
Scenario 2	129	34	81	104	125	149	191	1%
Scenario 3	201	35	155	177	199	223	262	0%
Boys, 13-year-olds (n=332)								
Current	123	60	50	81	114	161	236	19%
Scenario 1	129	45	71	98	122	154	213	6%
Scenario 2	144	43	86	114	138	169	224	1%
Scenario 3	217	44	160	187	213	242	298	0%
Girls, 9-year-olds (n=341)								
Current	102	46	45	70	94	128	188	28%
Scenario 1	107	34	62	83	102	126	169	14%
Scenario 2	119	33	74	96	115	138	179	5%
Scenario 3	175	33	130	152	172	194	232	0%
Boys, 9-year-olds (n=295)								
Current	121	38	68	91	114	144	189	8%
Scenario 1	120	32	74	96	115	139	178	5%
Scenario 2	132	33	86	109	128	152	192	1%

	Mean	SD	P05	P25	P50	P75	P95	<AR
Scenario 3	187	33	139	164	185	208	246	0%
Girls, 4-year-olds (n=195)								
Current	121	44	61	87	113	143	200	8%
Scenario 1	105	30	65	84	101	123	156	6%
Scenario 2	116	29	76	95	112	133	167	2%
Scenario 3	161	29	119	141	158	179	211	0%
Boys, 4-year-olds (n=204)								
Current	127	46	66	95	119	153	214	4%
Scenario 1	112	30	69	89	108	130	168	3%
Scenario 2	122	32	78	99	118	141	180	1%
Scenario 3	168	30	126	145	165	187	223	0%

Current fish intake, and fish intake in the scenarios are presented in Table 9.1-1.

Table 9.3.3-3 Total iodine intake ($\mu\text{g/day}$, including intake from food supplements) and proportion below the average requirement (AR) among 1- and 2-year-olds (Spedkost 3 and Småbarnskost 3) based on weighted observed individual mean (OIM) estimates of current (habitual) intake, and fish scenario 1, 2, and 3.

	Mean	SD	P05	P25	P50	P75	P95	<AR
2-year-olds, girls and boys (n=1413)								
Current	156	67	69	109	148	190	285	4%
Scenario 1	136	59	65	95	125	164	246	5%
Scenario 2	144	59	73	103	133	173	254	4%
Scenario 3	179	59	108	138	168	208	289	0%
1-year-olds, girls and boys (n=1957)								
Current	133	74	48	83	118	163	276	14%
Scenario 1	118	67	44	73	105	143	244	19%
Scenario 2	125	67	50	80	112	149	250	14%
Scenario 3	152	67	78	107	139	177	277	1%

Current fish intake, and fish intake in the scenarios are presented in Table 9.1-1.

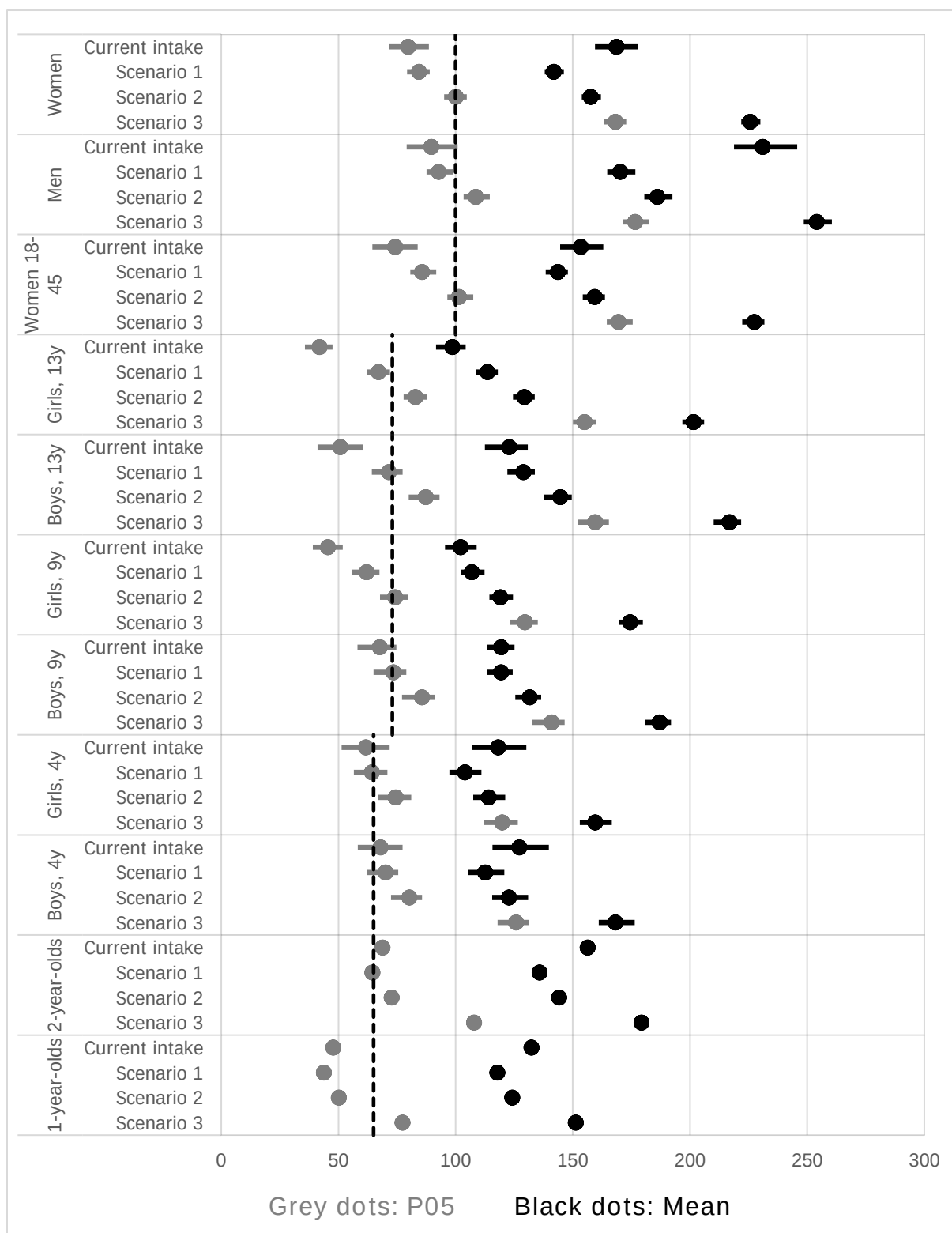


Figure 9.3.3-1 Distribution of the estimated habitual intake of iodine in the different age groups, at the current level of fish intake and in the three fish scenarios. Black dots show mean intakes, and grey dots show the 5th percentile, both in µg/day. The horizontal lines around the dots show confidence intervals (5% and 95%). The results are mixed model based, except for 1- and 2-year-olds, for whom weighted OIMs are reported, and, thus, no confidence intervals are available. The dashed vertical lines are ARs used in this assessment.

9.3.3.1 *Benefit characterisation at current intakes*

At current intake levels, women of childbearing age (18-45 years) have the lowest estimated intakes among adults, and 20% have intakes below the AR at 100 µg/day. The mean (median) iodine intake is 152 (142) µg/day, and the estimated intake in the 5th percentile is 74 µg/day. The higher requirements in pregnancy and during lactation is not covered in these data, and the percentage of pregnant and lactating women with intakes below AR is even higher. Thirteen-year-old girls have the lowest estimated iodine intake, and 33% of these girls have intakes below the AR at 73 µg/day. The mean (median) estimated iodine intake in 13-year-old girls is 100 (89) µg/day, and the estimated intake in the 5th percentile is 41 µg/day. Among the 9-year-old girls, 28% have intakes below the AR at 73 µg/day.

It should be mentioned that in a previous benefit and risk assessment of iodization of salt, it was estimated that 26% of women of childbearing age had intakes below AR (VKM, 2020). This discrepancy from the current estimates may be because we in the present benefit and risk assessment of fish have calculated all fish in fish products as haddock fish fillet. Haddock contains high concentrations of iodine. Fish products contains other fish species as well as haddock, and our iodine estimates may be overestimated.

Groups at risk of low iodine intakes

Generally, individuals and population groups that for various reasons have few iodine-rich sources in their diet, e.g., people with low intakes of lean fish and milk and other dairy products are at risk of low iodine intakes.

The findings that especially young girls and women of childbearing age have low intakes are, however, in accordance with studies in Norway on iodine status in women of childbearing age (Henjum et al., 2019).

9.3.3.2 *Benefit characterisation at altered fish intake in the fish scenarios*

For scenario 2, the iodine intake among women in childbearing age was increased to on average mean (median) of 158 (153) µg/day, and the estimated intake in the 5th percentile is 100 µg/day. The proportion having an intake below the AR was reduced to 5%. For scenario 3, the iodine intake was increased to on average mean (median) 227 (224) µg/day, and the proportion having an intake below the AR was reduced to null.

Among the 13-year-old girls, the intake of iodine in scenario 2 was increased to on average mean (median) of 129 (125) µg/day, and the estimated intake in the 5th percentile is 81 µg/day. The proportion having an intake below the AR was reduced to 1%. In scenario 3 none of the 13-year-old girls had an intake below AR. The same pattern was observed for the 9-year-old girls.

In scenario 1, the mean iodine intakes are slightly decreased in most age groups except for in the 13- and 9-year-olds. The intakes in the 5th percentile are, however, increased in all age groups except for the youngest age groups, and the proportion below AR decreases

among 13- and 9-year-olds. This is probably because this scenario represents a small increase in fish/lean fish in those age groups with the current lowest fish intake, whereas it represents a small decrease in fish intake in other age groups.

9.3.3.3 Evidence for health benefits related to intake of iodine

In a systematic literature review prior to NNR (2012), the scientific basis for the previous iodine recommendation in the Nordic countries were summarized (Gunnarsdottir and Dahl, 2012).

In Chapter 5.4 we evaluated health risk related to iodine intake. It is well established that severe iodine deficiency will impair growth and neurodevelopment through lower production of thyroid hormones. The most severe effect is damage to the foetus resulting in irreversible brain damage (VKM, 2020). It is, however, less clear to which degree mild to moderate iodine deficiency may affect growth and development in infancy and childhood. In a recent systematic literature review by VKM (2020) using the same tools and criteria for evidence as in this benefit and risk assessment of fish consumption, it was concluded that the evidence that mild to moderate maternal iodine deficiency in pregnancy is associated with reduced neurodevelopment in the child is "limited, suggestive" (VKM 2020) (in pregnant women mild-to-moderate iodine deficiency is defined as median UIC in the range 50-149 µg/L (Zimmermann, 2007)). Moreover, it was concluded that the evidence was "limited, no conclusive" for an association between mild to moderate iodine deficiency and reduced thyroid function and adverse birth outcomes and fertility (VKM, 2020).

9.3.3.4 Summary of benefit characterisation of iodine

In summary, it is well established that severe iodine deficiency will impair growth and neurodevelopment through lower production of thyroid hormones. However, the evidence that mild to moderate iodine deficiency in pregnancy may affect neurodevelopment in infancy/childhood is found to be "limited suggestive", and for thyroid function and birth outcomes and fertility it is found to be "limited, no conclusion".

As shown above, groups at highest risk of low intakes are women of childbearing age and young girls, and at current intake 20% of women of childbearing age and 33% of 13-year-old girls had an intake below AR.

The scenario estimations indicate that increasing intake of fish from the current intake to the lower range of recommended intake (scenario 2) would reduce the proportion having a relatively high probability of inadequate iodine intake to about 5% for all age groups and gender. In the upper range of recommended fish intake (scenario 3), all age groups and genders have iodine intakes above AR except for 1-year-olds.

Lean fish is one of few sources to iodine.

9.3.4 Selenium

The recommended daily selenium intake for adults is 50 µg/day for women and 60 µg/day for men, and AR is 30 and 35 µg/day for women and men, respectively (NNR, 2012). AR is higher during pregnancy and lactation, 49 µg/day and 59 µg/day, respectively (NNR, 2012). ARs for children and adolescents 1 to 18 years are in the range 17-45 µg/day (IOM, 2000).

The contributions to selenium from various food groups in adults are fish and seafood 30%, food supplements 9%, meat 17%, dairy 12%, eggs 10%, grains 16%, and other food groups 6%, and the contributions to selenium from various food groups in 13-year-olds are fish and seafood 16%, food supplements 2%, meat 23%, dairy 19%, eggs 9%, grains 25%, and other food groups 6%. Both lean and fatty fish are selenium sources.

In Tables 9.3.4-1-9.3.4-3, we have presented how selenium intake is distributed in the different age groups both at the current level and in the three scenarios. The data includes intake from food supplements. This is also illustrated in Figure 9.3.4-1. For the age groups 18 to 70 years, we have used the AR for adults, but not included the higher ARs during pregnancy and lactation.

Table 9.3.4-1 Total selenium intake (µg/day, including intake from food supplements) and proportion below the average requirement (AR) among adult women and men (Norkost 3) based on mixed model estimates of current (habitual) intake, and fish scenario 1, 2, and 3.

	Mean	SD	P05	P25	P50	P75	P95	<AR
Women, 18-70 years (n=925)								
Current	55	17	29	41	52	65	86	5%
Scenario 1	50	14	31	40	48	57	74	4%
Scenario 2	59	13	40	50	58	67	83	0%
Scenario 3	67	13	48	58	66	75	91	0%
Women, 18-45 years (n=466)								
Current	52	17	28	39	50	62	83	6%
Scenario 1	51	14	32	41	49	59	76	3%
Scenario 2	60	13	41	51	59	68	84	0%
Scenario 3	68	13	49	59	67	76	92	0%
Men, 18-70 years (n=862)								
Current	79	25	38	54	69	86	117	3%
Scenario 1	61	17	38	49	59	71	92	3%
Scenario 2	71	17	47	59	69	80	101	0%
Scenario 3	79	16	55	67	77	88	108	0%

Current fish intake, and fish intake in the scenarios are presented in Table 9.1-1.

Table 9.3.4-2 Total selenium intake ($\mu\text{g}/\text{day}$, including intake from food supplements) and proportion below the average requirement (AR) among 4-, 9- and 13-year-old girls and boys (Ungkost 3) based on mixed model estimates of current (habitual) intake, and fish scenario 1, 2, and 3.

	Mean	SD	P05	P25	P50	P75	P95	<AR
Girls, 13-year-olds (n=355)								
Current	32	11	18	25	31	38	52	66%
Scenario 1	34	8	23	29	33	39	49	58%
Scenario 2	42	8	30	36	41	46	56	20%
Scenario 3	47	8	36	42	47	52	61	3%
Boys, 13-year-olds (n=332)								
Current	39	13	22	30	37	46	63	42%
Scenario 1	40	10	26	33	39	45	57	34%
Scenario 2	47	10	33	40	46	53	64	8%
Scenario 3	53	10	39	46	52	59	70	1%
Girls, 9-year-olds (n=341)								
Current	31	9	19	25	30	36	47	73%
Scenario 1	32	7	22	27	31	36	45	70%
Scenario 2	38	7	27	33	37	42	50	38%
Scenario 3	42	7	32	37	42	47	55	13%
Boys, 9-year-olds (n=295)								
Current	37	11	22	29	35	43	57	47%
Scenario 1	37	9	25	31	36	42	53	45%
Scenario 2	43	9	30	36	42	48	58	19%
Scenario 3	47	9	35	41	47	53	62	5%
Girls, 4-year-olds (n=195)								
Current	30	8	17	23	29	36	44	24%
Scenario 1	28	7	18	23	27	32	41	28%
Scenario 2	32	7	22	27	32	37	45	7%
Scenario 3	36	7	26	31	36	40	48	1%
Boys, 4-year-olds (n=204)								
Current	32	9	19	25	31	38	47	16%
Scenario 1	30	7	20	25	30	34	43	13%
Scenario 2	35	7	25	30	34	39	48	2%
Scenario 3	39	7	29	34	38	43	51	0%

Current fish intake, and fish intake in the scenarios are presented in Table 9.1-1.

Table 9.3.4-3 Total selenium intake ($\mu\text{g/day}$, including intake from food supplements) and proportion below the average requirement (AR) among 1- and 2-year-olds (Spedkost 3 and Småbarnskost 3) based on weighted observed individual mean (OIM) estimates of current (habitual) intake, and fish scenario 1, 2, and 3.

	Mean	SD	P05	P25	P50	P75	P95	<AR
2-year-olds, girls and boys (n=1413)								
Current	36	15	18	27	33	43	67	4%
Scenario 1	34	13	19	26	32	39	60	3%
Scenario 2	39	13	24	31	37	44	64	0%
Scenario 3	42	13	26	34	39	47	67	0%
1-year-olds, girls and boys (n=1957)								
Current	31	15	13	22	28	38	61	12%
Scenario 1	30	13	14	21	27	34	56	11%
Scenario 2	33	13	18	25	30	38	60	4%
Scenario 3	35	13	20	27	33	40	62	2%

Current fish intake, and fish intake in the scenarios are presented in Table 9.1-1.

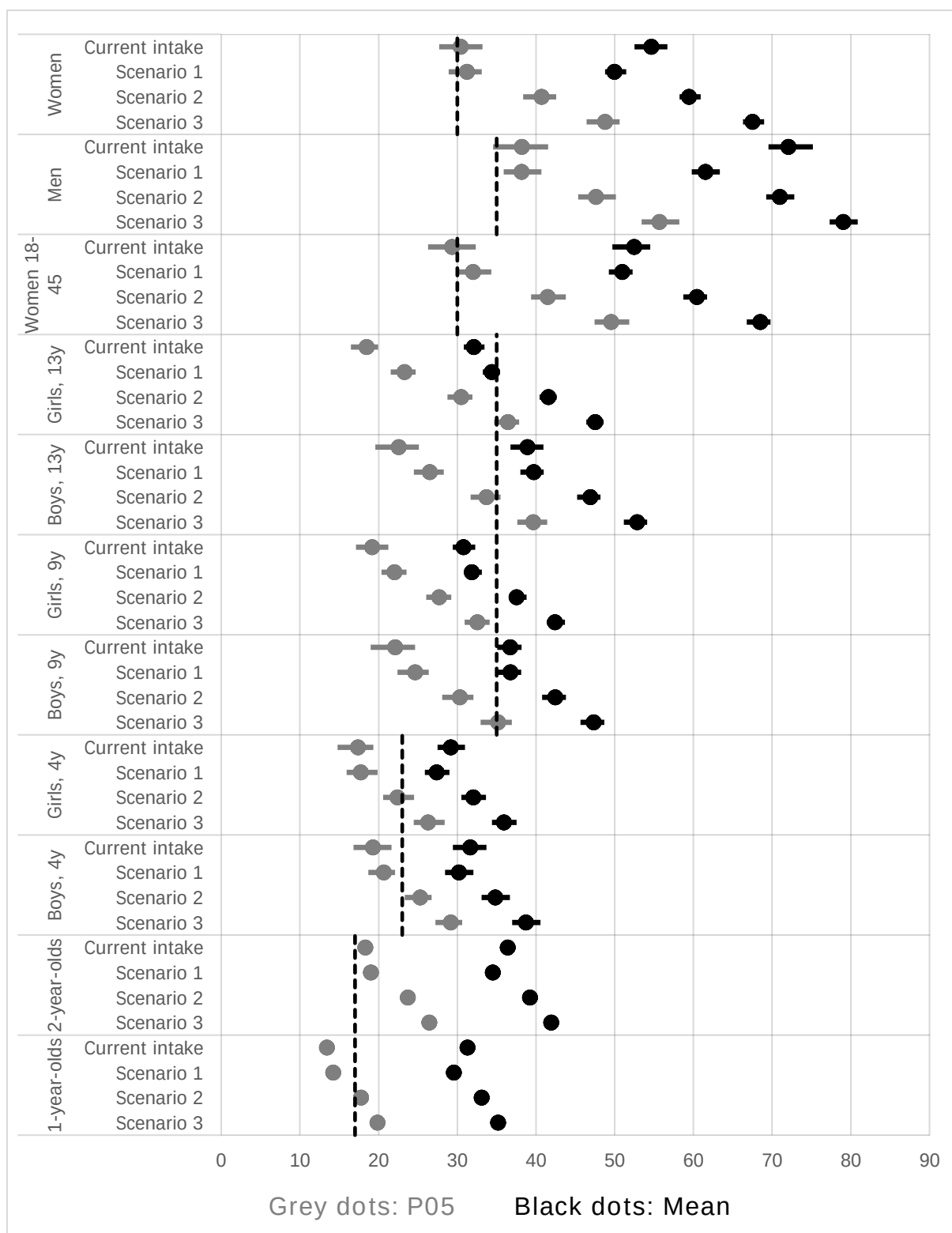


Figure 9.3.4-1 Distribution of the estimated habitual intake of selenium in the different age groups, at the current level of fish intake and in the three fish scenarios. Black dots show mean intakes, and grey dots show the 5th percentile, both in µg/day. The horizontal lines around the dots show confidence intervals (5% and 95%). The results are mixed model based, except for 1- and 2-year-olds, for whom weighted OIMs are reported, and, thus, no confidence intervals are available. The dashed vertical lines are ARs used in this assessment.

9.3.4.1 *Benefit characterisation at current intakes*

At current intake levels, women of childbearing age (18-45 years) have the lowest estimated selenium intakes among adults, and 6% have intakes below the AR at 30 µg/day. The mean (median) selenium intake is 52 (50) µg/day, and the estimated intake in the 5th percentile is 28 µg/day. The higher requirements in pregnancy and during lactation is not covered in these data, and the percentage of pregnant and lactating women with intakes below AR is higher. Nine-year-old girls have the lowest estimated selenium intake, and 73% of these girls have intakes below the AR at 35 µg/day. The mean (median) estimated selenium intake in 9-year-old girls is 31 (30) µg/day, and the estimated intake in the 5th percentile is 19 µg/day. Among the 13-year-old girls, 66% have intakes below the AR at 35 µg/day. It should be noted that for the AR for children and adolescents we have used ARs from IOM, who established generally higher ARs for selenium than did NNR (2012). The ARs for children ≥9 years above are therefore consequently equal or higher than for adults (see Table 2.2.7-1 in Chapter 2).

The AR from selenium is currently under debate, and there are studies indicating that the AR for a northern European population may be higher than the established AR from NNR (2012). It could be assumed that the proportions below AR may be underestimates rather than overestimates.

Groups at risk of low selenium intakes

Generally, protein-rich foods such as foods from animals are good selenium sources, so people with low intakes of such food may be at risk of low selenium intakes. Women of childbearing age and 13- and 9-year-olds are at highest risk of low selenium intakes. Overt selenium deficiency is rare (Alexander et al., 2020).

9.3.4.2 *Benefit characterisation at altered fish intake in the fish scenarios*

For scenario 2, the selenium intake among women in childbearing age was increased to on average mean (median) 60 (59) µg/day, and the estimated intake in the 5th percentile is increased to 41 µg/day. The proportion having an intake below the AR was reduced to null. For scenario 3, the selenium intake was increased to on average mean (median) 68 (67) µg/day, and 49 µg/day in the 5th percentile.

Among the 9-year-old girls, the intake of selenium in scenario 2 was increased to on average mean (median) 38 (37) µg/day, and the proportion having an intake below the AR was reduced to 38%. In scenario 3, 13% of the 9-year-old girls had an intake below AR. The same pattern of reduction in proportions below AR was observed for the other boys and girls in Ungkost 3.

In scenario 1, the mean selenium intakes are slightly decreased in most age groups except for in the 9-year-olds. The intakes in the 5th percentile changes slightly in both directions, and the proportion below AR decreases slightly or remains unchanged in most age groups except for in men and 4-year-old girls.

9.3.4.3 *Evidence for health benefits related to intake of selenium*

Keshan disease is the selected indicator to form the basis for reference values for selenium intake in both NNR (2012) and IOM (2000). The daily requirement for selenium is set according to a level in which the selenoproteins are optimally expressed, using the plasma concentration of selenoprotein P and glutathione peroxidase as indicators.

In Chapter 5.5, we evaluated health risk related to selenium intake. Based on previous work with dietary reference values and the health outcomes relevant for fish consumption, we have evaluated inclusion of associations between several health outcomes and selenium, but judged that it was not necessary to conduct updated systematic literature search and weight of evidence for associations between any specific health outcome and selenium.

9.3.4.4 *Summary of benefit characterisation of selenium*

No specific health outcomes associated with selenium have been evaluated in the literature searches for this benefit and risk assessment of fish consumption.

As shown above, young girls and women of childbearing age have the lowest selenium intakes, and at current intake 6% of women of childbearing age and 73% of 9-year-old girls have an intake below AR. The scenario estimations show that increasing fish intake to the lower range of recommended fish intake (scenario 2) would reduce the proportion below AR to null in women of childbearing age and to 38% in 9-year-olds. Increasing fish intake to the upper range of recommended fish intake (scenario 3) would reduce the proportion below AR to 13% among 9-year-olds.

It should be noted that the ARs for adult men and women are based on NNR (2012) conclusion, whereas the ARs for children and adolescents are based on conclusions from (IOM, 2000), which are generally higher than the ARs from NNR (2012).

Fish and fish products are the most important single source of selenium in the adult Norwegian population. The scenario estimations based on the national dietary surveys indicate that increasing intake of fish from the current intake to the recommended intake would reduce the proportion having a probability of inadequate selenium intake to null for most age groups and genders.

9.3.5 Vitamin B₁₂

The recommended daily vitamin B₁₂ intake for adults is 2 µg/day for adults, and AR is 1.4 µg/day (NNR, 2012). AR is higher during pregnancy and lactation, 2.2 µg/day and 2.4 µg/day, respectively (NNR, 2012). ARs for children and adolescents 1 to 18 years are in the range 0.7-2.0 µg/day (IOM, 2000).

The contributions to vitamin B₁₂ from various food groups in adults are fish and seafood 24%, food supplements 21%, meat 21%, dairy 26%, eggs 4%, and other food groups 4%, and the contributions to vitamin B₁₂ from various food groups in 13-year-olds are fish and

seafood 12%, food supplements 4%, meat 33%, dairy 42%, eggs 4%, and other food groups 5%.

In Tables 9.3.5-1-9.3.5-2 we have presented how vitamin B₁₂ intake is distributed in the different age groups both at current level and the three scenarios. The data includes intake from food supplements. This is also illustrated in Figure 9.1.5-1. For the age groups 18 to 70 years, we have used the AR for adults, but not included the higher ARs during pregnancy and lactation.

Table 9.3.5-1 Total vitamin B₁₂ intake (µg/day, including intake from food supplements) and proportion below the average requirement (AR) among adult women and men (Norkost 3) based on mixed model estimates of current (habitual) intake, and fish scenario 1, 2, and 3.

	Mean	SD	P05	P25	P50	P75	P95	<AR
Women, 18-70 years (n=925)								
Current	6.6	2.6	3.3	4.8	6.2	8.0	11.4	0%
Scenario 1	6.1	2.0	3.4	4.6	5.8	7.2	9.8	0%
Scenario 2	7.2	2.0	4.6	5.8	6.9	8.3	11.0	0%
Scenario 3	7.7	2.0	5.1	6.3	7.4	8.8	11.5	0%
Women, 18-45 years (n=466)								
Current	6.4	2.5	3.2	4.7	6.1	7.8	11.0	0%
Scenario 1	6.4	2.1	3.6	4.9	6.1	7.5	10.3	0%
Scenario 2	7.5	2.1	4.8	6.0	7.2	8.6	11.4	0%
Scenario 3	8.0	2.1	5.3	6.5	7.7	9.1	11.9	0%
Men, 18-70 years (n=862)								
Current	8.9	2.5	5.4	7.1	8.6	10.4	13.5	0%
Scenario 1	7.8	1.9	5.2	6.5	7.6	8.9	11.2	0%
Scenario 2	8.9	1.9	6.3	7.6	8.7	10.0	12.3	0%
Scenario 3	9.4	1.9	6.8	8.1	9.2	10.5	12.8	0%

Current fish intake, and fish intake in the scenarios are presented in Table 9.1-1.

Table 9.3.5-2 Total vitamin B₁₂ intake (µg/day, including intake from food supplements) and proportion below the average requirement (AR) among 4-, 9- and 13-year-old girls and boys (Ungkost 3) based on mixed model estimates of current (habitual) intake, and fish scenario 1, 2, and 3.

	Mean	SD	P05	P25	P50	P75	P95	<AR
Girls, 13-year-olds (n=355)								
Current	4.6	1.5	2.4	3.5	4.4	5.5	7.4	0%
Scenario 1	4.8	1.4	2.8	3.8	4.6	5.6	7.4	0%
Scenario 2	5.6	1.4	3.7	4.6	5.5	6.5	8.2	0%
Scenario 3	6.0	1.4	4.0	5.0	5.9	6.9	8.6	0%
Boys, 13-year-olds (n=332)								
Current	5.6	2.1	2.7	4.1	5.3	6.9	9.5	0%
Scenario 1	5.7	1.8	3.1	4.3	5.4	6.7	9.1	0%
Scenario 2	6.5	1.8	4.0	5.2	6.3	7.6	9.9	0%

	Me an	SD	P05	P25	P50	P75	P95	<AR
Scenario 3	6.9	1.8	4.4	5.6	6.7	8.0	10.3	0%
Girls, 9-year-olds (n=341)								
Current	4.1	1.4	2.1	3.1	4.0	5.0	6.7	0%
Scenario 1	4.2	1.2	2.5	3.4	4.1	5.0	6.4	0%
Scenario 2	4.9	1.2	3.2	4.0	4.8	5.6	7.1	0%
Scenario 3	5.2	1.2	3.5	4.4	5.1	5.9	7.4	0%
Boys, 9-year-olds (n=295)								
Current	4.9	1.6	2.6	3.7	4.7	5.8	7.7	0%
Scenario 1	4.8	1.4	2.9	3.9	4.7	5.7	7.3	0%
Scenario 2	5.5	1.4	3.5	4.6	5.4	6.4	8.0	0%
Scenario 3	5.8	1.4	3.9	4.9	5.7	6.7	8.3	0%
Girls, 4-year-olds (n=195)								
Current	4.1	1.1	2.5	3.3	4.1	4.8	6.1	0%
Scenario 1	3.9	1.0	2.4	3.1	3.8	4.5	5.7	0%
Scenario 2	4.5	1.0	3.0	3.8	4.4	5.1	6.3	0%
Scenario 3	4.8	1.0	3.3	4.0	4.7	5.4	6.6	0%
Boys, 4-year-olds (n=204)								
Current	4.4	1.3	2.5	3.4	4.3	5.3	6.8	0%
Scenario 1	4.1	1.0	2.6	3.4	4.1	4.8	6.0	0%
Scenario 2	4.8	1.0	3.3	4.0	4.7	5.4	6.6	0%
Scenario 3	5.0	1.0	3.5	4.3	4.9	5.7	6.8	0%

Current fish intake, and fish intake in the scenarios are presented in Table 9.1-1.

Table 9.3.5-3 Total vitamin B₁₂ intake (µg/day, including intake from food supplements) and proportion below the average requirement (AR) among 1- and 2-year-olds (Spedkost 3 and Småbarnskost 3) based on weighted observed individual mean (OIM) estimates of current (habitual) intake, and fish scenario 1, 2, and 3.

	Me an	SD	P05	P25	P50	P75	P95	<AR
2-year-olds, girls and boys (n=1413)								
Current	4.3	1.8	2.1	3.1	4.0	5.2	7.5	0%
Scenario 1	4.1	1.7	2.0	3.0	3.8	4.8	7.1	0%
Scenario 2	4.6	1.6	2.6	3.5	4.3	5.3	7.5	0%
Scenario 3	4.8	1.6	2.8	3.7	4.5	5.5	7.7	0%
1-year-olds, girls and boys (n=1957)								
Current	3.3	1.5	1.4	2.3	3.0	4.0	5.9	1%
Scenario 1	3.0	1.2	1.4	2.2	2.8	3.7	5.3	0%
Scenario 2	3.4	1.2	1.8	2.6	3.2	4.1	5.6	0%
Scenario 3	3.6	1.2	1.9	2.7	3.4	4.2	5.7	0%

Current fish intake, and fish intake in the scenarios are presented in Table 9.1-1.

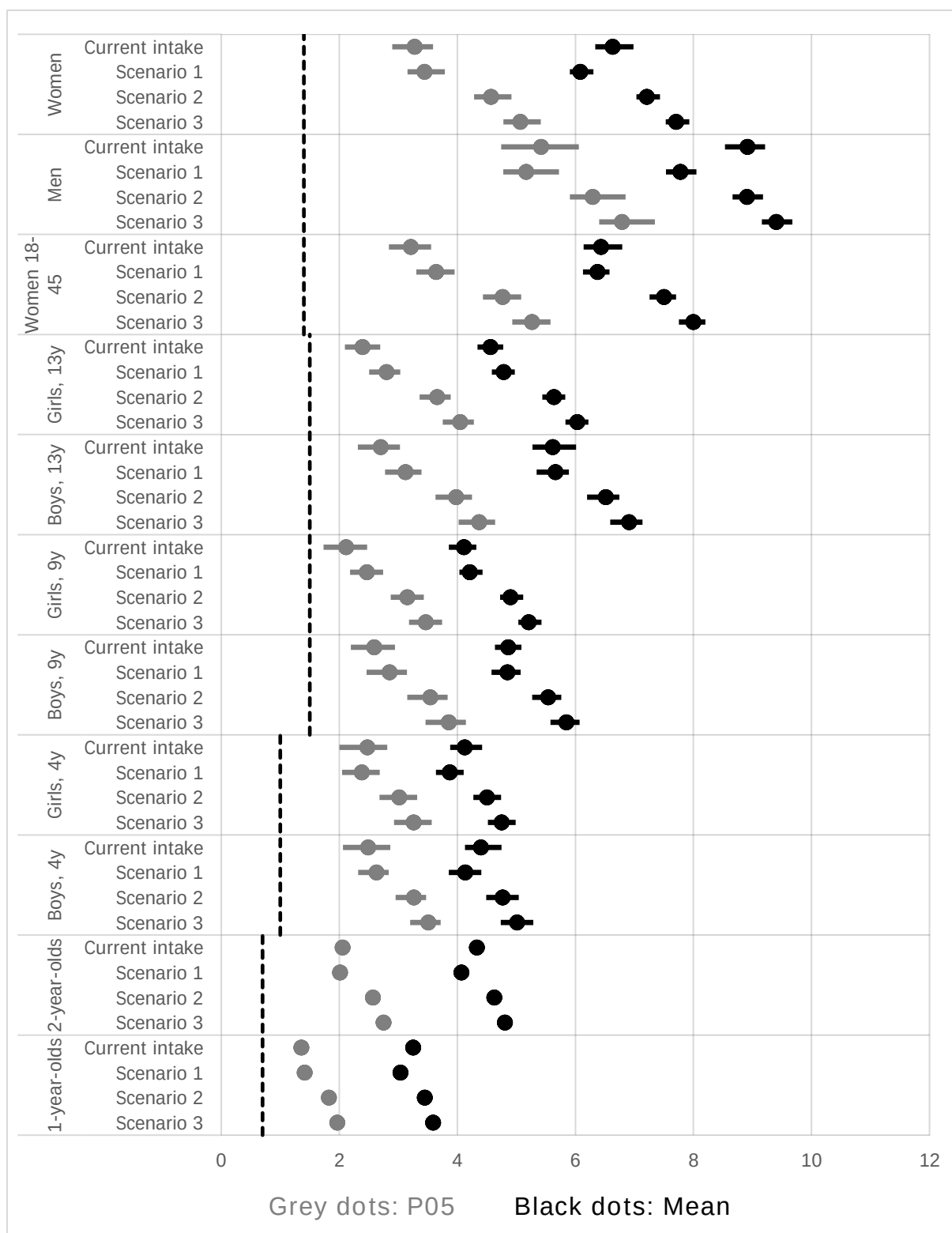


Figure 9.3.5-1 Distribution of the estimated habitual intake of vitamin B₁₂ in the different age groups, at the current level of fish intake and in the three fish scenarios. Black dots show mean intakes, and grey dots show the 5th percentile, both in µg/day. The horizontal lines around the dots show confidence intervals (5% and 95%). The results are mixed model based, except for 1- and 2-year-olds, for whom weighted OIMs are reported, and, thus, no confidence intervals are available. The dashed vertical lines are ARs used in this assessment.

9.3.5.1 *Benefit characterisation at current intakes*

At current intake levels, all age groups have estimated vitamin B₁₂ intakes above AR. The higher requirements in pregnancy and during lactation is not covered in these data.

Groups at risk of low vitamin B₁₂ intake

Intake below the average requirement is rare in the general population, but population groups who for various reasons do not include animal products in their diet are at risk of developing vitamin B₁₂-deficiency. Vegans and individuals that rarely consume products of animal origin e.g., eggs and dairy products, are accordingly at risk of developing deficiency. Vitamin B₁₂ deficiency causes macrocytic anaemia and may affect the brain and nervous system. Vitamin B₁₂ deficiency is seen in pernicious anaemia where the absorption of vitamin B₁₂ is impaired. Findings suggest that a substantial proportion of predominantly breastfed Norwegian infants have biochemical signs of suboptimal vitamin B₁₂ status/vitamin B₁₂ deficiency (Torsvik 2013, 2015, Bjørke Monsen 2008, Hay 2008). The clinical implications of these findings are under debate and uncertain.

9.3.5.2 *Benefit characterisation at altered fish intake in the fish scenarios*

All population groups have estimated vitamin B₁₂ intakes above AR in scenario 1, 2 and 3.

9.3.5.3 *Evidence for health benefits related to intake of vitamin B₁₂*

No single indicator forms the basis for reference values for vitamin B₁₂ intake in both NNR (2012) and IOM (2000). The daily requirement for vitamin B₁₂ is based on the amount of vitamin B₁₂ needed to maintain adequate haematological status in persons with pernicious anaemia.

In Chapter 5.6, we evaluated health risk related to vitamin B₁₂ intake. Based on previous work with dietary reference values and the health outcomes relevant for fish consumption, we have evaluated inclusion of associations between several health outcomes and vitamin B₁₂, but judged that it was not necessary to conduct updated systematic literature search and weight of evidence for associations between any specific health outcome and vitamin B₁₂.

9.3.5.4 *Summary of benefit characterisation of vitamin B₁₂*

No specific health outcome associated with vitamin B₁₂ has been evaluated in the literature searches for this benefit and risk assessment of fish consumption.

As shown above, with present fish consumption no specific age groups are at risk of having vitamin B₁₂ below the AR.

Fish and fish products are the most important source of vitamin B₁₂ in the adult Norwegian population. The estimated intakes for present fish consumption and the scenarios 2 and 3

indicate that no specific age groups are at risk of having vitamin B₁₂ below the AR. However, vegans and elderly might be at risk of developing vitamin B₁₂ deficiency known as pernicious anaemia and studies in breastfeed Norwegian infants show that a high proportion have signs of suboptimal vitamin B₁₂ status/B₁₂ deficiency, but the implication of these findings are uncertain.

9.4 Semi-quantitative risk characterisation of contaminants

This chapter presents semi-quantitative risk assessments of contaminants in fish (related to tolerable weekly intakes (TWI) described in Chapter 2 and contaminant intake estimates from Chapter 8).

The proportion of the population exceeding the TWIs and the degree of exceedance at mean and high (P95) exposure is considered. The contribution of fish to the exposure is also addressed, as well as the consequence of a change in fish consumption for exposure by help of scenarios of fish consumption (see scenarios Chapter 9.1).

A TWI should be interpreted as a safe upper level of intake, and when chronic intake of a contaminant is below a TWI there is no appreciable risk for adverse health effects. When exposure is above the TWI, the risk of adverse effects increases by increasing exceedance, but the increase in risk is not quantified.

9.4.1 PCDD/F and DL-PCB

As described in Chapter 2, VKM applies the TWI set by EFSA in 2018 at 2 pg TEQ/kg bw/week for the sum of PCDD/Fs and DL-PCBs for risk characterization.

The critical effect for PCDD/Fs and DL-PCBs is reduced sperm concentration in boys following pre- and postnatal exposure. In the critical study identified by EFSA (Minguez-Alarcon et al., 2017) there was a non-linear dose-response association for the sum of PCDD/Fs with a decrease in sperm concentration of about 40% already in the second quartile that did not decrease further. Similar effect size was observed in other studies. A 40% decrease in sperm concentration may affect fertility in men with an already reduced sperm production. However, although probability of a decrease in sperm concentration increase by higher exceedance of the TWI, the decrease in sperm concentration is not expected to decrease more than approximately 40%. EFSA (2018) stated that the available evidence indicates that there may be a postnatal period of sensitivity that might expand into puberty. The exposure for women from birth, during childhood and up to childbearing age (18-45) is therefore of particular interest, as well as exposure during childhood for boys, since the exposure in utero, via breastmilk and via food in childhood into puberty affect the blood concentration in young boys. Concentration levels in breastmilk are affected by intake up to childbearing age, as PCDD/Fs and DL-PCBs will accumulate in the body.

In the risk characterisation of PCDD/Fs and DL-PCBs, VKM applies a conservative approach using upper bound (UB) estimates for exposure. Further, VKM applies only exposure based on concentrations in food from Norway, combined with European data when concentration data are missing for Norwegian food (see Chapter 7, for a more thorough explanation). The use of Norwegian data is chosen because it is considered more likely to be representative in Norway as most of the fish, meat, dairy, and egg consumed is domestically produced. Lastly, contribution from fruit and vegetables to the total exposure is not included due to uncertainty in the available data (see Chapter 7 and Chapter 11).

Food is the main source of exposure to PCDD/Fs and DL-PCBs, anticipated to contribute with about 90% of the total exposure in Europeans (EFSA, 2018). Other sources include polluted air, soil and drinking water.

How the estimated exposure to the sum of the 29 congeners of PCDD/F and DL-PCBs is distributed in the different age groups, both at current level of fish intake, and in the three scenarios (described in Chapter 9.1.) is shown in Tables 9.4.1-1 to 9.4.1-3 below. This is also illustrated in Figure 9.4.1-1.

Table 9.4.1-1 Total PCDD/F and DL-PCB exposure (pg TEQ_{WHO2005}/kg bw/week, upper bound) and proportion exceeding the TWI in all adults and women in childbearing age (Norkost 3) presented as current (habitual) intake based on mixed model data, and with altered fish intake (scenario 1, 2, and 3).

	Mean	SD	P05	P25	P50	P75	P95	>TWI
18-70 years, women and men (n=1787)								
Current	4.6	1.7	2.3	3.4	4.4	5.6	7.9	98%
Scenario 1	3.9	1.0	2.6	3.2	3.8	4.5	5.7	100%
Scenario 2	5.3	1.1	3.8	4.6	5.2	6.0	7.2	100%
Scenario 3	5.6	1.1	4.0	4.8	5.5	6.3	7.5	100%
Women, 18-45 years (n=466)								
Current	4.4	1.6	2.3	3.2	4.2	5.3	7.5	97%
Scenario 1	4.1	1.0	2.8	3.5	4.0	4.7	5.9	100%
Scenario 2	5.7	1.1	4.2	5.0	5.6	6.4	7.6	100%
Scenario 3	6.1	1.1	4.5	5.3	6.9	6.7	7.9	100%

Table 9.4.1-2 PCDD/F and DL-PCB (29 congeners) exposure (pg TEQ_{WHO2005}/kg bw/week, upper bound) and proportion exceeding the TWI among all 13-, 9- and 4-year-olds (Ungkost 3) presented as current (habitual) intake based on mixed model data, and with altered fish intake (scenario 1, 2, and 3).

	Mean	SD	P05	P25	P50	P75	P95	>TWI
13-year-olds, girls and boys (n=687)								
Current	4.7	2.1	2.2	3.2	4.3	5.7	8.5	96%
Scenario 1	4.9	1.5	3.0	3.9	4.7	5.8	7.6	100%
Scenario 2	6.6	1.5	4.5	5.5	6.4	7.5	9.4	100%
Scenario 3	6.8	1.6	4.7	5.7	6.7	7.7	9.6	100%
9-year-olds, girls and boys (n=636)								
Current	6.6	2.3	3.5	5.0	6.3	7.9	11	100%
Scenario 1	6.8	1.7	4.3	5.6	6.7	7.9	9.9	100%
Scenario 2	8.8	1.8	6.2	7.6	8.7	10	12	100%
Scenario 3	9.2	1.8	6.4	7.9	9.0	10	12	100%

	Mean	SD	P05	P25	P50	P75	P95	>TWI
4-year-olds, girls and boys (n=399)								
Current	11	3.0	6.7	8.8	11	13	16	100%
Scenario 1	10	2.3	6.9	8.7	10	12	14	100%
Scenario 2	14	2.4	10	12	13	15	18	100%
Scenario 3	14	2.5	10	12	14	15	18	100%

Table 9.4.1-3 PCDD/F and DL-PCB (29 congeners) exposure (pg TEQ_{WHO2005}/kg bw/week, upper bound) and proportion exceeding the TWI among 1- and 2-year-olds (Spedkost 3 and Småbarnskost 3) presented as weighted OIM data for current (habitual) intake, and with altered fish intake (scenario 1, 2, and 3).

	Mean	SD	P05	P25	P50	P75	P95	>TWI
2-year-olds, girls and boys (n=1413)								
Current	12	6.2	5.4	8.3	11	15	22	100%
Scenario 1	12	4.0	6.7	8.9	11	14	18	100%
Scenario 2	16	4.3	10	13	15	18	23	100%
Scenario 3	16	4.3	11	13	15	18	23	100%
1-year-olds, girls and boys (n=1957)								
Current	12	7.5	4.3	7.8	11	15	24	100%
Scenario 1	11	3.8	5.7	8.2	10	13	18	100%
Scenario 2	15	4.0	9.4	12	14	17	22	100%
Scenario 3	15	4.0	9.7	12	15	17	23	100%

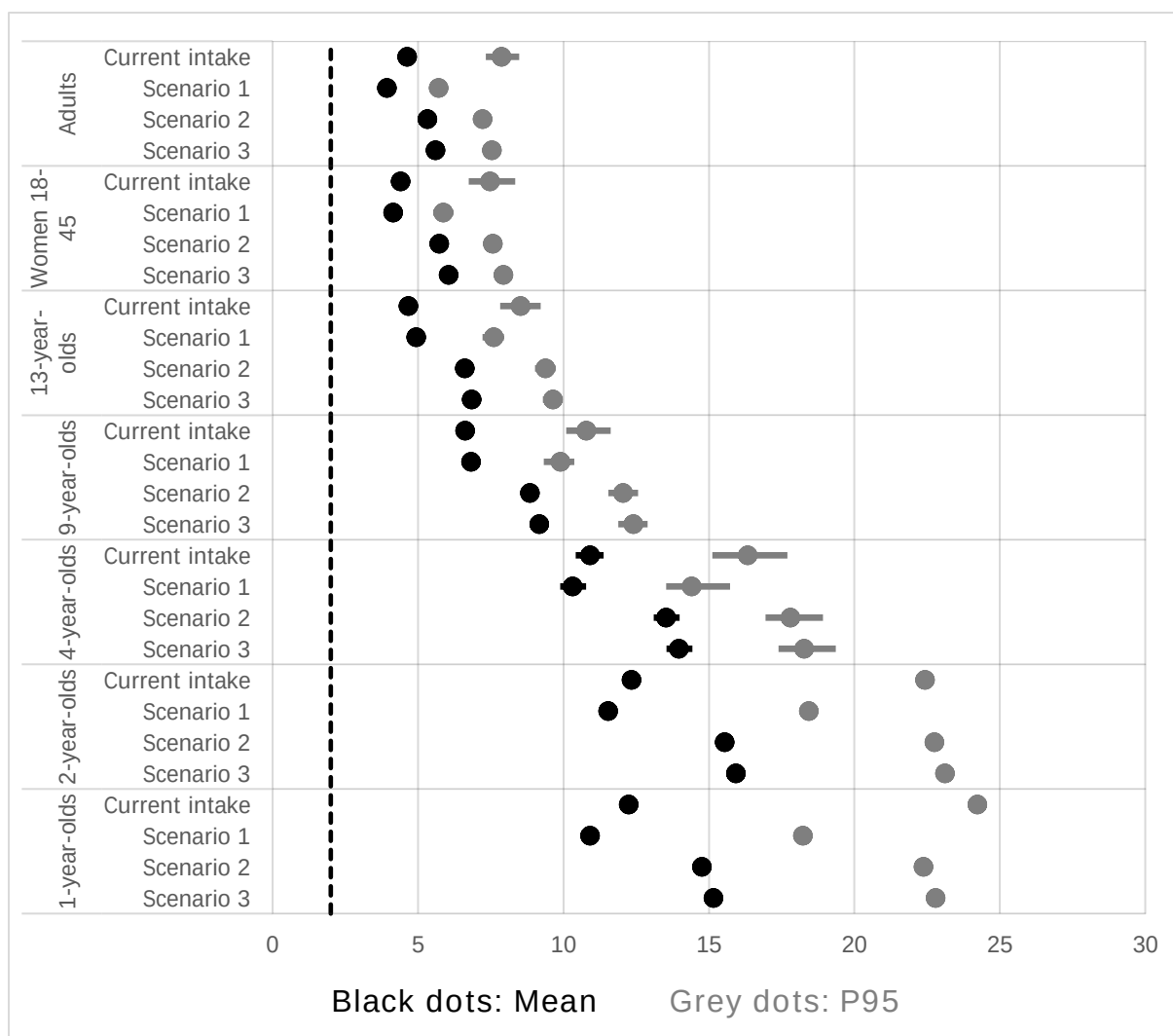


Figure 9.4.1-1 Distribution of the estimated exposure to the sum of the 29 congeners of PCDD/F and DL-PCBs in the different age groups, both at current level of fish intake and in the three scenarios. Black dots show mean exposure, grey dots show P95, both in pg total TEQ/kg bw/week. The horizontal lines around the dots show confidence intervals (5% and 95%). The results are mixed model based, except for 1- and 2-year-olds, for whom weighted OIMs are reported, and, thus, no confidence intervals are available. The dashed vertical line is the TWI used in this assessment (2 pg total TEQ/kg bw/week).

9.4.1.1 Risk characterisation at current intake

Fish intake contribute 39% to PCDD/F and DL-PCB exposure in the adult population, of which lean species (< 5% fat) contribute 6%, fatty species (> 5% fat) 28% and liver and roe 5% to the total PCDD/F and DL-PCB exposure. Exposure estimates presented in Chapter 8 indicate that other food groups also contribute considerably to the total exposure, in particular milk and dairy products (20%) and meat (12%) in adults.

Fish contributes 38% to the mean total intake for women in the age group 18 – 45 years (childbearing age), 21% for 13-year-olds, 21% for 9-year-olds, 28% for 4-year-olds, 30% for 2-year-olds and 33% for 1-year-olds.

The exposure estimates for the 29 congeners (total TEQ, UB) show that 98% of the adult population exceed the TWI. For adults, the mean exposure is 4.6 pg TEQ/kg bw/week, which is 2.6 pg TEQ/kg bw/week above the TWI, and the estimated exposure in the 95th percentile is 7.9 pg TEQ/kg bw/week.

As shown in Table 9.4.1-1, most (97%) of the women in the age group 18 – 45 years are estimated to exceed the TWI, and the mean exposure is 4.4 pg TEQ/kg bw/week. Moreover, the mean estimated intake in the 95th percentile is 7.5 pg TEQ/kg bw/week, which is about 3.7 times higher than the TWI.

The mean estimated exposures for children (1- 9 years) range from 6.6 to 12 pg TEQ/kg bw/week whereas the high (P95) exposure is in the range 10 – 24 pg TEQ /kg bw/week for these age groups. A two-fold higher intake in children up to age 9 years was taken into account by EFSA in the toxicokinetic modelling when setting the TWI (see Chapter 6 for the main principles of this toxicokinetic modelling). The mean exposure in Norwegian children is however higher than twice the TWI. In 13-year-olds, the mean intake is similar as in adults, and thus higher than the TWI.

Exceedance of the TWI is a health concern. Fish, and in particular fatty fish, is an important contributor to the dietary exposure, with 39% and 28% respectively for adults, but other food groups are also important sources (dairy 20%, meat 12%). The overall health risk from exposure to PCDD/Fs and DL-PCBs needs to be interpreted in the context of nutrient content in fish and the potential benefits from fish consumption (Chapter 10).

When considering the exposure to PCDD/Fs alone, the TWI is still exceeded, but the exceedance is lower. This is shown and discussed in Chapter 14, Appendix I.

Groups at risk of high of PCDD/F and DL-PCB exposure

At the current level of fish intake, all age groups are at risk of high PCDD/F and DL-PCB exposure. Since the critical effect is reduced sperm concentration in men after pre- and postnatal exposure, high exposure in women in childbearing age as well as in children and adolescents, both male and female, are of particular concern.

The concentration of PCDD/F and DL-PCB is particularly high in some specific food items like seagull eggs, crabs and fish liver. The Norwegian Food Safety Authority have issued special warnings for children and pregnant women not to eat these items, but for certain groups intake of these foods may cause particularly high exposure. Concentrations in fish may also vary with species and the geographical area where the fish was caught. Rarely consumed species are not captured in Norkost 3 and high consumers of such species might exceed the TWI.

9.4.1.2 Risk characterisation at altered fish intake in the fish scenarios

Tables 9.4.1-1 to 9.4.1-3 show the estimated PCDD/F and DL-PCB exposure in the scenarios of fish intake described in Chapter 9.1 in different age groups in relation to the intake at

current fish consumption. They also show the proportion of the population exceeding the TWI in the different scenarios.

For most age groups, scenario 1 represents a decrease in (mean) fish consumption. Since fish is an important contributor, the mean exposure to PCDD/F and DL-PCB is also decreased for scenario 1 compared to the current situation for most groups. The exception is 9- and 13-year-olds, for whom the mean exposure is slightly increased. The estimated exposure in the 95th percentile is however decreased in scenario 1 for all age groups. Thus, reducing the fish intake will reduce the exceedance of the TWI for those in the high end of exposure in all age groups. On the other hand, the proportion exceeding the TWI does not decrease in any of the scenarios for any age groups, and for some groups it increases, so for all age groups the proportion is 100% even if the fish intake is decreased.

In scenarios 2 and 3 where the fish intake is increased, the mean exposure is also increased for all age groups. The highest increase is from current to scenario 3 for 13-year-olds where the mean exposure increases from 2.4-fold the TWI to 3.4-fold. For women in childbearing age the increase entails an increase in exceedance from about two times the TWI at current to almost three times the TWI in scenario 2, and slightly above three times the TWI in scenario 3.

For the smallest children (1- and 2-year-olds) the highest increase is from six times the TWI to eight times the TWI. In the high end of the exposure (P95), the estimated change in exposure from current to scenario 2 and 3 is either a reduction (adults and 1-year-olds) or a lower increase than the described increase in estimated mean exposure. This is because at current intake some high consumers eat more than 450 grams fish per week (i.e. more than scenario 3).

9.4.1.3 Summary of risk characterisation of PCDD/F and DL-PCB

A high proportion of the Norwegian population exceed the TWI of 2 pg TEQ/kg bw/week. One- and 2-year-old children have the highest exposure. The mean and 95th percentile intakes are respectively 4.4 and 7.5 pg TEQ/kg bw/week for women in childbearing age, and the exceedance of the TWI is highest in children aged 1 to 4 years. The estimated exposures indicate a risk of adverse effects.

Reducing the fish intake will reduce the exceedance of the TWI for both the mean exposure and for those in the high end of exposure (P95). The proportion exceeding the TWI does, however, not decrease for any age group in any scenario, meaning that the proportion is 100% for all age groups, even if the fish intake is decreased. If the fish intake is increased, the mean exposure to PCDD/Fs and DL-PCBs is also increased for all age groups. The highest increase is from current to scenario 3 for 13-year-olds where the mean exposure increases from 2.4-fold the TWI to almost 3.4-fold.

9.4.2 PFASs

VKM applies the TWI for the sum of the four poly- and perfluoroalkyl substances (PFASs) PFOS, PFOA, PFNA and PFHxS of 4.4 ng/kg bw/week set by EFSA in 2020 (EFSA, 2020) in the risk characterisation in this opinion.

EFSA set the TWI based on PFAS concentration (sum of 4 PFASs) in 1-year-old children with reduction in vaccine response as critical effect. In the critical study used by EFSA (Abraham et al., 2020) the decrease in antibody titres after vaccination was up to 63% (for Hib-antibodies) in the highest quintile compared to the lowest quintile and there was no indication in the dose-response relationships that the effects on vaccination responses level off at higher exposure levels. A reduction in vaccination response is a marker of impaired immune response, which is adverse. There are however according to EFSA (2020) *"some data suggesting that PFAS exposure is associated with increased infection risk"*. The potential increase in infection risk has not been quantified. The 4 PFASs accumulate in the body and are transferred from mother to child over the placenta and via breast milk. The TWI was therefore set so that exposure below the TWI prevents that maternal serum concentrations lead to concentrations in breastmilk that results in exceedance of the critical serum concentration of PFASs in breastfed children (see Chapter 6.1.2).

The TWI is set to be protective also against potential adverse effects other than impaired vaccine responses observed in humans (see Chapter 6.1.2), and for all population groups.

Food and beverages are the main source of exposure for these four PFASs in the European population (EFSA, 2020). House dust and cosmetics (including toothpaste and mouthwash) are also important sources to a variable degree. House dust is of specific relevance in small children who play on the floor.

There is high uncertainty in the concentration data for PFASs in food, with huge differences between LB and UB, and a large proportion of undetected samples (described in Chapters 7 (Occurrence) and 8 (Exposure) and 11 (Uncertainty)). EFSA reported that lower bound (LB) exposure gave a better prediction than the upper bound (UB) estimates when comparing biomonitoring data with estimated dietary intakes (EFSA, 2020). Therefore, the intake estimates based on lower bound (LB) concentrations are used for risk characterization.

For all age groups, fish is the main contributor to sum of these four PFASs (about 38% for adults). Lean and fatty fish contribute approximately equally across age groups, with a little higher contribution from lean fish in adults. In addition to fish, fruit and vegetables contributes about 18%, and meat about 14%, to the sum of the four PFASs for adults (see Figures 8.4.2-2 and 8.4.2-3, Chapter 8).

PFOS constitutes 64% of the mean dietary PFAS exposure in adults and is the main contributor, followed by PFOA (22%). PFNA and PFHxS contribute less (10% PFHxS, 4% PFNA in adults), see Figure 8.4.2-1, Chapter 8. Fish is an important contributor to PFOS, PFOA and PFNA, but is not a source of PFHxS.

Table 9.4.2-1 PFAS exposure (sum of 4 PFASs, ng/kg bw/week, lower bound) and proportion exceeding the TWI in all adults and women in childbearing age (Norkost 3) presented as current (habitual) intake based on mixed model data, and with altered fish intake (scenario 1, 2, and 3). Data is presented with intake based on concentrations from the EFSA database only.

	Mean	SD	P05	P25	P50	P75	P95	>TWI
18-70 years, women and men (n=1787)								
Current	7.4	3.1	3.4	5.2	6.9	9.0	13	86%
Scenario 1	5.9	1.7	3.5	4.7	5.7	6.3	8.9	81%
Scenario 2	7.1	1.7	4.6	5.8	6.9	8.1	10	96%
Scenario 3	8.4	1.8	5.7	7.1	8.2	9.5	12	100%
Women, 18-45 years (n=466)								
Current	6.5	2.4	3.3	4.7	6.1	7.8	11	80%
Scenario 1	6.0	1.4	4.0	5.0	5.8	6.8	8.6	89%
Scenario 2	7.4	1.5	5.3	6.3	7.2	8.2	10	99%
Scenario 3	8.9	1.6	6.6	7.8	8.8	9.8	12	100%

Table 9.4.2-2 PFAS exposure (sum of 4 PFASs, ng/kg bw/week, lower bound) and proportion exceeding the TWI in 13-, 9- and 4-year-olds (Ungkost 3) presented as current (habitual) intake based on mixed model data and with altered fish intake (scenario 1, 2, and 3). Data is presented with intake based on concentrations from the EFSA database only.

	Mean	SD	P05	P25	P50	P75	P95	>TWI
13-year-olds, girls and boys (n=687)								
Current	4.5	2.3	1.8	3.0	4.1	5.6	8.8	44%
Scenario 1	4.8	1.4	3.0	3.9	4.6	5.5	7.3	57%
Scenario 2	6.4	1.5	4.4	5.4	6.3	7.3	9.1	95%
Scenario 3	8.2	1.6	5.9	7.1	8.0	9.1	11	100%
9-year-olds, girls and boys (n=636)								
Current	6.5	2.6	3.1	4.7	6.1	7.8	11	79%
Scenario 1	6.4	1.6	4.1	5.3	6.2	7.4	9.4	91%
Scenario 2	8.4	1.8	5.8	7.1	8.2	9.4	11	100%
Scenario 3	10	1.9	7.5	9.0	10	12	14	100%
4-year-olds, girls and boys (n=399)								
Current	14	5.6	7.0	10	13	17	25	100%
Scenario 1	11	3.2	7.2	9.2	11	13	17	100%
Scenario 2	14	3.2	10	12	14	16	20	100%
Scenario 3	17	3.4	13	15	17	19	24	100%

Table 9.4.2-3 PFAS exposure (sum of 4 PFASs, ng/kg bw/week, lower bound) and proportion exceeding the TWI in 1- and 2-year-olds (Spedkost 3 and Småbarnskost 3) presented as OIM weighted data for current (habitual) intake, and with altered fish intake (scenario 1, 2, and 3). Data is presented with intake based on concentrations from the EFSA database only.

	Mean	SD	P05	P25	P50	P75	P95	>TWI
2-year-olds, girls and boys (n=1413)								
Current	18	8.9	7.0	12	17	22	34	99%
Scenario 1	14	5.6	7.6	11	13	17	25	100%
Scenario 2	18	5.7	11	14	17	20	28	100%
Scenario 3	21	5.9	14	17	20	23	31	100%
1-year-olds, girls and boys (n=1957)								
Current	16	9.8	4.8	9.4	14	21	35	96%
Scenario 1	13	6.2	6.0	8.7	12	16	25	99%
Scenario 2	16	6.3	9.2	12	15	19	29	100%
Scenario 3	19	6.4	12	15	18	22	32	100%

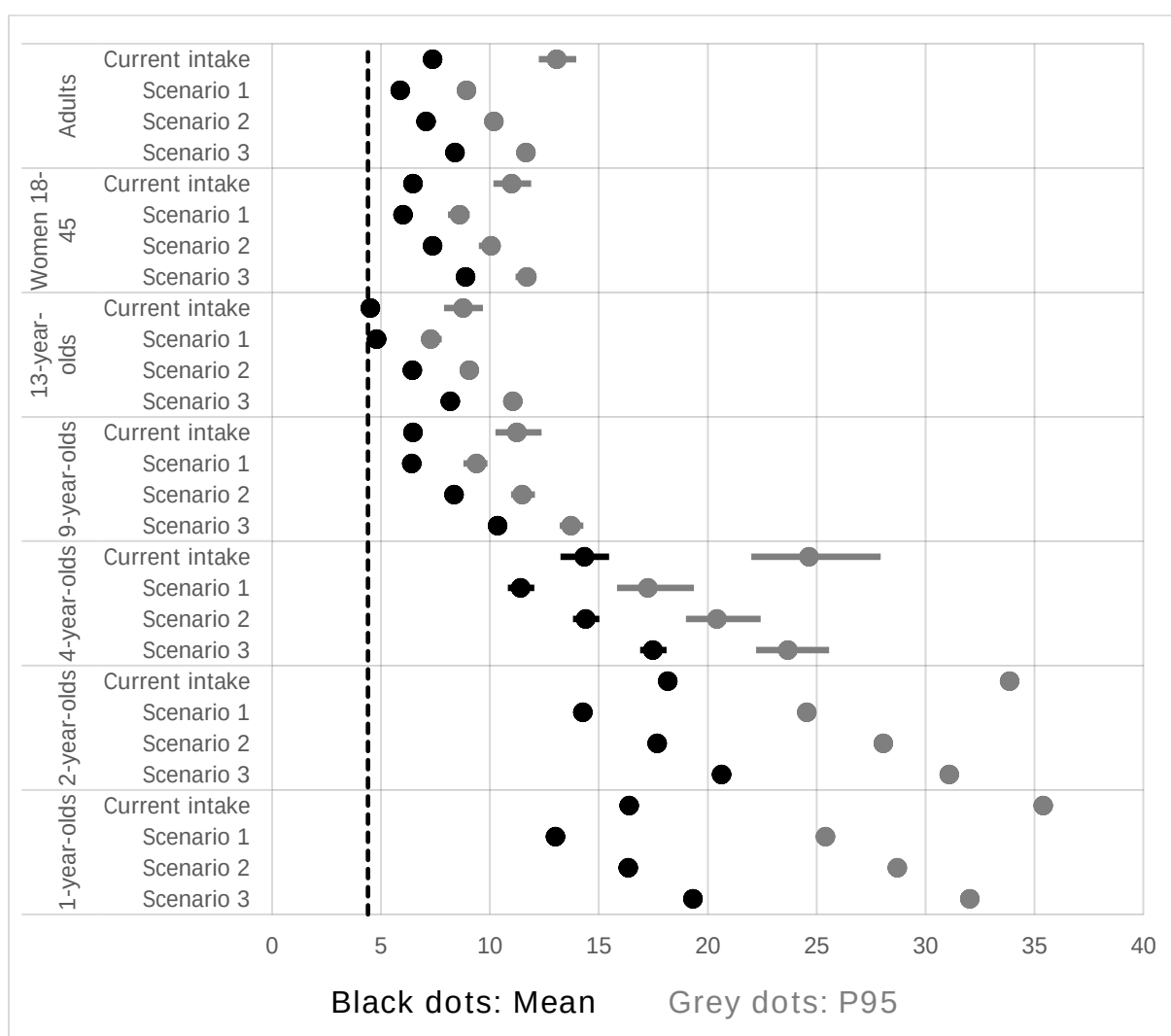


Figure 9.4.2-1 Distribution of the estimated exposure to the sum of 4 PFAS in the different age groups, both at current level of fish intake and in the three scenarios. Black dots show mean exposure, grey dots show P95, both in ng/kg bw/week. The horizontal lines around the dots show confidence intervals (5% and 95%). The results are mixed model based, except for 1- and 2-year-olds, for whom weighted OIMs are reported, and, thus, no confidence intervals are available. The

dashed vertical line is the TWI used in this assessment (4.4 ng/kg bw/week). Estimated exposure is presented with intake based on concentrations from the EFSA database.

9.4.2.1 Risk characterisation at current intake

Tables 9.4.2-1 to 9.4.2-3 show the current estimated PFAS exposure in the different age groups and the proportion of the population that has estimated intake exceeding the TWI for PFAS of 4.4 ng/kg bw/week (EFSA 2020). This is also illustrated in Figure 9.4.2-1. These estimates show that for adults the mean intake is 7.4 ng/kg bw/week, which is 1.7 times the TWI, and 86% of Norwegian adults exceed the TWI. The high (P95) exposure in adults is 13 ng/kg bw/week which is up to 3-fold the TWI. The exposure to PFASs varies considerably between age groups, with an estimated mean and 95th percentile intake of 18 and 34 ng/kg bw/week, respectively, in 1-year-olds. In the age group children ≤ 4 years, more than 95% exceed the TWI. The exposure level for 9- and 13-year-olds are in the same range as adults, while 4-year-olds have an estimated mean exposure about twice that of adults. The generally higher level of exposure in children is partly due to the higher intake of food per kg bw. In addition, the food group fruit and vegetables contribute more to the total PFAS exposure in children than in adults.

These exposure estimates, together with available biomonitoring data (see Chapter 6.1.2.1) indicate that parts of the population exceed the TWI and are at risk of having reduced immune response due to PFAS intake. Fish is one of several contributors to PFAS exposure, but other food groups are also important sources. The overall health risk from exposure to PFAS from fish needs to be interpreted in the context of nutrient content in fish and the benefit from fish consumption (Chapter 10).

Groups at risk of high PFAS exposure

PFASs are present in all food groups, and all contribute to the total exposure. Consumers of food and drinking water from areas with particularly high levels of contamination are at risk of high exposures. PFAS can accumulate in fish (especially PFOS) and the Norwegian Food Safety Authority has issued warnings on consumption of fish from specific areas due to PFAS pollution (www.matportalen.no).

9.4.2.2 Risk characterisation at altered fish intake in the fish scenarios

Table 9.4.2-1 to 9.4.2-3 show the estimated PFAS exposure in the scenarios with altered fish intake described in Chapter 9.1 for different age groups in relation to the intake at current fish intake. The tables also show the estimated proportion of the population exceeding the TWI in the different scenarios.

For the adult population, the mean PFAS exposure is decreased in both scenario 1 and 2, and the estimated exposure in the 95th percentile is decreased in all three scenarios. The proportion exceeding the TWI is decreased in scenario 1 but increased in scenario 2 and 3. The slight decrease in PFAS exposure (from 7.4 to 7.1 ng/kg bw/week) despite increased fish intake may be explained by the high proportion of fatty fish in the scenarios, given the

somewhat lower contribution of fatty fish to the total PFAS exposure, in addition to the lower fish intake among high consumers in the scenarios compared to high consumers at current fish intake.

In scenario 3, the proportion of the adult population exceeding the TWI increase from 86% (current) to 100%, while the mean exposure increases from 7.4 ng/kg bw/week (current) to 8.4 ng/kg bw/week. Thus, even though both the mean estimated exposure and the proportion exceeding the TWI increase, the difference between current intake and the highest scenario for adults is small, especially taking into consideration that the scenarios is calculated without substitution.

For 13-year-olds, the estimated mean exposure (4.5 ng/kg bw/week) and the proportion exceeding the TWI (44%) at current level is somewhat lower, but in the two scenarios with increased fish intake the levels of exposure is similar to the levels for adults, and hence for this age group an increased fish intake will increase the risk of adverse effects.

Also for 9-year-olds, the mean exposure and the proportion exceeding TWI increases with increased fish intake. The higher increase in estimated mean exposure for 9- and 13-year-olds than in the other age groups can be explained by the relatively low fish intake for these age groups at the current level.

For the younger children where the mean exposure and the proportion exceeding TWI is already high at current fish consumption levels, the altered fish intake in the scenarios affects the exposure estimates less.

9.4.2.3 Summary of risk characterisation PFAS

Exposure estimates for PFAS are uncertain. Biomonitoring data indicate that LB dietary exposure estimates are relatively reasonable but are still likely to underestimate true exposure.

Based on these estimates the adult population have a mean PFAS exposure that is 1.7-times the TWI and at high exposure (P95) up to three times the TWI at current fish intake.

The estimates show that decreasing or increasing the fish intake as described for scenarios 1 and 2 will probably lead to relatively small changes in exposures to PFAS for the adult population. Increasing the fish intake up to scenario 3 will cause an increase in the proportion exceeding the TWI, leading to an exceedance for all adults, but the increase in exceedance will be low.

Children have a high estimated exposures both in the current situation and in the calculated scenarios. This may indicate risk of adverse effects on the immune system for these children and for the next generation.

9.4.3 Methyl mercury

EFSA has established a TWI for methylmercury of 1.3 µg/kg bw per week (expressed as mercury) based on human neurodevelopmental outcomes after prenatal exposure (EFSA, 2012).

VKM applies a conservative approach to exposure assessment of methyl mercury by assuming that all mercury found in fish and other seafood is methyl mercury (see Chapter 7 and 8).

The foetal brain is the most sensitive organ to methyl mercury exposure. Methyl mercury can cross both the placenta and the blood-brain barrier, and high prenatal exposure is of concern. As described in Chapter 6.1.3, unborn children constitute the most vulnerable group for developmental effects of methyl mercury exposure. The maternal intake in the last six months of pregnancy is of highest relevance. Women of childbearing age is therefore of particular interest.

People can be exposed to mercury through air, food, drink, and amalgam-treated teeth. Food is however the most important source. Fish was estimated to contribute with 89% of the exposure from the total diet, the remaining 11% is from other seafood. Lean fish contributed 64% and fatty fish 25% to the total intake (in adults).

Table 9.4.3-1 Methyl mercury exposure (µg/kg bw/week) and proportion exceeding the TWI in all adults and women of childbearing age (Norkost 3) presented as OIM estimates from current dietary intake, and with altered fish intake (scenario 1, 2, and 3)

	Mean	SD	P05	P25	P50	P75	P95	>TWI
18-70 years, women and men (n=1787)								
Current	0.3	0.5	0	0	0.1	0.4	1.2	4%
Scenario 1	0.2	0.2	0.1	0.1	0.1	0.2	0.3	0%
Scenario 2	0.3	0.2	0.2	0.2	0.2	0.3	0.4	0%
Scenario 3	0.4	0.2	0.3	0.3	0.4	0.5	0.6	0%
Women, 18-45 years (n=466)								
Current	0.2	0.4	0	0	0	0.3	0.9	2%
Scenario 1	0.2	0.2	0.1	0.1	0.1	0.2	0.3	0%
Scenario 2	0.3	0.2	0.2	0.2	0.3	0.3	0.4	0%
Scenario 3	0.5	0.2	0.3	0.4	0.5	0.5	0.6	0%

Table 9.4.3-2 Methyl mercury exposure ($\mu\text{g/kg bw/week}$) and proportion exceeding the TWI in 13-, 9- and 4-year-olds (Ungkost 3) presented as OIM estimates from current dietary intake, and with altered fish intake (scenario 1, 2, and 3).

	Mean	SD	P05	P25	P50	P75	P95	>TWI
13-year-olds, girls and boys (n=687)								
Current	0.1	0.2	0	0	0	0.2	0.6	0%
Scenario 1	0.2	0.1	0.1	0.1	0.2	0.2	0.2	0%
Scenario 2	0.3	0.1	0.2	0.2	0.3	0.3	0.4	0%
Scenario 3	0.5	0.1	0.4	0.5	0.5	0.6	0.7	0%
9-year-olds, girls and boys (n=636)								
Current	0.2	0.3	0	0	0.1	0.3	0.8	1%
Scenario 1	0.2	0.1	0.1	0.2	0.2	0.2	0.2	0%
Scenario 2	0.3	0.1	0.2	0.3	0.3	0.4	0.4	0%
Scenario 3	0.6	0.1	0.4	0.6	0.6	0.7	0.8	0%
4-year-olds, girls and boys (n=399)								
Current	0.4	0.6	0	0.1	0.3	0.6	1.3	5%
Scenario 1	0.3	0.1	0.2	0.3	0.3	0.3	0.4	0%
Scenario 2	0.5	0.1	0.4	0.5	0.5	0.5	0.6	0%
Scenario 3	0.9	0.1	0.7	0.8	0.9	1.0	1.1	1%

Table 9.4.3-3 Methyl mercury exposure ($\mu\text{g/kg bw/week}$) and proportion exceeding the TWI in 2- and 1-year-olds (Småbarnskost 3 and Spedkost 3) (Ungkost 3) presented as weighted OIM estimates from current dietary intake, and with altered fish intake (scenario 1, 2, and 3).

	Mean	SD	P05	P25	P50	P75	P95	>TWI
2-year-olds, girls and boys (n=1413)								
Current	0.6	0.4	0.1	0.3	0.5	0.7	1.2	4%
Scenario 1	0.3	0.0	0.3	0.3	0.3	0.4	0.4	0%
Scenario 2	0.6	0.1	0.5	0.6	0.6	0.7	0.8	0%
Scenario 3	1.0	0.1	0.8	0.9	1.0	1.1	1.2	1%
1-year-olds, girls and boys (n=1957)								
Current	0.6	0.5	0.1	0.2	0.5	0.8	1.4	7%
Scenario 1	0.3	0.0	0.3	0.3	0.3	0.4	0.4	0%
Scenario 2	0.6	0.1	0.5	0.6	0.6	0.7	0.7	0%
Scenario 3	1.0	0.1	0.8	0.9	1.0	1.1	1.2	1%

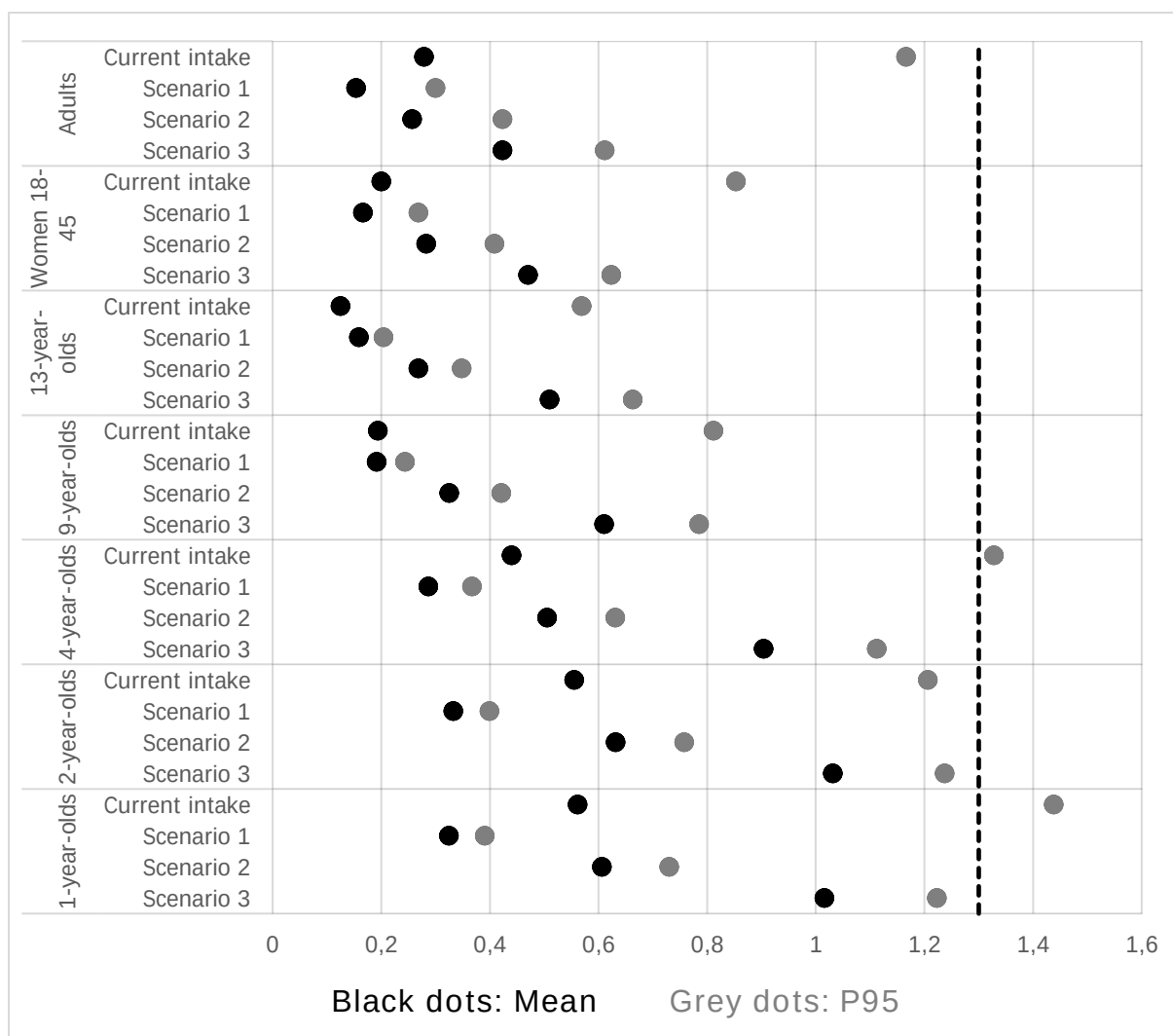


Figure 9.4.3-1 Distribution of the estimated exposure to methyl mercury in the different age groups, both at current level of fish intake and in the three scenarios. Black dots show mean exposure, grey dots show P95, both in $\mu\text{g/kg bw/week}$. The dashed vertical line is the TWI used in this assessment ($1.3 \mu\text{g/kg bw/week}$).

9.4.3.1 Risk characterisation at current intake

Tables 9.4.3-1 to 9.4.3-3 show the current methyl mercury intake in the different age groups and the proportion of the population estimated to exceed the TWI for methyl mercury of $1.3 \mu\text{g/kg bw/week}$ (EFSA, 2012). These estimates show that a low proportion of the Norwegian population exceed the TWI, with relatively small differences between age groups. The highest exposure is in 1- and 2-year-olds. For 1-year-olds 7% exceed the TWI. Also, at the high (P95) exposure the exceedance is low, again with the highest estimated exposure for 1-year-olds, and only 1-year-old and 4-year-olds exceed the TWI.

Women in childbearing age is of particular interests, and 2% in this group have estimated intake exceeding the TWI with the current fish intake. It should be noted that the dietary method used in Norkost 3 is likely to overestimate the OIM at higher intakes (P95) of foods that are not eaten on a daily basis, as is the case for fish and other seafood (see Chapter 8).

The assumption that all mercury in fish and other seafood is considered to be methyl mercury represents an overestimate. A low intake of methyl mercury in pregnant women, both in terms of the low proportion exceeding TWI and magnitude of exposure, is in accordance with biomonitoring data in pregnant women in Norway (See Chapter 6, Caspersen et al 2019).

Overall, the risk from dietary methyl mercury exposure at current intake is considered low.

Groups at risk of high of methyl mercury exposure

The concentration of methyl mercury in fish vary with species, size and the geographical area where the fish was caught. Certain fish species (large predatory fish such as e.g., large fresh tuna, skates, large brown trout) contain higher concentration of mercury than commonly consumed species. Rarely consumed species are not captured in Norkost 3 and high consumers of such species might exceed the TWI.

The total exposure estimate (Chapter 7) was based on mean concentration in food and did not take into consideration that fish caught at places with local pollution, might contain higher concentrations of mercury. Consumers of fish caught at places with local pollution might be at risk of exceeding the TWI.

The Norwegian Food Safety Authority has issued warnings to the general population and specifically to pregnant women against consumption of species containing high mercury concentrations or fish from particular fjords and harbours (www.matportalen.no).

9.4.3.2 Risk characterisation at altered fish intake in the fish scenarios

Tables 9.4.3-1 to 9.4.3-3 show the estimated methyl mercury exposure in the scenarios of fish intake described in Chapter 9.1 for different age groups in relation to the intake at current fish consumption and shows the proportion of the population exceeding the TWI in the different scenarios.

For most age groups scenario 1 represents a decrease in mean fish consumption, and since fish is the dominating contributor to methyl mercury, this leads to a decrease in methyl mercury exposure relative to the present dietary intake estimate for these age groups. For 13-year-olds, however, a small increase in the mean methyl mercury exposure is estimated for scenario 1, and for 9-year-olds there is no change. The exposure is still below the TWI also for these age groups. For scenario 1, the proportion exceeding the TWI is zero for all age groups.

Fish intake in scenarios 2 and 3 represents an increase in total fish intake relative to the current intake in all age groups, and the exposure to methyl mercury also increase in both scenarios for all age groups, except for adults in scenario 2. Still, the population proportion exceeding the TWI decrease relative to the current fish consumption in all age groups.

For scenario 2, this can be explained partly by the high proportion of fatty fish in the scenarios compared to the composition of the current mean fish consumption, causing the

intake of lean fish to decrease in some age groups. Since fatty fish have lower concentrations of methyl mercury than lean fish (see Chapter 7), this also causes the methyl mercury exposure to decrease. For scenario 3, the intake of lean fish is increased in all age groups, but because all consumers in the scenario in each age group have been attributed the same fish intake, the intake of lean fish in high consumers (P95) decrease, and the proportion exceeding the TWI for methyl mercury is reduced. As for scenario 1, the proportion exceeding the TWI is zero for all age groups in scenario 2. In scenario 3, 1% of 4- , 2- and 1-year-olds exceed the TWI.

9.4.3.3 Summary of risk characterisation of methyl mercury

With the current fish intake in Norway, only a small proportion of the population exceeds the TWI for methyl mercury when applying a conservative approach assuming that all mercury in fish and shellfish is methyl mercury.

With altered fish intake in the scenarios (both decreased and increased), the estimated mercury intake decreases in general. This is because the scenarios are based on the most commonly consumed species that are low in mercury concentration. Furthermore, the high fish scenario (scenario 3) represents a decrease in fish consumption for high fish consumers. In summary, the proportion exceeding the TWI is either zero or very low for all age groups in all three scenarios.

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10 Benefit and risk comparison

The terms of reference for the present benefit and risk assessment of fish intake are to evaluate the potential health consequences for the Norwegian population if they:

- Continue with the same fish intake levels as of today,
- Increase the consumption of fish to match the recommendations given by the Norwegian Directorate of Health.

The present Norwegian food based dietary guidelines recommend eating fish 2-3 times per week for all age groups. This amounts to 300-450 g prepared fish per week for adults, of which at least 200 g should be fatty fish (Norwegian Directorate of Health, 2014). There is no corresponding recommendation for amounts of fish intake in children and adolescents. However, in the present benefit and risk assessment, recommendation of eating fish 2-3 times per week was recalculated into gram per day for children and adolescents, based on the fish recommendation for adults and the energy requirement for children and adolescents (see Table 2.1-1).

This benefit and risk assessment is based on:

- a systematic literature review of health outcomes associated with fish intake,
- a quantitative modelling with incidence and mortality as common metrics to estimate how changes in fish intake from current intake to three constructed scenarios may change disease incidence and mortality,
- a review of health outcomes associated with intake of the nutrients: LC n-3 FA, vitamin D, iodine, selenium, and vitamin B₁₂, where intake of fish is an important contributor,
- semi-quantitative evaluations of the estimated intakes of LC n-3 FA, vitamin D, iodine, selenium, and vitamin B₁₂ in different population groups in Norway at current fish intake and different fish intake scenarios, and comparison to established average requirements (AR),
- critical endpoints for tolerable weekly intakes (TWI) described in EFSA opinions of the contaminants PCDD/Fs and DL-PCBs, PFASs and methyl mercury, where intake of fish is an important contributor, and
- semi-quantitative evaluations of the estimated intakes of the contaminants PCDD/Fs and DL-PCBs, PFASs and methyl mercury in different population groups in Norway at current fish intake and different fish intake scenarios compared to the established TWIs.

This chapter aims to assess all previous chapters and sections together in a systematic way, to address and discuss benefits and risks from fish consumption. This will lead up to the conclusions and answers to the terms of reference.

Benefit is understood as reduced probability of disease or death related to fish intake or intake of components in fish such as nutrients, while *risk* is understood as increased probability of adverse health effects related to fish intake or intake of associated components in fish such as contaminants.

In our systematic literature review of health outcomes associated with fish intake, we have included health outcomes in the following categories: cardiovascular diseases (CVD) and mortality, neurodevelopmental outcomes in children and adults, birth outcomes, overweight and obesity, type 2 diabetes, bone health, rheumatoid arthritis, multiple sclerosis, vaccine response, immunological diseases, and semen quality.

For the benefit assessment, we have included nutrients where fish consumption contributes to at least 20% of the average total dietary intake of the specific nutrients (not including contribution from food supplements). In the Norwegian population, this applies to LC n-3 FAs, vitamin D, iodine, selenium, and vitamin B₁₂. For the risk assessment, we have included contaminants for which fish intake is an important contributor to exposure, and the exposure may be close to (or above) a health-based guidance value (HBGV). In the Norwegian population this applies to PCDD/Fs and dl-PCBs, PFASs and methyl mercury.

The health benefits and risks related to fish consumption and intake of the nutrients and contaminants in fish cover all life stages and both sexes. We discuss population groups that will benefit from fish consumption and population groups at risk of adverse health effects. We additionally identify groups vulnerable to low intake of nutrients and to high intakes of contaminants relevant for fish consumption.

Health effects related to fish intake may be associated with the fish directly or mediated through compounds such as nutrients and contaminants in fish (Fig. 10-1).

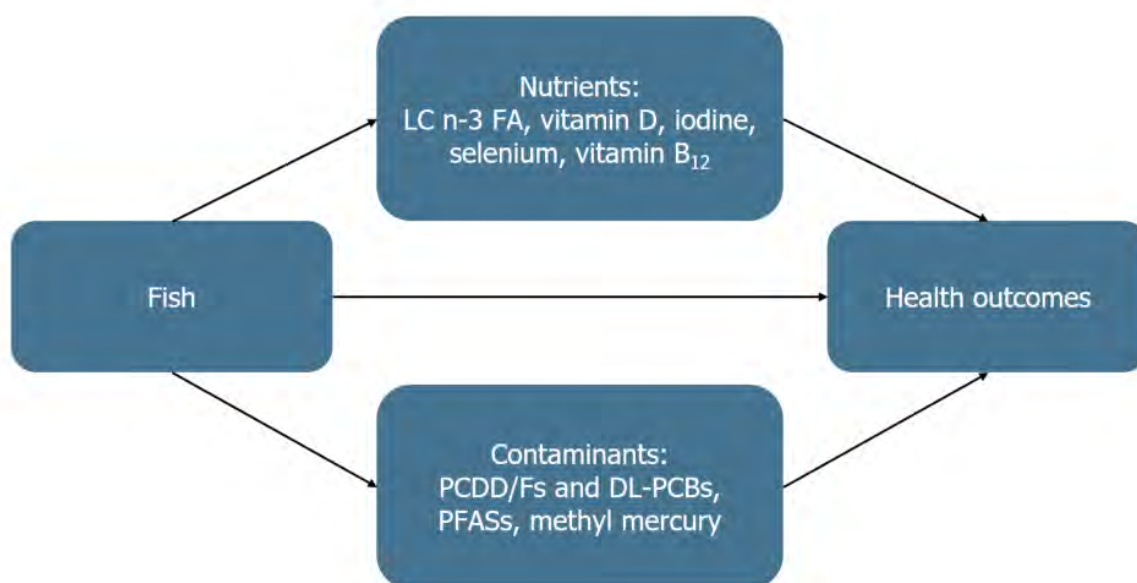


Figure 10-1 Illustration of how beneficial or adverse health effects from fish can be mediated through nutrients, contaminants or through unknown modes of action only ascribed to fish as such.

10.1 Benefit and risk assessment

EFSA's guidance on human health risk-benefit assessment of foods (EFSA, 2010a) and the later more refined assessment, BRAFO, Benefit and Risk Assessment of Foods (Hoekstra et al. 2012) suggest a tiered approach. This approach consists of a pre-assessment and problem formulation, followed by four tiers: (1) Individual assessments of benefits and risks, (2) Qualitative integration of benefits and risk, (3) Deterministic computation of a common health metric, and finally (4) Probabilistic computation. For the current benefit and risk assessment, we have used incidence and mortality rates as common health metric in tier 3, while tier 4 has not been implemented (see Chapter 3.4 for details).

VKM has performed systematic literature reviews and thorough evaluations of evidence for associations between fish and nutrient intake and different health outcomes. For grading of evidence VKM has used a predefined set of criteria prepared by World Cancer Research Fund (WCRF) (see Chapter 3.1 for details). The evaluation of evidence for associations between contaminants and their critical endpoints and setting of TWIs has, however, been performed by EFSA without formal grading of evidence. Thus, the level "probable" evidence that is used as inclusion criteria for health outcomes associated with fish or nutrients (Chapter 3.1.6), have no counterpart in the evaluation of evidence for contaminants. A direct comparison of the level of evidence for effects of fish and nutrients on one side and contaminants on the other side is therefore not possible.

The evidence considered sufficient to establish a TWI is different from that considered as sufficient for food-based recommendations. Beneficial health effects, e.g., from fish per se or from nutrients, are identified primarily from interventions or observational studies in humans, with systematic reviews and meta-analyses forming the basis for quantifying the

recommendations. In contrast, adverse health effects of the included contaminants have been deduced from experimental studies in animals together with results from human observational studies. Effects of contaminants are rarely studied in intervention studies for ethical reasons, and epidemiological studies of large populations rarely contain sufficient detail on contaminant exposure to make robust associations. A thorough risk assessment includes evaluation of evidence from several types of studies to establish causality for an effect. However, inherently from the methodology, often only the single study (“the critical study”) with effects at the lowest exposure level is used for setting a TWI. Consequently, “the critical study” has a lot of weight. This must be taken into consideration when comparing health effects from contaminant exposures above TWIs and nutrient intakes below AR, as well as when weighing the risks from contaminants in fish against the benefits from fish consumption.

A summary of the weight of evidence conclusions for different health outcomes and exposure to fish, nutrients, or contaminants from Chapters 4, 5 and 6 are presented in Table 10.1-1.

Table 10.1-1 Overview of the weight of evidence conclusions between exposure and different health outcomes for fish intake (Chapter 4), nutrient intake (Chapter 5) and intake of contaminants (Chapter 6).

Health outcome	Probable	Limited, suggestive	Limited, no conclusion	Established knowledge, basis for AR	Basis for TWI contaminants
CVD outcomes (adults)					
CVD incidence	LC n-3 FA (>1 g/day supplements) (protective)	Fish (protective) LC n-3 FA (<1 g/day supplements) (protective)	Fatty fish Lean fish		
CHD incidence	Fish (protective) LC n-3 FA (protective)	Fatty fish (protective) Lean fish (no assoc.)	Lean fish		
CHD incidence, secondary prevention		Fish (protective)	Fatty fish		
MI incidence	LC n-3 FA (protective)	Fish (protective) Fatty fish (protective)	Lean fish		
Stroke incidence	Fish (protective)	Fatty fish (protective) Lean fish (protective)	LC n-3 FA		
Ischemic stroke		Fish (protective)			
Haemorrhagic stroke		Fish (protective)			
Heart failure (HF)		Fish (protective)			
Atrial fibrillation (AF)		Fish (adverse) Lean fish (protective) LC n-3 FA (adverse)	Fatty fish		
Venous thromboembolism (VTE)			Fish		
Mortality outcomes (adults)					
Alzheimer mortality			Fish		
CVD mortality	Fish (protective) LC n-3 FA (protective)				
CHD mortality	Fish (protective) LC n-3 FA (protective)		Fatty fish Lean fish		

Health outcome	Probable	Limited, suggestive	Limited, no conclusion	Established knowledge, basis for AR	Basis for TWI contaminants
MI mortality	Fish (protective)				
Stroke mortality	Fish (protective)	Fatty fish (protective) Lean fish (protective)			
Stroke mortality, ischemic		Fish (protective)			
Stroke mortality, haemorrhagic		Fish (protective)			
T2D mortality			Fish		
All-cause mortality	Fish (protective) Vitamin D (protective)		LC n-3 FA Fatty fish Lean fish		
Neurodevelopment (children)					
Child neurodevelopment, maternal exposure		Fish (beneficial) Iodine (beneficial)	Fatty fish Lean fish LC n-3FA		Methyl mercury (adverse)
Child neurodevelopment, child exposure		Fish (beneficial) Fatty fish (beneficial)	Fatty fish Lean fish LC n-3FA		
Cognition and mental health (adults)					
Cognitive decline in adults, including Alzheimer's disease and dementia	Fish (protective)		Fatty fish Lean fish LC n-3FA		
Cognition in adults			LC n-3FA		
Mental health in adults (depression)		Fish (protective) LC n-3FA (protective)	Fatty fish Lean fish		
Postpartum depression		Fish (protective)	Fatty fish		
Other chronic diseases in adults					
Type 2 diabetes		Lean fish (no assoc.)	Fish Fatty fish LC n-3 FA		

Health outcome	Probable	Limited, suggestive	Limited, no conclusion	Established knowledge, basis for AR	Basis for TWI contaminants
Rheumatoid arthritis		Fish (protective)	Fatty fish Lean fish		
Bone health/hip fracture	Vitamin D (protective)	Fish (protective)			
Overweight in adults			Fish		
Overweight in children, maternal exposure		Fatty fish (no assoc.)	Fish Lean fish		
Overweight in children, child exposure			Fish		
Birth outcomes					
Preterm birth	Fish (protective)		Fatty fish Lean fish LC n-3FA		
Small for gestational age		Fish (protective)	Fatty fish Lean fish		
Low birth weight	Fish (protective)	LC n-3FA (protective) Vitamin D (protective)	Fatty fish Lean fish		
High birth weight		Fish (increase/adverse) Fatty fish (increase/adverse) Lean fish (increase/adverse)			
Birth weight (continuous)	LC n-3FA (positive assoc.)	Fish (positive assoc.) Fatty fish (positive assoc.) Lean fish (positive assoc.) Vitamin D (positive assoc.)			
Birth length (continuous)			Fish Fatty fish Lean fish		

Health outcome	Probable	Limited, suggestive	Limited, no conclusion	Established knowledge, basis for AR	Basis for TWI contaminants
Head circumference (continuous)			Fish Fatty fish Lean fish		
Asthma and allergies (children)					
Eczema in children, maternal exposure		Fish intake during pregnancy (protective)	Fatty fish pregnancy Lean fish pregnancy Fish intake lactation		
Eczema in children, child exposure		Fish first year of life (protective)	Fish later ages		
Wheeze first 2 years of life, maternal exposure		Fish (protective)	Fatty fish Lean fish		
Wheeze older age, maternal exposure		Fish (no assoc.)	Fish		
Asthma, maternal exposure			Fish pregnancy Fatty fish pregnancy Lean fish pregnancy Fish lactation		
Asthma, child exposure			Fish		
Allergic rhinitis, maternal exposure		Lean fish pregnancy (no assoc.)	Fish pregnancy Fatty fish pregnancy		
Allergic rhinitis, child exposure			Fish		
Allergic sensitization, maternal exposure			Fish		
Allergic sensitization, child exposure			Fish		
Other					
Multiple sclerosis		Fish (protective)			
Colorectal cancer		Fish (protective) Vitamin D (protective)			

Health outcome	Probable	Limited, suggestive	Limited, no conclusion	Established knowledge, basis for AR	Basis for TWI contaminants
Prostate cancer		Selenium (protective)			
Respiratory tract infection		Vitamin D (protective)			
Vaccine response					PFASs (adverse)
Sperm concentration/ semen quality		LC n-3 FA (beneficial) Selenium (beneficial)			PCDD/Fs (adverse)
Female fertility			Iodine		
Goiter				Iodine (protective)	
Keshan disease				Selenium (protective)	
Pernicious anaemia				Vitamin B ₁₂ (protective)	

10.1.1 Health effect of fish consumption

Quantitative assessments were performed for health outcomes where the evidence for an association with fish intake was categorized as strong ("convincing" or "probable"). The quantitative modelling for fish as a whole food (with its food matrix) and health outcome, includes both possible benefits and risks by fish consumption. All associations between fish consumption and health outcomes that were included in the quantitative modelling, were identified as beneficial.

Most of the studies included in the systematic literature review of fish intake and health outcome were prospective cohort studies. The weight of evidence analysis from the review showed that the evidence for a beneficial association between fish intake and 11 health outcomes were "probable" (see Table 10.1-1). "Probable" and "convincing" weight of evidence conclusions, means that the evidence is strong enough to give recommendations. Using the WCRF criteria, the evidence is graded "probable" that fish intake reduces CVD mortality, CHD mortality, stroke mortality, all-cause mortality, CHD incidence, stroke incidence, dementia, Alzheimer's disease, preterm birth, and low birth weight. It should be noted that some health outcomes are overlapping, e.g., CHD mortality is a subcategory of CVD mortality, and Alzheimer's disease is a subcategory of dementia. There was no evidence categorized as "convincing". The evidence that fish intake reduces CVD incidence, MI incidence, heart failure, atrial fibrillation, impaired child neurodevelopment, mental health (depression) among adults, small for gestational weight, birth weight and impaired bone health (measured as hip fracture) is graded as "limited, suggestive" in our systematic literature reviews (see Table 10.1-1). "Limited, suggestive" means that the evidence is too limited to permit "probable" or "convincing" causal judgement, but the evidence is suggestive of a direction of effect.

The systematic literature review on fish also included critical endpoints for the included nutrients and contaminants in fish. Sperm concentration was the critical endpoint for PCDD/Fs and DL-PCBs, however, no studies on fish intake and semen quality/male infertility were included in our systematic literature review as none of the identified papers fulfilled our inclusion criteria. Vaccination response was the critical endpoint for PFASs exposure, however, no studies on fish intake and vaccine response or on fish intake and suppression of immune response were included in our systematic literature review as none of the identified papers fulfilled our inclusion criteria.

A weight of evidence analysis of the literature on the associations between fatty fish and health outcomes was also performed. No associations between fatty fish intake and health outcomes are graded as "probable" (see Table 10.1-1). However, the evidence that fatty fish intake reduces the risk of CHD incidence, stroke and child neurodevelopment is graded as "limited, suggestive", and with the same direction as for total fish intake. Thereby, the quantitative modelling was only conducted for total fish intake and not separately for fatty fish intake.

The weight of evidence analyses on fish intake and health outcomes were mainly based on epidemiological studies analysing high vs. low fish intake. The analysis of high vs. low intake indicates the direction of the association but does not take the amount of fish intake expressed as gram or servings associated with the outcome into consideration. Where possible, we have computed pooled estimates for included primary papers, but used published dose-response relationships.

Hence, in the quantitative modelling of benefit and risk from fish consumption, we have applied dose-response relationships identified from meta-analyses in the systematic literature review, and disease occurrence data from publicly available sources, including national health registries (see Chapter 9.2 for details).

Quantitative estimations were conducted for adult women and men separately for all outcomes, except coronary heart disease (CHD) incidence, for which outcome data was only available for sexes combined. Quantitative estimation for preterm birth (PTB) was naturally only done for women. No quantitative modelling was done for low birth weight (LBW), as the underlying cause of LBW appeared to be PTB in studies of maternal fish intake during pregnancy. Stroke and myocardial infarction mortality were not included in the modelling even if the evidence is graded “probable”. This is because VKM did not identify any dose-response meta-analyses of mortality only. These outcomes were also nested within other mortality outcomes (MI nested within CHD, and stroke within CVD) that were included in the quantitative modelling.

Table 10.1.1-1 shows that changing the weekly fish intake among men from the current mean intake at 350 g per week to 300 g per week (scenario 2) results in an increase in annual numbers of incident cases or deaths estimated for all outcomes included in the quantitative assessment except for CVD mortality (0%). The increase was largest for dementia and Alzheimer’s disease with about 10% and 16% yearly increase in cases, respectively (which is equivalent to an increase of 459 and 416 cases, respectively). Since Alzheimer’s disease is a subcategory of dementia, these values cannot be added together. When changing from current fish intake to 450 g per week (scenario 3) among men, a decrease in annual numbers of incident cases or deaths was found for all outcomes except for CVD mortality (0%). The decrease was most prominent for incidence of CHD, stroke, and dementia with 4.5%, 2.1%, and 4.6% decreases in cases yearly, respectively (equivalent to a prevention of 1636, 59, and 214 cases, respectively).

For women, changing from the current mean intake at 238 g per week to 300 g per week (scenario 2), a small decrease in annual numbers of incident cases or deaths was found for all outcomes included in the quantitative assessment see Table 10.1.1-2. The decrease was largest for dementia and preterm birth with 2.9% and 6.3% decrease in cases yearly, respectively (equivalent to a prevention of 171 and 185 cases, respectively). When changing from current fish intake to 450 g per week (scenario 3) among women, the decrease was largest for dementia with a 9.5% decrease in cases yearly (equivalent to a prevention of 565 cases) and preterm birth with a 7.1% decrease in cases yearly (equivalent to a prevention of 208 cases).

In general, the percentage change in annual number of incident cases or deaths estimated for a change in fish intake from the current intake to 450 g per week was higher among women than among men. This was also the case when looking into mean change in annual number of incident cases and deaths. However, an increase in fish intake from the current mean level (350 g in men and 238 g in women per week) had no large impact on CVD or CHD mortality in neither men nor women. This is because the underlying dose-response relationship was flat for intakes >40 g per day, implying no changes in risk for intake that exceed 280 g fish per week. No dose-response curves were available to model the impact of stroke- or myocardial infarction mortality separately.

There are multiple assumptions behind the modelling and the quantitative estimates, and uncertainties around the presented estimates. These are thoroughly discussed in Chapter 9.2.

Table 10.1.1-1 Potential impact fractions (PIF) for men represented by percent change in annual number of incident cases or deaths and mean change in annual number of incident cases and deaths estimated for change in fish intakes from the current mean intake to 150, 300 or 450 g/week for each health effect. The negative signs indicate an expected percentwise decrease in number of cases or deaths due to the change in fish intake and the positive signs indicate an increase.

Current mean intake 350 g/week	Potential impact fraction			Mean annual change of incident cases/deaths		
	Scenario 1 150 g	Scenario 2 300 g	Scenario 3 450 g	Scenario 1 150 g	Scenario 2 300 g	Scenario 3 450 g
All-cause mortality	+1.5%	+0.4%	-0.8%	+59	+14	-29
CVD mortality	+3.9%	0	0	+36	0	0
CHD mortality	+6%	+1.5%	-2.0%	+29	+7	-10
CHD, incidence*	+4.3%	-0.2%	-4.5%	+1578	-64	-1636
Stroke, incidence	+4.4%	+1.1%	-2.1%	+121	+30	-59
Dementia, incidence	+9.9%	+2.4%	-4.6%	+459	+111	-214
Alzheimer's disease, incidence	+16%	0	0	+416	0	0

*Total population (both sexes).

Note: Uncertainty ranges on the numbers are presented in Chapter 9.2.

Table 10.1.1-2 Potential impact fractions (PIF) for women represented by percent change in annual number of incident cases or deaths and mean change in annual number of incident cases and deaths estimated for change in fish intakes from the current mean intake to 150, 300 or 450 g/week for each

health effect. The negative sign indicates an expected percentwise decrease in number of cases or deaths due to the change in fish intake and the positive signs indicate an increase.

Current mean intake 238 g/week	Potential impact fraction			Mean annual change of incident cases/deaths		
	Scenario 1 150 g	Scenario 2 300 g	Scenario 3 450 g	Scenario 1 150 g	Scenario 2 300 g	Scenario 3 450 g
All-Cause mortality	+0.7%	-0.5%	-1.6%	+18	-13	-44
CVD mortality	+2.6%	-1.3%	-1.3%	+9	-5	-5
CHD mortality	+2.6%	-1.8%	-5.2%	+3	-2	-7
CHD, incidence*	+4.3%	-0.2%	-4.5%	+1578	-64	-1636
Stroke, incidence	+1.9%	-1.3%	-4.5%	+28	-20	-65
Dementia, incidence	+4.2%	-2.9%	-9.5%	+252	-171	-565
Alzheimer's disease, incidence	+14%	-1.8%	-1.8%	+481	-61	-61
Preterm birth	+1.8%	-6.3%	-7.1%	+52	-185	-208

*Total population (both sexes).

Note: Uncertainty ranges on the numbers are presented in Chapter 9.2.

Most of the outcomes included in the quantitative assessments (all-cause, CVD- and CHD-mortality, CHD incidence, stroke incidence, dementia and Alzheimer's disease) are diseases where incidence increase substantially with age, and most cases are found in people older than 70 years. By increasing age, both men and women are at higher risk and will benefit from fish consumption. As shown in the table above, women would gain more from changing their intake from current intake to the recommended intake (both 300 and 450 g per week), since their current mean fish intake is lower than men's. The evidence that fish intake during pregnancy protects against preterm birth and low birth weight is also strong ("probable"), and the negative health effects that follow these conditions, may last throughout life.

Women of childbearing age (women in the age group 18-45 years) have a lower fish intake than women in the age group 18-70 years (182 g vs. 238 g per week) and their children will therefore benefit from maternal fish consumption, and benefit from increasing intake from current intake to recommended intake (both 300 g and 450 g per week).

As mentioned above, although the outcomes included in the quantitative assessment are generally diseases affecting the older age groups (except for preterm birth and low birth weight), primary preventions of chronic diseases need to start early. In addition, studies have shown that establishing healthy eating pattern early in life is of importance, given that these behaviours tend to continue into adulthood (Kelder et al., 1994; Lien et al., 2001; Lytle et al., 2000; Mikkila et al., 2005; Wang et al., 2002). Hence, supporting recommended fish intake already in young age may be of importance for intake later in life and for later health benefit. It is not known for how long a specific fish intake is required, or if specific time periods of life are more important than others, to obtain the beneficial effects of the intake of fish later in life.

10.1.2 Benefit characterization of nutrients in fish

The beneficial associations between fish consumption and the various health outcomes are assumed to be partly mediated through nutrients in fish. As illustrated in Figure 10-1 fish *per*

se may also have a direct beneficial effect on health outcomes not mediated through the nutrients. We eat foods, foods consist of a variety of nutrients and other substances, and it is challenging to evaluate the role of isolated, individual nutrients.

The weight of evidence for health effects of intake of nutrients for which fish is an important contributor were based on systematic literature reviews and meta-analyses, using the WCRF criteria (for more details see Chapter 3.2).

We did semi-quantitative assessments of vitamin D, iodine, selenium, and vitamin B₁₂ to investigate the proportion below AR at current fish intake, and in the three fish scenarios.

The nutrients LC n-3 FA, vitamin D, and iodine have very few natural sources in the diet (especially LC n-3 FA and vitamin D). Among adults, fish and seafood contribute to 66, 23, and 44% of the intake, respectively. Fatty fish is the main source of LC n-3 FA and vitamin D, and lean fish is the main source of iodine. Fish is also a main contributor to the total intakes of selenium, and vitamin B₁₂. However, these nutrients are also naturally present in numerous other foods. An increased fish intake would probably reduce the intake of selenium, and vitamin B₁₂ from other sources. This is not accounted for in the scenario calculations.

LC n-3 FA

There is no established AR for the LC n-3 FA, but a semi-quantitative assessment has been conducted using an adequate intake (AI) at 250 mg/day for EPA plus DHA set by EFSA (2010b).

Our literature review shows that there is “probable” evidence that LC n-3 FA intake reduces the probability of CVD mortality, CHD mortality, CHD incidence, and MI incidence. Moreover, the evidence is graded “probable” that LC n-3 FA is associated with increased probability of higher birth weight (see Table 10.1-1). Except from birth weight and MI incidence, these health outcomes are already included in the quantitative modelling of fish intake and health outcomes. The evidence that LC n-3 FA intake is associated with CVD incidence (at doses <1 g/day), mental health in adults, low birth weight and semen quality is graded as “limited, suggestive” (see Table 10.1-1).

At current fish intake level, 18% of the women of childbearing age (18-45 years), and 10% of adults (18-70 years) have intakes of EPA plus DHA below AI. Fish, and especially fatty fish is one of very few natural sources to LC n-3 FA, and consequently, increasing intake of fish will have high impact on the total intake of LC n-3 FA. In the fish scenarios, in which all participants in the food dietary surveys are assigned a fixed daily intake of fish, all adults have estimated intakes of EPA plus DHA above the adequate intake.

Vitamin D

The evidence that vitamin D has a beneficial effect on bone health and reduced risk of all-cause mortality is “probable” in NNR (2012). Most intervention studies leading to these

conclusions intervened with calcium in addition to vitamin D. The evidence that vitamin D intake is associated with lower probability of low birth weight (<2500 g) and higher birth weight is graded as “limited, suggestive” in our updated reviews. We also concluded that the evidence is “limited, suggestive” for the notion that vitamin D intake lowers the risk of acute respiratory tract infections. This was based on conclusions from the SACN report from 2020, where it is stated that vitamin D *may* reduce the risk of respiratory tract infection, but the size of any *potential benefit* of vitamin D in reducing acute respiratory tract infection risk may be small (SACN, 2020) (see Table 10.1-1).

At current intake levels (including supplements), all included age groups have a relatively high proportion of individuals with an intake of vitamin D below AR. Women of childbearing age (18-45 years) have the lowest estimated intakes among adults, and 59% have intakes below the AR at 7.5 µg/day. Among children, the 13- and 9-year-old girls have the lowest estimated vitamin D intake, and 67% and 65%, respectively, have intakes below the AR at 7.5 µg/day. The age groups with the lowest proportion below AR within current intake of vitamin D were 1-year-olds and adult men, where 16% and 35%, respectively, had an intake below AR.

For most individuals, scenario 1 represents a reduction in fish consumption. Reduced fish intake will imply a reduced vitamin D intake, and the proportion of the population with intakes below AR increases in most age groups and both genders.

Because the increase of fatty fish intake is small from current fish consumption to the estimated scenarios 2 and 3, the increase in vitamin D intake is moderate (see Figure 9.1.2-1). The scenario estimations indicate that increasing intake of fish from the current intake to the recommended intake would lead to a moderate increase in vitamin D intake at the population level and may be of special importance for those with a very low dietary intake of vitamin D where even a small increase may be of substantial importance. In women of childbearing age, the proportion below AR was reduced from 59% to 51% when changing from current intake to scenario 2 and to 46% when changing from current intake to scenario 3. For the 13- and 9-year-olds, the proportion below AR was reduced from 67% to 53% and from 65% to 53%, respectively, when changing from current intake to scenario 2. The proportion below AR was further reduced to 50% for 13-year-olds and to 51% for 9-year-olds when changing to scenario 3.

Although not included in the semi-quantitative benefit characterisation of vitamin D, age groups >70 years have higher requirements for vitamin D than adults <70 years. It is reasonable to assume that in the older age groups, the proportions with intakes below the requirements will be much larger.

Iodine

It is well established that severe iodine deficiency during pregnancy will impair child growth and neurodevelopment through lower production of thyroid hormones. However, the

evidence that mild to moderate iodine deficiency in pregnancy may affect neurodevelopment in infancy/childhood is found to be “limited, suggestive” (see Table 10.1-1).

At current intake levels (including supplements), women of childbearing age (18-45 years) have the lowest estimated intakes among adults, and 20% have intakes below the AR at 100 µg/day. The higher requirements in pregnancy and during lactation are not covered in these data, and the percentage of pregnant and lactating women with intakes below AR is even higher. Thirteen-year-old girls have the lowest estimated iodine intake, and 33% of these girls have intakes below the AR at 73 µg/day. Among the 9-year-old girls, 28% have intakes below the AR at 73 µg/day (see Figure 9.3.3-1).

For most individuals, scenario 1 represents a reduction in fish consumption. As lean fish is rich in iodine, whereas fatty fish is not, a reduced lean fish intake will imply a reduced iodine intake, and the proportion of the population with intakes below AR increases in most age groups and both genders.

The scenario estimations indicate that increasing intake of lean fish from the current intake to the recommended intake would reduce the proportion having a relatively high probability of inadequate iodine intake to almost zero for all age groups and genders (see Figure 9.3.3-1). For groups at highest risk of low intake at current fish intake, women of childbearing age and 13-year-old-girls, the proportions having a relatively high probability of inadequate iodine intake were almost removed when increasing from current fish intake to the scenarios 2 and 3.

Selenium

One health outcome associated with selenium have been evaluated in the literature searches for this benefit and risk assessment of fish consumption. It was found that the evidence for an association between selenium intake and semen quality is graded as “limited, suggestive” (see Table 10.1-1).

At current intake levels (including supplements), women of childbearing age (18-45 years) have the lowest estimated selenium intakes among adults, and 6% have intakes below the AR at 30 µg/day. The higher requirements in pregnancy and during lactation are not covered in these data, and the percentage of pregnant and lactating women with intakes below AR is higher. Nine-year-old girls have the lowest estimated selenium intake, and 73% of these girls have intakes below the AR at 35 µg/day. Among the 13-year-old girls, 66% have intakes below the AR at 35 µg/day.

For most individuals, scenario 1 represents a reduction in fish consumption. As fish is the most important single source to selenium, reduced fish intake will most likely imply a reduced selenium intake.

The scenario estimations indicate that increasing intake of fish from the current intake to the recommended intake would reduce the proportion having a probability of inadequate selenium intake to null for most age groups and both genders. For groups at highest risk of

low intake, like 13- and 9-year-old girls, increasing from current intake of fish to scenario 3, the proportion having a relatively high probability of inadequate selenium intake was reduced from 73% to 13% and from 66% to 3%, respectively.

Vitamin B₁₂

No specific health outcomes associated with vitamin B₁₂ have been evaluated in the literature searches for this benefit and risk assessment of fish consumption (see Chapter 3).

At current intake levels (including supplements), all age groups have estimated vitamin B₁₂ intakes above AR. The higher requirements in pregnancy and during lactation are not covered in these data. All population groups have estimated vitamin B₁₂ intakes above AR in the scenarios.

Fish and dairy products are the most important sources of vitamin B₁₂ in the adult Norwegian population. The estimated intakes for current fish consumption and the scenarios 2 and 3 indicate that no specific age groups are at risk of having vitamin B₁₂ below the AR. However, vegans and elderly for which we do not have specific dietary intake data, might be at risk of developing vitamin B₁₂ deficiency known as pernicious anaemia due to low intake of animal food including fish or low dietary intake in general. Moreover, studies in breastfeed Norwegian infants show that a high proportion have signs of suboptimal vitamin B₁₂ status/B₁₂ deficiency, however, the implication of these findings are uncertain.

10.1.3 Risk characterization of contaminants in fish

The risk associated with fish consumption may be understood as increased probability of adverse health effects related to contaminants in fish.

We have performed semi-quantitative risk assessments of PCDD/Fs and DL-PCBs, PFASs, and methyl mercury (see Chapter 9.4) based on an evaluation described in Chapter 2.

Fish may contain a variety of contaminants, and their concentrations vary depending on species and geographical area. As mentioned in Chapter 10.1.1, the quantitative modelling for fish as a whole food and health outcomes includes both possible benefits and risks by fish consumption. Therefore, all contaminants included in the present benefit risk assessment, as well as others, may also have affected the health effects described in 10.1.1 from fish as a whole food, as very few studies have adjusted for the effect of contaminants.

The TWIs used in the present benefit and risk assessment (see Chapter 2) are based on different critical endpoints where evidence has been found between intake of contaminants and the outcome. The critical effect for PCDD/Fs and DL-PCBs is reduced sperm concentration following pre- and postnatal exposure. For PFASs, it is reduced vaccine response in children following pre- and postnatal exposure, and for methyl mercury, it is child neurodevelopment related to prenatal exposure (maternal intake).

The child neurodevelopment outcome was included in the systematic literature review of fish intake and health outcomes. The evidence for a beneficial effect on cognition and/or a protective effect against mental health problems were considered "limited, suggestive". Child neurodevelopment is thus not included in the quantitative modelling.

The contaminants have not been included in the quantitative modelling for a variety of reasons. There is a lack of consensus for the use of linear no-threshold dose-response model for methyl mercury published in the Global Burden of Foodborne Disease (GBFD) project, which is not in line with EFSA's TWI. The dioxin model (also from the GBFD project) has not been updated with the 2018 EFSA Scientific opinion on TWIs for dioxins and DL-PCBs. Furthermore, no model exists for PFASs and would have to be developed from scratch.

PCDD/Fs and DL-PCBs

The critical effect for PCDD/Fs and DL-PCBs set at 2 pg TEQ/kg bw/week, is reduced sperm concentration in men following pre- and postnatal exposure. In addition to postnatal exposure in the boys, the exposure in females during childhood and adolescence up to and during pregnancy is of particular relevance. This is because the resulting maternal blood concentration and breast milk concentration determines the exposure in young boys.

At current fish intake level, a high proportion of the Norwegian population exceed the TWI for PCDD/Fs and DL-PCBs (>96% in all age groups). Ninety-seven percent of the women of childbearing age exceed the TWI.

Fish contributes on average 20-40% to the total PCDD/F and DL-PCB exposure in the current situation for the various age groups. In scenarios 2 and 3, where the fish intake is increased from current to 300 g or 450 g per week, respectively, the mean exposure to PCDD/Fs and DL-PCB is also increased for all age groups. The increase is highest for women of childbearing age, 13- and 9-year-olds. For women in childbearing age, this increase in exposure entails an increase in exceedance from about two times the TWI at current intake, up to three times the TWI in scenario 3. For 13-year-olds, it increases from 2.4-fold to 3.4-fold. For 9-year-olds, the increase is from 3.3-fold to 4.6-fold the TWI.

In adults, the mean estimated exposure to PCDD/F and DL-PCB is 2.3-fold the TWI and the 95th percentile is almost 4-fold the TWI at current intake, and the degree of exceedance is 2.8 and 3.8-fold for the same age group in scenario 3, respectively. Thus, there is only a small change in degree of exceedance by increased fish consumption in the fish scenarios 2 and 3 (increased for mean, reduced for 95th percentile).

PFASs

There are large uncertainties associated with the exposure calculations for PFASs due to limitations in the available occurrence data and low sensitivity of the analytical methods applied. Concentrations measured in human blood indicate that the TWI, set at 4.4 ng/kg bw/week, in parts of the population, both in EU and in Norway, is exceeded (EFSA, 2020, see also Chapter 6.1.2.1).

The semi-quantitative risk characterisations using estimated exposures show that for adults the mean intake is 7.4 ng/kg bw/week, which is 1.7 times the TWI, and 86% of Norwegian adults exceed the TWI at current fish intake.

For women in childbearing age, the proportion exceeding the TWI is 80% at current intake. For children and adolescents, the proportion exceeding the TWI, as well as the mean intake, decreases with increasing age, except for 1-year-olds. For children ≤ 4 years, the proportion with an estimated intake above TWI is $\geq 79\%$.

Both the scenarios with increased fish intake cause increased PFASs exposure in the following three groups: women in childbearing age, 13- and 9-year-olds. These groups are of special interest as the critical effect is reduced vaccine response in children, bearing in mind that PFASs accumulate in the body and are transferred from mother to child over the placenta and via breast milk. The highest increase in estimated mean PFASs exposure from current fish intake compared to the highest recommended fish intake is for 13-year-olds, for whom the increase is from 4.5 ng/kg bw/week, which is almost similar to the TWI, to 8.2 ng/kg bw/week, which is 1.8 times the TWI. Generally, however, the increase in exposure from current fish consumption to the recommended intake is low.

As already mentioned, the uncertainty in the calculations is high. Moreover, the contribution from fish to PFASs exposure varies between age groups from about 30% (women in childbearing age) to 40% (1- and 2-year-olds), and consequently about 60-70% of the PFASs exposure comes from other sources. Increasing the intake of fish may therefore not have a particularly high impact on the total PFASs exposure, assuming that an increase in fish intake will cause reduced intake from other sources.

Methyl mercury

At current intake of fish, 2% of women in childbearing age exceed the TWI of methyl mercury, set at 1.3 $\mu\text{g/kg}$ bw/week. The proportion exceeding the TWI was somewhat higher for 4-year-olds (5%) and 1-year-olds (7%). Overall, the risk from dietary methyl mercury exposure at current intake is considered low.

The mean estimated exposure to methyl mercury in scenario 1 is below the TWI for all. When fish intake is increased towards recommended intake (scenarios 2 and 3), the exposure to methyl mercury is also increased but still the population proportion exceeding the TWI decrease relative to the current fish consumption. This is because in the scenarios, everyone will have the same fish intake. The intake of lean fish in high consumers (95-percentile) is therefore decreased, and lean fish is the main source for methyl mercury. Hence, in our exposure estimates, both decreased and increased total fish intake may result in reduced exposure to methyl mercury, given that the increase is mainly in the consumption of fatty fish.

With the current fish intake in Norway, only a very small proportion of the population exceeds the TWI for methyl mercury when applying a conservative approach assuming that

all mercury in fish and shellfish is methyl mercury. Moreover, the proportion exceeding the TWI is either zero or very low for all age groups in all three scenarios.

10.2 Comparison benefit and risk

The objective of this benefit and risk assessment of fish is to weigh the beneficial effects against the adverse effects of fish intake both at current levels of fish intake, and if we increase the intake of fish towards the recommended intakes.

10.2.1 Overall results on the benefit and risk from fish consumption

The quantitative modelling for fish as a whole food (matrix) and health outcome (Chapter 9.2), includes both possible benefits and risks. As outlined previously (Chapter 9.2.2), we identified 11 beneficial effects and no adverse effects graded as “probable”. Hence, for the outcomes studied, the net effect of fish (containing both nutrients and contaminants) is beneficial.

Tables 10.1.1-1 and 10.1.1-2 show estimated health effects of fish intake based on the quantitative modelling for fish and health outcome when changing fish intake from current intake to recommended intakes. A change from current intake of fish towards a recommended intake would result in an overall beneficial effect. Among women, an increase from current intake to recommended intake (both scenarios 2 and 3) decrease the expected number of annual cases of CHD incidence, stroke, dementia, and Alzheimer’s disease, as well as number of preterm births. Among men, the expected numbers of cases of stroke, dementia, and Alzheimer’s disease were also reduced, but only for the scenario of 450 g (scenario 3). The explanation is that scenario 2 would be a reduction in intake of fish for Norwegian men. Increase in fish intake from the current level had no large impact on CVD or CHD mortality in neither men nor women. This is because the underlying dose-response curve was flat for intakes >40 g/day, implying no changes in risk for intake exceeding 280 g per week.

The results from the semi-quantitative assessments for nutrients and contaminants should also inform the total weighing of the benefit and risk associated with fish intake. As shown above, low intake of vitamin B₁₂ is in general not a problem in the Norwegian population and will therefore not be included in the total judgment of the benefit and risk associated with fish intake. The proportion exceeding the TWI for methyl mercury is either zero or very low for all age groups both at current intake and in all three scenarios and will also not be included further in the total judgment of the benefit and risk associated with fish intake.

There was strong overlap in CVD outcomes where the evidence is graded “probable” for a protective association with both fish intake (quantitative modelling) and LC n-3 FA intake (semi-quantitative assessment). There was overlap for CVD mortality, CHD mortality, and CHD incidence (Table 10.2.3-1). For other outcomes, the evidence is graded “probable” for a protective association with LC n-3 FA intake and “limited, suggestive” for fish intake (MI incidence) or vice-versa (low birth weight). These findings support that a beneficial effect of fish is related to LC n-3 FA. The literature on fatty fish was generally more limited than for

total fish, which reduced the statistical power of summary estimates for fatty fish (and other sub-types of fish). The evidence for a protective association with fatty fish is not graded higher than “limited, suggestive” for any health outcome, although “probable” associations for many CVD outcomes with total fish appeared to be driven more by fatty fish than by lean fish.

Additionally, we need to weigh the beneficial effects of intake of vitamin D, iodine, and selenium and the adverse effects of intake of PCDD/Fs and DL-PCBs, and PFASs.

According to our estimations based on the national dietary surveys, low intake of vitamin D is a challenge especially among women of childbearing age and young girls. These groups have at the current intake level of fish, the highest probability of a too low intake compared to AR. The percent of the population with lower vitamin D intake compared to AR did not change considerable when the fish intake increased to the recommended intake. The proportion with a vitamin D intake below AR at scenario 3 were still 36% and 67% among women of childbearing age and 13-year-old-girls, respectively. However, the increase in fish intake may be of special importance for those with a very low dietary intake of vitamin D, where even a small increase may be of substantial importance. It should be noted that an amount of 200 g of fatty fish were chosen for both the scenarios 2 and 3 included in this benefit and risk assessment. If the amounts of fatty fish were increased, the proportion with vitamin D intake below AR would be reduced.

As mentioned above, the evidence that vitamin D has a beneficial effect on bone health is “probable”, and the AR for vitamin D is based on bone health. This underlines the importance of having sufficient vitamin D in the diet.

At current intake of fish, low intake of iodine is a challenge among women of childbearing age and young girls (9- and 13-year-olds). Increasing intake of fish from the current intake to the lower range of recommended intake (scenario 2) would reduce the proportion having a relatively high probability of inadequate iodine intake to about 5% for all age groups and gender. In the upper range of recommended fish intake (scenario 3), all age groups and genders have iodine intakes close to or above AR.

At current intake of fish, low selenium intake is a challenge especially among children and adolescents, especially females. Increasing fish intake to the lower range of recommended fish intake (scenario 2) would reduce the proportion below AR to 39% in 9-year-old girls, which is the population group with the highest probability of an intake below AR. Increasing fish intake to the upper range of recommended fish intake (scenario 3) would reduce the proportion below AR to 14% among 9-year-old girls.

In the interpretation of nutrient intake, it is important to be aware that it is reasonable to assume that our estimated nutrient intakes in adults and especially in the 13-year-olds may be somewhat lower than the true intakes due to underestimation of intake in the dietary surveys (see Chapter 7.3.2).

In summary, women of childbearing age and female children/adolescents are the groups with highest probability of an intake below AR for vitamin D, selenium, and iodine at current intake of fish. These groups may benefit from increasing the fish intake from current intake to recommended intake (both 300 g and 450 g per week), especially for intake of iodine and selenium. However, increasing fish intake towards the recommended fish intake still resulted in a relatively high proportion with a high probability of a too low intake of vitamin D if the intake of fatty fish is not increased above 200 g per week. It is important to mention that the population groups included in our estimations have been limited to those present in the national dietary surveys. Unfortunately, they do not include information about vulnerable groups for specific nutrients, e.g., like specific immigrant groups and elderly (older than 70 years) where studies have shown low vitamin D status due to low intake of vitamin D and/or limited sun exposure. For these groups, an increase in intake of fatty fish may contribute to a more adequate vitamin D intake and the associated health benefits.

For PCDD/Fs and DL-PCBs more than 95% of the population have an estimated exposure above the TWI at current fish intake, and the estimated mean intake is above the TWI in all age groups. With an increased fish intake, 100% of the population is estimated to have an exposure above the TWI, and the mean exposure increases for all. For women in childbearing age, this increase in exposure entails an increase in exceedance from about two times the TWI at current exposure to almost three times the TWI in scenario 2, and slightly above three times the TWI in scenario 3.

Fish contributes 20-40% to the total TEQ exposure, meaning 60-80% is from other sources (mainly dairy and meat). An increased fish intake would probably reduce the exposure from other sources. This is not accounted for in the scenario calculations.

An exceedance of the TWI for PCDD/Fs and DL-PCBs indicates an increased risk of reduced sperm concentration, which in turn may result in infertility in men with an already reduced sperm production. Infertility affects about 15% of all couples worldwide. Male factors such as decreased semen quality contribute to around 40% of the cases (Falsig et al., 2019). VKM notes that there are many environmental and genetic factors that can lead to decreased semen quality, and exposure to PCDD/Fs and DL-PCBs above the TWI of 2 pg TEQ/kg bw/week is regarded as a contributing factor but not sufficient by itself to result in male infertility.

For PFASs, the current intake will give an estimated mean intake in adults that is 1.7 times the TWI. Increasing the fish intake up to scenario 3 will cause an increase in the proportion exceeding the TWI, leading to an exceedance for all adults, but the increase in exceedance will be low. Children have a high estimated exposure both in the current situation and in the calculated scenarios.

The critical endpoint for PFASs is reduced immune response, measured as reduction in vaccine response in children. Due to the maternal accumulation and the impact of maternal concentrations for both pre- and postnatal exposure of the child, exposure of girls during childhood and adolescence and women in childbearing age are of particular concern.

However, as for the PCDD/F and DL-PCBs, several sources other than fish contribute substantially to the exposure, and an increased fish intake would probably reduce the exposure from other sources.

10.2.2 The effect of the health outcomes associated with fish intake

From a public health perspective and according to The Global Burden of Disease database, the following diseases cause the most death and disability in Norway (ranked according to death and disability combined (DALYs)); 1. ischemic heart disease; 2. low back pain; 3. falls (including hip fractures); 4. chronic obstructive pulmonary disease (COPD); 5. stroke; 6. lung cancer; 7. diabetes; 8. headache disorders; 9. colorectal cancer; 10. anxiety disorders (<https://www.healthdata.org/norway>).

Analyses from the Global Burden of Disease analyses also report that smoking followed by high blood pressure, high fasting plasma glucose and unhealthy diet are the most important risk factors for death and disability combined (as DALYs) in Norway.

The burden of disease that can be attributed to low fish consumption has not been estimated. According to recent data, the list of single food items having the highest attribution to the burden of disease (DALYs) is diets low in whole grains, high in red/processed meat, low in legumes, high in sodium, low in fruits, low in fibre, low in vegetables, low in nuts and seeds, high in trans fatty acids, low in seafood n-3 fatty acids (<https://www.healthdata.org/norway>).

Mathematical modelling indicates that increasing intake of fish to recommended intakes, and especially towards the upper range of recommended intake, 450 g per week in scenario 3 will reduce the probability of having stroke and CHD, non-communicable diseases that are important contributors the burden of disease in Norway. Moreover, increasing intake of fish towards recommended intake is estimated to reduce number of new cases of CHD, dementia and Alzheimer's disease, cognitive diseases which are increasing in the population as the proportion of elderly people is increasing. The proportion of the population with an intake below AR for selenium and iodine, and below AI for EPA plus DHA, will also be reduced. The low intake of vitamin D will not necessarily be solved by increasing fish intake alone but increasing the fish intake and especially fatty fish intake could be of importance for those with the lowest vitamin D intakes. In conclusion, all age groups would benefit from increasing from current intake to recommended intake of fish.

As shown in scenario 1, a reduction in intake of fish from current intake to 150 g per week is estimated to increase the risk of CVD and CVD mortality and the number of deaths from these diseases. This may indicate that a reduction in fish intake is not beneficial.

On the other hand, increasing fish intake would increase intake of PCDD/Fs and DL-PCBs, and PFASs. As shown above, when increasing towards recommended intake everyone in all age groups would exceed the TWI for PCDD/Fs and DL-PCBs, and almost everyone will also exceed the TWI for PFASs. The critical endpoints linked to intake of PCDD/Fs and DL-PCBs, and PFASs (sperm concentration and vaccine response in children, respectively) are

important, but the contribution of these endpoints to the combined death and disability burden (as DALYs) has not been estimated. The effect of reduced sperm concentration is potentially a reduction of male fertility, and infertility in general accounts for a minor part of the burden of disease in Norway (<https://www.healthdata.org/norway>). A reduced response to vaccination in children is commonly used as a marker of a reduced immune response, which may have consequences for the risk of infection. However, the general applicability of this endpoint as well as the size and severity of the effect is not known.

10.3 References

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11 Uncertainty

Overall, VKM emphasise that the methods used for the quantitative assessment in this opinion are well established and up to date, and the inclusion of health outcomes is based on a thorough systematic literature review. The intake and exposure estimates are based on the most updated and best reliable data available. The level of uncertainty in this assessment is therefore considerably lower than in previous assessments, although uncertainty still exist.

This benefit and risk assessment is composed of two parts. The main part is a quantitative assessment of health outcomes related to fish consumption based on the results of an extensive systematic literature review and fish intake estimates. The other part consists of a semi-quantitative assessment of nutrients based on intake estimates and comparisons with an established reference value, i.e., average requirements (AR), and a similar semi-quantitative assessment of contaminants based on exposure estimates and comparisons with established tolerable weekly intakes (TWIs).

The uncertainty chapter is structured in the same manner, with uncertainties related to the quantitative assessment with the most prominent effect on the conclusion presented first. Uncertainties related to the semi-quantitative assessment of nutrients and contaminants are presented last.

11.1 The quantitative assessment of fish intake and health outcomes

11.1.1 Uncertainty related to the evidence for associations between fish consumption and health effects

Of the outcomes that have been evaluated, VKM decided that only effects for which the total body of evidence (across all types of studies) is graded as “probable” or “convincing” (highest grade) according to the WCRF grading criteria were included in the quantitative benefit and risk assessment. Consequently, whether the association for a health effect was graded “probable” or not has a major impact on the final conclusions of the benefit and risk assessment (no outcomes were graded “convincing”). VKM has applied several precautionary measures to minimize uncertainties and avoid grading evidence as “probable” by mistake, and thereby overinterpret the benefits of fish consumption. In this respect, VKM has both calculated their own pooled estimates based on data extracted from quality assessed primary studies and compared these estimates with previously published summary estimates for the same outcomes, from the systematic literature review. Further, VKM has systematically reviewed the literature on nutrients in fish and evaluated whether associations also for nutrients supported the associations between fish consumption and the health outcomes.

The evidence for effects of fish intake on different health outcomes is based on a systematic review of epidemiological studies in humans. The hierarchy of epidemiological evidence

places randomized controlled trials (RCTs) above other study designs, but there are few RCTs that have investigated the role of fish intake in relation to development of chronic diseases. RCTs are usually infeasible in this context because study participants cannot be blinded to their intake or adhere to intervention diets for the long latency period (years or decades) for chronic diseases to develop or deaths to occur. Therefore, dietary interventions are usually in the form of dietary advice about fish intake and not fish intake *per se*.

Intervention studies of dietary intake have been performed for outcomes with shorter follow-up time, such as neurodevelopmental outcomes in children, but these studies have been limited by low compliance to the intervention diets. Due to these practical difficulties, the main body of evidence on fish intake and health impacts consists of studies with an observational design, such as cohort studies. These studies have other limitations than intervention studies that must be considered when interpreting the data.

One limitation of observational studies is the potential for confounding factors. If study results are confounded, it means that the indicated result is caused (at least in part) by another factor that influences both the exposure and outcome being studied. Examples of confounding factors that may impact both fish intake and risk of disease are general health awareness and socioeconomic status. Chronic diseases are generally multifactorial, and other risk factors may be confounding factors if they also have an influence on fish intake.

Epidemiological studies will adjust for known confounding factors during analysis to the extent possible, but there is always the risk of residual confounding through that confounders were measured imperfectly. In addition, unmeasured confounding can occur if data is lacking for important confounders or if confounders remain unidentified. Due to potential confounding, observational studies cannot establish finite causal effects of fish intake. To reflect this uncertainty, the results of observational studies are commonly referred to as “associations” rather than effects, and causal inferences must be made based on multiple sources of data. Due to the overweight of observational studies in the body of evidence, the highest grade “convincing” was not given for any health outcome in the current assessment.

Dietary intake is generally difficult to measure accurately at the level of individuals, and the resulting misclassification or measurement errors may introduce bias in estimates of disease risk. Most large, epidemiological studies have measured self-reported dietary intake, including fish intake, using food frequency questionnaires (FFQs). The method has been designed to capture habitual diet, which is usually of interest in relation to chronic diseases. However, validation studies have demonstrated that intake may be over- or underreported, in particular for foods perceived to be healthy, or unhealthy, respectively. Fish is often viewed as a healthy food and may therefore be prone to overreporting in many populations. Also, FFQs should primarily be used to rank study participants in order of intake. Absolute intake levels are often calculated on the basis of FFQ data but are more uncertain and should be interpreted with caution. In prospective studies with a long follow-up, dietary intake may change over time. Many diseases develop over a long period of time, and often it is not known whether there are critical windows where diet influences the disease process more. Although some studies include repeated measurements of diet, most studies have only measured fish intake at study inclusion. Therefore, lack of updated exposure/intake data is also a source of error in many studies of long-term dietary intake. In addition, type of fish,

as well as methods for preparation of fish meals are unknown in many studies. Consumption of fish often occurs after culinary treatment or processing. Different processing methods may affect the content of both nutrients and contaminants in fish, and hence the health effects measured in the studies.

Eight outcomes from four different categories are both graded “probable” and included in the quantitative benefit and risk assessment. These are in the categories CVD, mortality, neurodevelopment (adults) and birth outcomes. As described above, VKM is especially concerned about uncertainty related to these outcomes as they have the highest impact on the outcome of the assessment.

Outcomes for which the total body of evidence (across all types of studies) was graded as “limited suggestive” according to the WCRF was not included in the quantitative assessment. Therefore, VKM is particularly concerned about outcomes that were on the borderline between the two categories, “limited, suggestive” and “probable”. Grading an outcome “too low” might underestimate the beneficial effect of fish intake. If the same is true for an adverse effect, the beneficial effect of fish intake might have been overestimated.

For the neurocognitive and mental health outcomes in adults, the outcome measures differ. Some are based on clinical diagnoses (both registry-based and clinical assessments in the study), others are based on different self-report measures. Although the objective of the studies is to measure a common phenomenon (e.g., dementia, Alzheimer’s disease, cognitive decline, or depression), there are uncertainties as to whether the modes of assessment are comparable. This can lead to large between-study variations in the results on fish intake and neurodevelopmental outcomes in adults.

For the neurodevelopmental outcomes in children, the same uncertainties apply as for the neurodevelopmental outcomes in adults. In addition, for the neurodevelopmental outcomes in children, a number of studies were also limited by multiple comparisons and lack of defined primary outcomes, contributing to additional uncertainties in this health outcome. Therefore, the evidence for neurodevelopmental outcomes in children is graded “limited, suggestive”, while evidence for neurocognitive and mental health outcomes in adults is graded “probable” due to a higher number of studies, and a generally lower level of uncertainty.

For mortality, the predominant causes of death may vary between populations and geographical areas, and fish intake may not have a role in all causes. Additionally, not all studies of all-cause mortality were limited to mortality caused by disease. This can lead to large between-study variations in results on fish intake and all-cause mortality. Still, the total body of evidence was graded “probable”.

For the outcomes *male fertility (sperm concentration)* and *immune response (vaccine antibody response)* no studies of acceptable quality were identified, and these outcomes are therefore not included in the quantitative assessment of fish. This is a limitation for the benefit and risk assessment, as male fertility is the critical endpoint for PCDD/Fs and DL-PCBs, and immune response is the critical endpoint for PFASs. VKM was asked to include

these groups of contaminants in the assessment. These contaminants would also have been included based on the flow scheme described in Chapter 2, because fish is an important source. It is uncertain whether increased fish intake can have a quantifiable impact on these outcomes.

A reduced response to vaccination may be used as a marker of an attenuated immune system, which may have a broader specter of health consequences, and important public health challenges are covered in VKM's systematic literature review on fish intake and health outcomes. Still, the lack of relevant studies on immune function is a limitation for the benefit and risk assessment.

Due to the choice of limiting the inclusion of literature to human studies, findings from animal studies and/or *in vitro* studies were not included in this benefit and risk assessment. Moreover, since VKM did not perform an open search for fish intake and all possible health outcomes but restricted the search to a defined list of outcomes, there is a possibility that endpoints having a plausible biological effect might have been left out from the assessment. However, due to the vast amount of literature that has been reviewed, including systematic literature reviews, and the broad expertise of the project group, VKM considers this to be unlikely, and a minor source of uncertainty.

11.1.2 Uncertainty related to the evidence for associations between nutrient intake and health outcomes

For nutrients and health outcomes, the evidence for an association is graded "probable" for LC n-3 FA and five different outcomes. These are CVD and CHD mortality, CHD and MI incidence, and birth weight. The evidence for an association between LC n-3 FA and the five outcomes might strengthen the mechanistic plausibility of an association between fish and these outcomes. There are, however, uncertainties related to these associations. Short-term effects of high doses of LC n-3 FA on chronic diseases tested in RCTs may differ from long-term effects of lower doses, similar to those from fish intake, observed in prospective cohort studies.

The latest large RCT with LC n-3 FA, STRENGTH from 2020, is not included in the meta-analysis of the CVD outcome, except for atrial fibrillation (Kow et al., 2021). This study did not observe any effect of LC n-3 FA on a composite outcome of major adverse cardiovascular events in statin-treated patients at high cardiovascular risk. Thus, including this trial in future meta-analysis may reduce the protective effect of LC n-3 on CVD outcomes.

Another nutrient where the evidence for associations with health outcomes are graded "probable" is vitamin D. "Probable" associations were found for all-cause mortality and for bone health. The beneficial effect of increased fish intake in relation to increased dietary intake of vitamin D is included in the semi-quantitative assessment. Uncertainties related to these associations may therefore be of relevance. Most of the RCTs leading to the conclusions have intervened with a combination of vitamin D and calcium, complicating the interpretation of the independent effect of vitamin D. In recent years, nearly all trials have

intervened with high doses of vitamin D (pharmacological doses) without co-supplementation with calcium, either given daily, weekly, monthly, yearly or as one mega-dose. In general, these trials have found few effects of vitamin D on various health outcomes. However, effects of such high doses, especially when not given daily, might be different from daily low doses. In addition, most participants in the trials were not vitamin D deficient at baseline. There are strong suggestions in the literature for no additional benefit by increasing the 25(OH)D concentrations above 50 nmol/L and individuals with low 25(OH)D concentrations are underrepresented in RCTs.

11.1.3 Uncertainties in the assumptions made for the quantitative modelling

In the assessment, a change in fish intake was modelled by shifting the population mean of current intake to the alternative intake scenarios represented as point estimates. By doing so, the variation in intakes in the population is disregarded, and this assumption is a source of unquantified uncertainty around the size of the estimated effect. This assumption might introduce bias because the difference between true intake and population mean differ for different age groups or sex.

The mean intake is a simple estimate of the population intake which ideally should be closest to, or the best representation of, the individual intakes. The limitation lies in the fact that different age groups among the population have significantly different intakes. For example, the mean is pushed upwards due to very high fish consumption in the senior age groups as compared to the lower age groups where the consumption is significantly lower and the relative risk (RR) for most of the diseases has an inverse proportionality with the consumption (as can be seen from the dose-response curves). Thus, the estimated RR of the "young cohorts" is higher than what it should be and for the "senior cohorts" are lower than what it should be for current consumption and that in turn affects the impact fraction calculations. Considering the scope of the project, the time constraints, and the data constraints, modelling of the mean was chosen, but it is recommended to explore more sophisticated models in future projects.

Estimating the expected number of new cases and deaths in each alternative intake scenario was done under the assumption that the current intake of fish is reflected in the current disease incidence and mortality. Under this assumption, the lag-time between exposure and onset of disease is ignored (i.e., it is disregarded that non-communicable diseases develop over time due to exposure to modifiable risk factors). This assumption may possibly cause an overestimation of the effect (or the importance) of fish intake.

It is also assumed that there has been little change in fish consumption over time, e.g., that a 30-year-old today will have the same consumption pattern as a 30-year-old 10 years ago. The latter can be considered a crude assumption and will introduce uncertainty that may influence estimates in any direction.

A considerable source of uncertainty in the assessment is the assumption on the structure and parameters linking relative risks to fish intake. As an indication of the parameter uncertainty of the relative risks, the annual number of new cases and deaths, and potential impact fractions in each alternative intake scenario, were estimated by applying the lower and upper limits of the 95% confidence interval around the relative risks (Tables 9.2.6-1, 9.2.6-2 and 9.2.6-3). However, additional unquantified uncertainty originating from the relative risks is likely to affect the quantitative estimates due to e.g., residual confounding, extrapolation of relative risks between populations with different distribution of risk factors, mortality rates, etc. Additionally, the structure of the assumed relationship between intake and risk (mostly log-linear, Table 9.2.4-1), as well as the overall assumption of fish intake as the sole changing factor, will potentially affect outcome estimates both in magnitude and direction.

11.2 Methodological challenges for estimating dietary consumption of fish, nutrient intake, and contaminant exposure

Self-reporting instruments are frequently used in nutrition research. All methods used to either assess long-term or short-term diet, prospectively or retrospectively, have associated measurement errors. For example, dietary assessment methods for long-term retrospective intake challenges the memory of the participants and their ability to take into account the variability of intake by day or season. Many participants also find it challenging to estimate portion sizes and frequencies of intake. Some of these inherent methodological challenges are more prominent in the 24-hour recall method, while others are more prominent when using FFQs.

11.2.1 Uncertainties in fish intake estimates with regard to study population and age group

In the Norkost 3 survey the response rate was 37%. The study population included more women and less men than in the general population. There were less participants below 39 years of age and more above 39 years of age than in the general population. And there were less participants from single households in the study population than in the general population. Also, there were fewer smokers and a higher share of participants with high education than in the general population. The Norkost study report concluded that the differences seen between the study population and the general population resulted in a slightly healthier average habitual diet for survey participants than what was probably the true habitual diet in the general population. As fish is considered a healthy food group it is likely that the intake of fish was somewhat overestimated in the survey.

Norkost 3 was conducted in 2011. Now, more than ten years later, the patterns of intake of fish in particular, and the diet as a whole, may have changed. In addition, dietary intake data in sub-populations, such as groups with immigrant background, were not well represented.

The Ungkost 3 survey for 8-9 years and 12-13 years was conducted as a national, school-based survey, using an online dietary diary, with participation rates of 55% and 53%, respectively. For the 4-year-olds in Ungkost 3 the participation rate was 20%. Although the surveys were designed to be nationally representative, the study population had fewer boys than girls included and more children with parents with higher education than in the general population. Thus, the results may be biased and the Ungkost 3 reports concluded that the results probably showed a slightly healthier average habitual diet than what was the true habitual diet in these age groups, and thus an overestimation of fish intake.

In both Småbarnskost 3 and Spedkost 3 the participation rates were 47% and 66%, respectively. The study populations consisted of a higher share of children with parents with a high level of education and a lower share of children with parents with primary school as the highest level of education, as compared to the general population. This may have introduced bias towards a healthier average diet than what was the true habitual diet in these age groups, and thus an overestimation of fish intake.

In general, social desirability may influence participants to underestimate the intakes of foods perceived as “undesirable/unhealthy” and overestimate the intake of healthy foods. In all the studies upon which this risk assessment is based, the social desirability bias may have influenced the intake estimations of fish and fish products towards overestimation.

In addition, cultural differences may have influenced the intake estimates. The proportion of fish in the habitual diet may be influenced by geographical and cultural differences and the changes or no-changes in traditional diet over time. Data from the Tromsø Study suggest a higher fish intake in older age groups. However, due to the data format, data on fish intake from the Tromsø Study was not compatible with the data from the national studies and was thus not included in the analyses.

11.2.2 Uncertainties in the estimates of fish intakes with regard to general methodological limitations

Self-reported assessment methods such as FFQs rely on the participant’s memory, the ability to remember what he/she ate and/or drank during a certain time period and correctly translate this into frequencies and amounts. This is a challenge and may introduce uncertainties in the data when it comes to fish intake. FFQs also rely on the participants’ ability to understand the questions as intended by those who design the assessment/survey.

The 24-hour recall method is prone to underestimate energy intake, which may indicate a general underestimation of all food groups or food group specific underreporting. In addition, two 24-hour recalls will with high probability introduce uncertainty in the intake estimates of food eaten seldom or not frequently. Fish is for many people such a food item. Foods seldom eaten may be better assessed using long-term dietary tools, such as frequency questionnaires.

Direct comparison of diet or dietary components, assessed in different age groups and with different dietary assessment methods, is challenging without validation and calibration

studies for the population, food group or substance in question. Direct comparisons between results from different dietary methods have to be made with caution. Spedkost 3 and Småbarnskost 3 used FFQs, while the other studies used 2-4 days of recall/record.

For the estimates on how large a share of the adult Norwegian population that eat according to the recommendations, VKM used a propensity questionnaire from Norkost 3. This questionnaire only gives an estimate of the number of fish meals per week, and not the exact amount in grams. It is therefore uncertain how large a share that actually eat >300 grams of fish per week.

11.2.3 Misreporting in the dietary surveys

Calculated energy intake can be used as an indicator of the extent to which dietary survey participants underreport or overreport food intake (Black et al., 2000). The dietary assessment methods used in Norkost 3 and Ungkost 3 tend to underestimate the energy intake, but the extent varies between the age groups. In Norkost 3, about 16% of the participants underreported their energy intake, with similar incidence in men and women (Totland et al., 2012). In Ungkost 3, 33% of the 13-year-olds, 12% of the 9-year-olds, and 5% of the 4-year-olds underreported energy intake (Hansen et al., 2016; Hansen et al., 2015), while respectively 1%, 2% and 2% overreported energy intakes. Misreporting of energy intake can shed some light on inaccuracy in the nutrient and contaminant intakes. However, this varies by nutrient and contaminant. In Norkost 3 and Ungkost 3, nutrients and contaminants which intake is dependent on only a few food sources with high content, are differently affected by underreporting than substances with many food sources. However, due to the relatively large underreporting of energy in adults and 13-year-olds (16% and 33%, respectively), it is reasonable to assume that the mean intakes of all nutrients and contaminants in adults and especially in the 13-year-olds are, to some degree, underestimated at the group level. It should be noted that under- and overreporting of energy is not corrected for by either the observed individual means (OIM) or the mixed model approach.

To explore the differences between the dietary assessment methods used in 1-, 2- and 4-year-old children, a comparison of energy intake was performed. The underreporting of energy intake in the 4-year-olds was 5%. According to NNR (2012), estimated energy requirement increases by approximately 25% in children from 2 to 4 years of age. However, the mean calculated energy intakes in 2-year-olds and 4-year-olds were less than 25% apart. The estimated energy intakes were 5.3 MJ/day and 5.5 MJ/day, respectively, in 2- and 4-year-old girls, and 5.6 MJ/day and 6.1 MJ/day, respectively, in 2- and 4-year-old boys. The estimated mean energy intake in the 4-year-olds in Ungkost 3 was in line with the estimated daily energy requirements given in Table 8.6 in NNR (2012), whereas the calculated energy intake in 2-year-olds in Småbarnskost 3 exceeds the estimated daily energy requirements for this age group. For example, for 2-year-old girls, the estimated requirement is 4.14 MJ/day and the intake in Småbarnskost 3 is 5.3 MJ/day. This is indicative of an overestimation of intakes in the 2-year-olds. For 1-year-olds, the mean estimated energy requirement is 3.4 MJ/day, while the reported energy intake is 4.8 MJ/day. Furthermore, since the breastmilk

intake data for the 1-year-olds was unavailable, breastmilk was not included in the total dietary intake estimates. Forty eight percent of the 1-year-olds were breastfed.

The present benefit and risk assessment cannot conclude that the observed overreporting of energy translates equally into higher intake of fish, nutrients and contaminants. Nevertheless, the discrepancies between the estimated energy intake and the estimated requirements, and the fact that mean intake of nutrients and contaminants were the same or higher in 2-year-olds than in 4-year-olds show that nutrients and contaminants were overestimated in 1- and 2-year-olds. Furthermore, breastmilk contains both nutrients and contaminants, and not taking breastmilk into account lead to an underestimation of intake for 1-year-olds.

11.2.4 Statistical correction of uncertainties

In Norkost 3, diet was recorded on 2 days. The low number of days increases the risk that these days are unrepresentative for the habitual intake of an individual and may not capture food items eaten seldom or seasonally. Due to the low number of sampled days in the dietary surveys that used 24-hours recall and food diary (2 days in Norkost 3 and 3-4 days in Ungkost 3), exposure distribution estimates based on OIMs for the age groups from 4-year-olds and above, may overstate the variability in the true exposure. These assessment methods are particularly prone to overestimation of the tails of an intake distribution. In addition, the degree of unrepresentativeness among survey respondents may lead to a bias in the estimated intakes. In an attempt to alleviate these problems, the adopted statistical modelling approach can be used to directly estimate and account for different sources of variability. The mixed modelling approach was used to describe the total intake of nutrients and contaminants (except MeHg), both at the current level of intake and for the scenarios. The approach corrects for day-to-day (within person) variability, variability between individuals and for unrepresentativeness of survey respondents (for further details, see Chapter 7.5).

Among the 1- and 2-year-olds the dietary intake was assessed by a FFQ, the parent/caretaker estimated the habitual intake of the child during the last two weeks. The day-to-day variability is thus accounted for by the survey design. Weighted OIMs were used for 1- and 2-year-olds when reporting the total intake of nutrients and contaminants, both for the current exposures and for the scenarios. The weighted OIM distributions correct for unrepresentativeness in survey respondents.

11.2.5 Uncertainties with regard to nutrient and contaminant content in fish

The nutrient and contaminant composition in fish, as in any food, is influenced by many factors, such as feed, seasonal variations, geographical region, storing conditions and genetics (for nutrients), and may vary over time. The occurrence data for nutrients in fish used in the present benefit and risk assessment was compiled according to guidelines for food composition compiling. The majority of the nutrient data from fish were compiled from

analytical projects conducted at the Institute of Marine Research in Norway. This means that the best estimates available were used for the nutrient intake estimation. However, when the present assessment was conducted the VKM did not have food composition data for Alaskan pollock and smelt, two fish species frequently used as ingredients in fish products in Norway. The use of food composition data from cod as approximate values may have introduced uncertainty in the nutrient estimations.

Values for iodine and single fatty acids were not available in a small number of food items consumed by adults as reported in the Norkost 3 survey. This may have introduced underreporting of these nutrients.

11.2.6 Intake estimates for nutrients

Nutrient and contaminant intake estimates are affected by the same issues of study design, population bias and methodological uncertainties as described above for fish intake. It is known that OIMs, and particularly weighted OIMs, do a reasonably good job in estimating the mean of the intake distribution for the population, while overestimating the standard deviation, with too low levels for low percentiles and too high levels for high percentiles. The uncertainty in mixed models is harder to estimate. It is known that mixed models improve on weighted OIM-based habitual intake distributions by adjusting for the day-to-day (within person) variation. The survey data for 4-, 9-, and 13-year-olds are 3-4 consecutive days. The potential correlation between days introduced by this study design may increase uncertainty in the day-to-day variability used in the mixed model.

11.2.7 Exposure estimates for contaminants

11.2.7.1 PCDD/Fs and DL-PCBs

The data sets on PCDD/Fs and DL-PCBs contained few samples from Norway, in particular samples from Norwegian farm animals (sheep, cattle, pork, and chicken), and the representativity of samples analysed is not known. This can lead to both overestimation and underestimation of the true exposure.

The number of samples from milk and egg is higher, and hence there is less uncertainty related to these. The number of samples from fish were sufficient.

For fruit and vegetables, there were few samples from the EFSA database and no samples from Norway. In addition, the concentrations found in some of the samples were very much higher than expected in these food groups. As PCDD/Fs and DL-PCBs are lipid-soluble and accumulate in the food chain, whereas fruits and vegetables generally have low fat content (0.1-0.4%) and are low on the food chain, the reported concentrations are hard to explain. One possibility could be that these fruits and vegetables were contaminated locally, e.g., by remnants of contaminated earth or deposition from local air pollution. It is not known whether the samples were washed before analysis. As there were no good explanations for

such high concentrations, VKM decided not to include any data on fruits and/or vegetables. Food groups missing/not included lead to underestimation of the true exposure.

Analytical results from recent (2022) samples of apples, banana, carrots, cauliflower, broccoli, cabbage, and potatoes on the Norwegian market that became available after the exposure was calculated by VKM, confirm that the concentrations are low, and that fruit, vegetables and potatoes are not major contributors to exposure in Norway (NFSA 2022, results made available to VKM).

As a conservative approach, VKM decided to use the upper bound (UB) concentration values for PCDD/Fs and DL-PCBs (see Chapter 7.2.1 for explanation of upper and lower bound). Use of UB exposure represents an overestimation of the true exposure in the benefit and risk assessment.

Regional differences in contaminant concentrations (Ho et al., 2021) and possibly higher concentration in locally caught fish have not been included and may underestimate the true exposure in some population groups (high consumers of self-caught fish).

11.2.7.2 PFASs

For the PFASs, there are large differences between lower bound (LB) and UB concentrations, indicating large uncertainties in the reported data. Due to many undetected samples (because of high LOQs, see Chapter 7.2.1), using UB values would be a huge overestimation, and based on biomonitoring data, VKM chose to use LB for risk characterisations. But use of LB in risk characterisations is an underestimation of the true exposure.

There is a general lack of occurrence data, which can cause both underestimation and/or overestimation of exposure. The food group “food for infants and small children” was omitted due to few samples and one “suspicious” sample with very high concentrations (particularly of PFNA). Omitting this food group causes an underestimation of exposure. For other food groups, measured concentrations in a subgroup are assumed to be representative for the whole group, which may not always be correct, and therefore cause uncertainty. Lack of representativeness may cause either underestimation or overestimation.

The severity in the lack of occurrence data, as well as high LOQs in the methods for analysis, differs between the various compounds included in the group PFASs, causing higher uncertainty in the concentration data for PFHxS and PFNA than for PFOS and PFOA. This may cause both under- and/or overestimation.

11.2.7.3 Methyl mercury

For methyl mercury, the concentrations in fish may vary substantially between geographical areas (Ho et al., 2021). VKM has not assessed where the fish was caught, or to what extent people eat locally caught fish. Using average occurrence data may underestimate exposure in some population groups.

Contribution from fish liver and roe was not included in the calculations of exposure to methyl mercury. The concentrations of methyl mercury in these foods are not high and the consumption is low, hence, this uncertainty is considered small.

In the VKM assessment, all measured mercury concentrations in fish and other seafood are assumed to be methyl mercury. This is a conservative assumption representing an overestimation of the true exposure. Normally, 70% - 100% of total mercury in fish fillet is methyl mercury.

11.2.8 Uncertainty when using recipes and recalculation into fish fillet equivalents

Valid information about the composite fish dishes and fish products was important in this benefit and risk assessment. The recipes used have been compiled using standard food composition guidelines. When recalculating the fish dishes into fish fillet equivalents, an average weight yield factor was used for all fish species and all preparation methods. Furthermore, retention factors were not applied for nutrient and contaminant content of prepared fish. This may have introduced uncertainty in the estimated amounts, and contaminant and nutrient intake, but VKM considers this uncertainty to be low.

Also, nutrient and contaminant concentrations in raw fish were used, which may lead to uncertainty of the intake of these compounds.

11.3 The semi-quantitative assessment of nutrients and contaminants and values for comparison

11.3.1 Intake- and exposure estimates in the scenarios for changed fish consumption

VKM estimated total intakes of nutrients and exposure to contaminants based on changes in fish consumption, without adjustment for changed intake of other parts of the diet or energy adjustment. This will most probably cause an underestimation of the nutrient intakes and contaminant exposures in scenario 1, where fish consumption is reduced (for most groups), and an overestimation in scenario 2 and 3, where fish consumption is increased (for most groups), for nutrients and contaminants where other sources than fish contribute to the dietary intake.

11.3.2 Reference values for comparison

VKM has made semi-quantitative benefit and risk assessments of nutrients and contaminants based on comparisons of exposure to established thresholds, i.e. ARs and TWIs. This approach has limitations. Due to the inherent differences between traditional benefit and risk assessments in the fields of nutrition and toxicology, the results of these semi-quantitative assessments are not directly comparable

The TWIs aim at protecting all parts of the population against adverse effects of chemical contaminants. On the other hand, the ARs used for nutrients, defined as an intake that is estimated to meet the requirement of approximately half the population of healthy individuals in a group, are set such that parts of the population with nutrient intakes below the AR are likely to have inadequate intakes, but not all will.

The differences in the approaches taken for deriving an AR for a micronutrient and TWI for a contaminant can lead to an imbalance between the acceptability of benefits and risks when used in this benefit and risk assessment. Whereas there should be no appreciable risk of toxicity due to a contaminant in any parts of the population by exposure below the TWI, only half of the population benefit from the adequacy of a nutrient (low risk of inadequate intake) with an intake at the AR.

11.3.2.1 ARs for nutrients

By definition, the nutrient requirements are covered for only 50% of the population at AR. For all included nutrients, we have used the ARs established by NNR for adults (NNR, 2012). ARs for children for vitamin D is from NNR (2012) and ARs for the other nutrients are from IOM. According to NNR "there is substantial uncertainty in several of these values so they should be applied with caution and, if possible, related to clinical and biochemical data. Furthermore, intake of nutrients above these values is no guarantee that deficiency symptoms could not occur in certain individuals" (NNR, 2012). For specific uncertainties related to the individual reference values we refer to the publications from NNR and IOM (NNR, 2012; IOM, 2001, 2000 and 1998).

Generally, there is more uncertainty related to ARs for children and adolescents than for adults. The ARs for children and adolescents are extrapolated from ARs for adults. The ARs for adults from IOM for selenium and vitamin B₁₂ are higher than those from NNR (2012). As we use AR for adults from NNR (2012) and AR for children from IOM for iodine, selenium, and vitamin B₁₂, the ARs we use for children are disproportionate to those we use for adults for selenium and vitamin B₁₂.

The evidence for the critical endpoint (bone health) for AR for vitamin D is "probable". However, ARs established based on balance studies have limitations. NNR report limited data on the uptake of vitamin D from natural sources and express uncertainty related to measurements of serum 25(OH)D concentrations. For iodine, AR in adults is based on studies of thyroid iodine accumulation and turnover in euthyroid and supported by balance studies. These have limitations, e.g. differences in long-term iodine intake prior to inclusion, iodine equilibrium relies on thyroidal store and not only intake and excretion, the studies were conducted at a time when key indicators such as serum TSH were not available.

However, as the semi-quantitative assessment of nutrients in fish is not the main contributor to the main conclusions in this report, the impact of any uncertainty regarding ARs is not considered to be significant for the main conclusions.

11.3.2.2 TWIs for contaminants

The TWIs for PCDD/Fs, PFASs and methyl mercury used in the semi-quantitative risk assessment are set by EFSA, and for specific uncertainties related to each value we refer to the EFSA Opinions. The main uncertainties associated with the TWIs are summarized as follows:

“The CONTAM Panel considered that the impact of the uncertainties on the risk assessment of PCDD/Fs in food is moderate. For the sum of PCDD/F and DL-PCBs, due to the uncertainty in the relative potency of PCB-126 in humans, the impact of the uncertainties on the risk assessment is high. Overall, the assessment is likely to be conservative” (EFSA, 2020).

“Overall, the CONTAM Panel considered that the impact of the uncertainties on the risk assessment for the sum of PFOA, PFNA, PFHxS and PFOS is high” (EFSA, 2018).

“The CONTAM Panel concluded that the impact of the uncertainties on the risk assessment of exposure to methylmercury and inorganic mercury is considerable and that the assessment is likely to be conservative.” (EFSA, 2012). Additionally, the Opinion from EFSA is from 2012, and although VKM performed a literature review to investigate whether an updated assessment is needed, only systematic reviews and meta-analyses were checked, and hence updated primary studies might contain data on adverse effects of methyl mercury exposure that is not considered.

11.4 References

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12 Conclusions and answers to the terms of reference

The Norwegian Food Safety Authority requested that the Norwegian Scientific Committee on Food and Environment (VKM) performed a benefit and risk assessment of fish intake to evaluate which health consequences that will occur for the Norwegian population if the population:

- (1) Continues with the same consumption levels of fish as of today
- (2) Increases the consumption of fish to match the recommendations given by the Norwegian Directorate of Health

In the assignment letter, VKM was requested to also include an evaluation of how changes in fish intake affect exposure to nutrients and contaminants in fish.

The benefit and risk assessment of fish is based on an extensive systematic literature review to evaluate the epidemiological evidence for associations between fish consumption and health outcomes, and a weight of evidence process based on criteria defined by the World Cancer Research Fund. To answer the terms of reference, a quantitative model using incidence and mortality as common metrics to estimate the effect of changes in fish intake from current intake to three constructed scenarios is applied. In the modelling of benefits and risks from fish intake, only health outcomes categorized with strong evidence according to the World Cancer Research Fund's grading system (i.e., "convincing" or "probable" evidence) for an association with fish intake is included. There was no strong evidence for an impact of children's fish consumption on any health outcomes in the children (neurodevelopment, mental health challenges, overweight/obesity, asthma and allergy), and consequently the quantitative analysis only includes adults. Moreover, there was no evidence for adverse associations between fish intake and health outcomes that were categorized as strong, and consequently, the modelling of benefits and risks related to fish intake includes only beneficial outcomes. In this benefit and risk assessment the term *current intake/exposure* refers to intake reported in the national dietary surveys Norkost 3 (2010-2011), Ungkost 3 (2015-2016), Småbarnskost 3 and Spedkost 3 (2019). The fish intake scenarios are simple constructed scenarios in which all participants in the food dietary surveys are assigned a fixed daily intake of fish and a fixed amount of fatty and lean fish (see Table 9.1-1). In both scenario 2 (300 g/week fish) and 3 (450 g/week fish), the amount of fatty fish is kept steady at 200 g per week, and only the amount of lean fish is increased from scenario 2 to scenario 3.

Our quantitative analyses/model does not include critical health outcomes relevant for the contaminants due to limited available data. Moreover, a quantitative modelling approach with common metrics is not applied for contaminants and nutrients due to limitations in available models. As described in Chapter 3.4, the dioxin (PCDD/Fs and DL-PCBs) model, published in the Global burden of foodborne disease project has not been updated with the 2018 EFSA

Scientific opinion on TWIs for PCDD/Fs and DL-PCBs. For PFASs there is no existing model, and there is a lack of consensus for the use of linear no-threshold dose-response model for methyl mercury. Moreover, for the included nutrients in the present benefit and risk assessment there are available models for LC n-3 FA and vitamin B₁₂, but not for vitamin D, iodine, or selenium. To avoid possible imbalance from including some single compounds (contaminants and/or nutrients) and not others, VKM decided not to integrate any single compounds found in fish in the quantitative modelling.

The evaluation of all nutrients and contaminants relevant for fish intake has been performed using a semi-quantitative approach. The exposures/intakes of nutrients are evaluated as proportions of the populations with intakes below average requirements (ARs), and the exposures to contaminants are evaluated as proportions of the population with intakes above the TWIs.

It should be noted that the quantitative modelling with common metrics generally is the preferred methodology for a benefit and risk assessment and considered to be at a higher tier than a semi-quantitative approach without common metrics.

In the present benefit and risk assessment, the beneficial health effects of fish intake are weighed against the adverse health effects of fish intake. *Benefit* is understood as reduced probability of adverse health effects related to fish intake or intake of components in fish such as nutrients, while *risk* is understood as increased probability of adverse health effects related to fish intake or intake of components in fish such as contaminants.

The recommendation of eating fish 2-3 times per week is based on a report with a thorough review of systematic summaries of knowledge done in 2011 by a project group on behalf of the Norwegian National Council for Nutrition ("Nasjonalt råd for ernæring"). The report from 2011 included the conclusions from the first benefit and risk assessment of fish in Norway from 2006. The report from 2011 concluded that there is "convincing" causal relationship between intake of LC n-3 FA and reduced CHD mortality, a "probable" causal relationship between intake of two portions of fatty fish per week (about 200 g per week) and reduced risk of CHD mortality, and between selenium intake and prostate cancer.

A VKM opinion on benefit and risk of fish from 2006 was updated in 2014, and VKM then came to similar conclusions as in 2006; the benefits from fish intake outweighs the risks. Since then, new data and evidence relevant for evaluation of benefits and risks from fish intake have emerged. EFSA has revised the TWIs for two important contaminants to which fish is a major dietary contributor, i.e., PCDD/Fs and DL-PCBs, and PFASs.

12.1 Terms of reference 1: Which health consequences will occur for the Norwegian population if the population continues with the same consumption levels of fish as of today?

From our systematic literature review it is concluded that the evidence is “probable”, and consequently is categorized as strong evidence, for a beneficial association between total fish intake and 11 health outcomes. These outcomes are CHD incidence, stroke incidence, CVD mortality, CHD mortality, myocardial infarction mortality, stroke mortality, all-cause mortality, dementia, Alzheimer’s disease, preterm birth and low birth weight. Eight of these were included in the quantitative modelling of fish intake and health outcomes. Preterm birth was included in the modelling, but not low birth weight, as the underlying cause of low birth weight appeared to be preterm birth in studies of maternal fish intake during pregnancy. Stroke and myocardial infarction mortality were not included in the model even if the evidence is graded “probable”, because no dose-response meta-analysis was found that included studies of stroke or myocardial infarction mortality only. For fatty fish, our systematic literature review does not conclude with a “probable” association between intake and any of the health outcomes. However, for several outcomes for which the association is judged as “probable” for total fish intake, the association with fatty fish is “limited, suggestive” (CHD incidence, stroke incidence, and stroke mortality) and in the same beneficial direction as for total fish intake. Furthermore, in our systematic literature review of nutrients in fish it is concluded that the evidence for a beneficial association between LC n-3 FA on CVD mortality, CHD mortality, CHD incidence, and myocardial infarction incidence is categorised as strong (“probable”). The evidence is also “probable” that LC n-3 FA is positively associated with birth weight (continuous). These results on LC n-3 FA support that fatty fish has a beneficial health effect.

Overall, the findings from our weight of evidence analyses show the impact of the beneficial effects of fish intake on several important public health challenges, and our findings align with the conclusions made in the above-mentioned report from 2011. Moreover, the systematic review of fish intake evaluates the association between fish as a whole food and health outcome, including both possible benefits and risks associated with fish intake.

According to Norkost 3 (conducted in 2010-2011), a large share of Norwegian women and men consumes less fish than recommended. Thirty-eight percent of women and 42% of men 18-70 years report that they consume less than 2 fish meals per week, and 62% and 66%, respectively, less than 3 meals per week (see Table 8.2-3). Among women of childbearing age (18-45 years), 45% report to consume less than 2 fish meals per week, and 70% less than 3 meals per week. As indicated by the mathematical modelling in the quantitative benefit and risk assessment (Chapter 9.2), changing the weekly fish intake from the current mean intake among adult men (350 g/week) and women (238 g/week) to 150 g per week (scenario 1), results in an increase in annual numbers of incident cases or deaths estimated for all outcomes included in the quantitative assessment (CVD mortality, CHD mortality, all-cause mortality, incidence of CHD, stroke, dementia, Alzheimer’s disease, and preterm birth).

This indicates that a lower fish consumption than the recommended intake is a potential health risk. This also indicates that population groups with a current intake below the mean intake of today may be at particular risk.

Intakes of nutrients and contaminants included in the present benefit and risk assessment were compared with the ARs for vitamin D, iodine, selenium, and vitamin B₁₂, and AI for the LC n-3 FAs EPA plus DHA, and with the TWIs for the contaminants. The AR is defined as the level of a nutrient intake that is sufficient to cover the requirement for half of a defined group of individuals, assuming a normal distribution of the requirement. Therefore, with an intake equal to AR, still, half of the population will not have a sufficient intake and be at risk of too low intakes. Even with 100% of the population having an intake above AR, there is no guarantee that all individual requirements are met. For contaminants, the TWIs cover the whole population and is defined as a safe level of exposure that a person can have throughout the whole life without appreciable risk for adverse health effects. When exposure is above the TWI, the risk of adverse effects may increase by increasing exceedance, but the increased risk is not quantified in the present benefit and risk assessment. As shown, ARs and TWIs are based on different prerequisites and are not comparable parameters.

Intake of fish is the major natural dietary source of LC n-3 FAs, vitamin D, iodine, and selenium among adults, and at current fish intake it contributes with 66%, 23%, 44%, and 30% of the total intake, respectively (see Chapter 8 for more details). The contributions from fish to intake of vitamin D and iodine are somewhat lower among children and adolescents than among adults. Fish is also a major contributor to the total intake of vitamin B₁₂. For LC n-3 FAs, vitamin D, and iodine there are few other natural sources in the diet besides fish. Selenium and vitamin B₁₂ are naturally present in numerous other foods.

At current fish intake, all included age groups have a relatively high proportion of individuals with an intake of vitamin D below AR. Women of childbearing age (18-45 years) and the 9- and 13-year-old girls have the highest proportion with an intake below AR (59% in women of childbearing age and 67% and 65% in 13- and 9-year-old girls, respectively). These population groups also have the highest proportion with an intake below AR of iodine and selenium at current fish intake. For iodine, the proportion below AR is 19% in women of childbearing age and 34% and 29% in 13- and 9-year-old girls, respectively. For selenium, the numbers are 7% in women of childbearing age, and 65% and 71% in 13- and 9-year-old girls, respectively. At current fish intake, all age groups have an intake of vitamin B₁₂ above AR. At current fish intake, 18% of the women of childbearing age (18-45 years), and 10% of adults (18-70 years) have intakes of EPA plus DHA below an adequate intake.

In summary, at current intake of fish, several groups of the Norwegian population have an intake of vitamin D, iodine, and selenium below AR and below adequate intake for the LC n-3 FAs EPA plus DHA. The population groups with the highest proportion below AR are women of childbearing age and adolescent girls (9- and 13-year-olds).

Among adults, fish is the most important single contributor to PCDD/Fs and DL-PCBs, and PFASs. Fish intake contributes with approximately 40% of the intake of both these

contaminant groups, with some variation between age groups. However, these contaminants are also present in numerous other foods. At current fish intake, more than 96% of the population (all age groups) have an estimated exposure to PCDD/Fs and DL-PCBs above the TWI. For PFASs, 86% of Norwegian adults exceed the TWI at current fish intake, while for methyl mercury, the proportion exceeding the TWI at the current intake of fish is 4% for adults.

In summary, at the current intake of fish, a large proportion of the Norwegian population exceed the TWIs for PCDD/Fs and DL-PCBs, and PFASs, for which fish is an important dietary source. However, there are many dietary sources of these contaminants, so even though a reduction of fish intake probably will cause some reduction in the exposure, it may not suffice to get an exposure below the TWIs.

The critical endpoints linked to intake of PCDD/Fs and DL-PCBs, and PFASs, above TWIs (sperm concentration and vaccine response, respectively) are relevant health issues, but the contribution of these specific endpoints to the combined death and disability adjusted life year burden (as DALYs) has not been estimated. Reduced sperm concentration from PCDD/F and DL-PCB exposure is potentially a contributing factor to reduced male fertility, but infertility in general accounts for a minor part of the burden of disease in Norway. A reduced response to vaccination may be used as a marker of an attenuated immune system, which have several health consequences. The general applicability of this endpoint as well as the size and severity of the effect in terms of disease burden is not known.

We emphasize that the population groups included in our intake estimates have been limited to those included in the national dietary surveys. Unfortunately, they do not cover all groups at risk of low intake of nutrients, e.g., specific immigrant groups and elderly (older than 70 years) in which studies have shown low vitamin D status due to low intake of vitamin D and/or limited sun exposure.

VKM's conclusion is based on systematic reviews and weight of evidence analyses of associations between fish intake, fatty fish intake and health outcomes, and includes a quantitative assessment of fish intake and health outcomes with incidence rates and mortality as common metrics. Additionally, we have conducted systematic literature reviews and weight of evidence analyses for nutrients, and included semi-quantitative assessments of the nutrients LC n-3 FA, vitamin D, iodine, selenium, and vitamin B₁₂ and of the contaminants PCDD/Fs and DL-PCBs, PFASs and methyl mercury, all substances of which fish intake is an important source.

VKM concludes that fish intake is beneficial and protective against several health outcomes that present important public health challenges in Norway. For these outcomes, the evidence is **graded "probable" which is considered strong evidence** according to the World Cancer Research Fund's grading system. The evidence for beneficial effects of fatty fish intake was weaker than for total fish intake.

However, the evidence is substantiated by strong evidence (**"probable"**) for beneficial effects of LC n-3 FAs intake on several of the same health outcomes as for fish.

The outcomes included in the quantitative assessment are generally chronic non-communicable diseases affecting the older age groups (except for preterm birth). However, these diseases may have a long latency period. Also, dietary behaviour tends to track from young age into adulthood. These factors support that recommended fish intake already in young age may be of importance for intake later in life and for later health benefit.

VKM concludes that fish intake at the current mean level in Norway has beneficial health effects when compared to lower intakes (scenario 1, 150 g/week). However, current fish intake is below the recommended weekly intake at 300-450 g (including at least 200 g fatty fish) for large groups of the population. For these groups, increasing the intake to meet recommendations is estimated to have an additional benefit.

12.2 Terms of reference 2: Which health consequences will occur for the Norwegian population if the population increases the consumption of fish to match the recommendations given by the Norwegian Directorate of Health?

The Norwegian Directorate of Health recommends fish as dinner meal 2-3 times per week for all age groups. Fish as bread spread is also recommended. Six portions of bread spreads represent approximately one dinner portion. Translated into grams the recommendations represent 300-450 grams prepared fish filet per week for adults, of which at least 200 grams should be fatty fish. The recommendations are not specified in grams for children.

Mathematical modelling indicates that increasing intake of fish to recommended intakes, and especially towards the upper range of recommended intake, 450 g per week in scenario 3 will reduce the number of new cases of CHD and stroke, non-communicable diseases that are important contributors to the burden of disease in Norway. Increasing intake of fish towards recommended intake is also estimated to reduce the number of new cases of dementia and Alzheimer's disease, both cognitive disorders which are increasing in the population as the proportion of elderly is increasing. The modelling indicates that an increase in fish intake from the current level to the recommended level will lead to a small reduction in all-cause mortality, but negligible reductions for CVD and CHD mortality. This is because the underlying dose-response relationship was flat for higher intakes. Moreover, the modelling indicates that incidences of preterm birth will decrease with increased intake of fish in scenario 2 and 3 for women.

The proportion of the population with an intake below AR for selenium and iodine, and below adequate intake for the LC n-3 FAs EPA plus DHA, will be reduced in the population if the intake of fish increases towards the recommendations. Although the low mean intake of vitamin D will not necessarily be rectified by increasing fish intake, an increasing intake of especially fatty fish could be of importance for those with the lowest vitamin D intakes.

Increased fish intake towards the recommended intake is estimated to increase exposure to PCDD/Fs and DL-PCBs, and PFASs, to a level where almost everyone in all age groups will exceed the TWIs. For adults, the increase in exceedance of the TWI with increased fish consumption is estimated to be moderate, i.e., from 2.3 times the TWI to 2.8 times the TWI for PCDD/Fs and DL-PCBs, and from 1.7 times the TWI to 1.9 times the TWI for PFASs. Our simple fish intake scenarios, give an overestimation of exposure as they are calculated with an addition of fish and no replacement of other foods.

VKM's conclusion is based on a systematic review and weight of evidence

analyses of associations between fish intake, fatty fish intake and health outcomes, and includes a quantitative assessment of fish intake and health outcomes with incidence rates and mortality as common metrics. Additionally, we have conducted systematic literature reviews and weight of evidence analyses for nutrients, and included semi-quantitative assessments of the nutrients LC n-3 FA, vitamin D, iodine, selenium, and vitamin B₁₂ and of the contaminants PCDD/Fs and DL-PCBs, PFASs, and methyl mercury, all substances of which fish intake is an important source.

VKM concludes that fish intake is beneficial and protective against several health outcomes that present important public health challenges in Norway. For these outcomes, the evidence is **graded "probable" which is considered strong evidence** according to the World Cancer Research Fund's grading system. The evidence for beneficial effects of fatty fish intake is weaker than for total fish intake. However, the evidence is substantiated by strong evidence (**"probable"**) for beneficial effects of LC n-3 FAs intake on several of the same health outcomes as for fish.

Increase in fish consumption up to recommended weekly intakes of 300-450 grams fish is expected to have a beneficial impact on several important public health challenges in the form of reduced incidence of CHD and stroke, dementia **including Alzheimer's disease**, preterm birth, and lower all-cause mortality.

The outcomes included in the quantitative assessment are generally chronic non-communicable diseases affecting the older age groups (except for preterm birth). However, these diseases may have a long latency period. Also, dietary behaviour tends to track from young age into adulthood. These factors support that fish intake already in young age may be of importance for intake later in life and for later health benefit.

VKM concludes that the benefits from increasing fish intake to the recommended two to three dinner courses per week (corresponding to 300-450 grams, including at least 200 grams fatty fish in adults) outweigh the risks for all age groups.

13 Data gaps detected in this benefit and risk assessment

In the subchapters below, we highlight data gaps related to specific parts of this benefit and risk assessment. First, to the quantitative assessment of fish intake and health outcomes (Chapter 13.1) which is considered the main part of this benefit and risk assessment, secondly to the semi-quantitative assessments of nutrients and contaminants (Chapter 13.2), and finally other data gaps revealed (Chapter 13.3).

Specific fish intake recommendations in terms of grams per week for children are missing.

13.1 Data gaps relating to the quantitative assessment of fish intake

13.1.1 Data on fish intake and health outcomes

The literature review revealed several shortcomings with the current basis for drawing conclusions about associations between fish consumption and health outcomes, and we want to highlight these data gaps:

- A limited number of epidemiological studies have investigated the health outcomes related to subgroups of fish such as fatty and lean fish
- For several of the included health outcomes there are too few studies to draw conclusions
- Few studies investigating fish consumption and health outcomes related to the critical endpoints for contaminants in fish, such as semen quality parameters and immune response
- Dose-response studies for fish consumption and health outcomes for intake of fatty and lean fish
- For neurodevelopmental outcomes there is a lack of consensus/standardization of test procedures
- A lack of coherent definitions of some disease endpoints, and lack of data on disease subgroups A lack of coherent definitions of some disease endpoints, and lack of data on disease subgroups (e.g., T2D)
- Of the 270 included primary studies, only 10 were graded A (see Appendix III, Chapter 16 for quality criteria)

With better quality and more detailed evidence on all relevant health outcomes, the strength and precision of the conclusions would have been better. A better understanding of the dose-response relationship between fish intake and the various health outcomes would have reduced the uncertainty in the quantitative assessment.

13.1.2 Methodology for the quantitative modelling

- Established method for an integrated quantitative modelling that includes food/food groups and single compounds such as nutrients and contaminants without doubling the beneficial or adverse effects from nutrients or contaminants
- Established models for inclusion of the single compounds PCDD/Fs and DL-PCBs, PFASs, vitamin D, selenium, and iodine, and their related health outcomes in the quantitative model

A better understanding of the dose-response relationship between fish intake and the various health outcomes would have reduced the uncertainty in the quantitative assessment.

Available established models for PCDD/Fs and DL-PCBs, PFASs, vitamin D, selenium and iodine, and their related health outcomes, would have enabled a fully integrated quantitative assessment, which is the preferred benefit-risk approach.

13.1.3 Fish intake data

- More validation studies of specific food items as fish intake are needed. This could be by relative comparison of different dietary assessment methods and by use of biological markers (plasma concentration of EPA and DHA)
- Norkost 3 is ten years old, more frequent updated dietary surveys are continuously needed. The next national dietary survey Norkost 4, is planned to be conducted in 2022
- Lack of intake data for special groups, i.e., elderly people, pregnant women, and immigrant groups. The next national dietary survey Norkost 4 will include elderly people and immigrant groups, and will contribute to fulfil these data gaps

More updated and precise data on fish intake, would have improved the estimations both for the quantitative assessment of fish, and also the semi-quantitative assessments of intakes of nutrients and exposure to contaminants

13.2 Data gaps related to the semi-quantitative assessments of nutrients and contaminants

13.2.1 Nutrient intake assessments

- For many foods (mostly other than fish), analysed concentration data are lacking, and only estimated or declared concentration data of nutrients are available
- In general, better dietary survey data and better concentration data on all included nutrients, i.e., vitamin D, LC n-3 FAs, iodine, selenium, and vitamin B₁₂ are wanted
- Concentration of nutrients in Alaskan pollock and Atlantic Argentine, usual ingredients in various fish products in Norway

Better data on nutrient intakes would have improved the semi-quantitative assessment of nutrients and reduced uncertainties.

13.2.2 Contaminant exposure assessments

- Concentration of contaminants in Alaskan pollock and Atlantic Argentine, usual ingredients in various fish products in Norway, are needed for all contaminants included (PCDD/F and DL-PCBs, PFASs and methyl mercury)
- In general, occurrence data on PCDD/F and DL-PCB and PFASs covering more food groups is needed
- In particular for PCDD/F and DL-PCB, there is a need for more and updated concentration data on cod roe-liver pâté and the bread spread "Kaviar" (consists of roe) frequently eaten by the youngest age groups
- There is a lack of biomonitoring data, i.e., concentrations in blood and breastmilk, of PCDD/Fs and DL-PCBs in the Norwegian population (the most recent are 16 years old). Especially data for women of childbearing age is needed
- For PFASs, concentration data in all food groups based on analytical methods with lower detection limits is needed
- There is a need for better data on consumption of seldomly eaten foods that may contain high concentrations of contaminants (e.g., brown crab meat, freshwater fish, and seagull eggs)

Better data on contaminant concentrations in foods and better consumption data would decrease the uncertainty in the exposure estimates and thereby the uncertainty in the semi-quantitative assessment of contaminants in various age groups. Particularly for PFASs, the uncertainties would have been reduced substantially.

Biomonitoring data for PCDD/F and DL-PCBs, would decrease the uncertainty by obtaining knowledge about whether Norwegians have higher exposure due to the high fish consumption relative to most European countries. Furthermore, continued biomonitoring of PCDD/Fs and DL-PCBs as well as PFASs are necessary to update time trends in exposure.

13.2.3 Other data gaps related to the semi-quantitative assessment of included nutrients and contaminants

13.2.3.1 *Nutrients and health outcomes*

- There is a lack of RCTs performed in populations with vitamin D deficiency
- Future RCTs investigating health effects of LC n-3 should assess participants habitual intake of these fatty acids before intervention to better understand why some respond better to intervention than others

Such data could have helped to evaluate the health effects related to vitamin D and LC n-3 FAs.

- Average requirement (AR) for LC n-3 FA is missing
- ARs for children from NNR are missing for iodine, selenium, and vitamin B₁₂ and several other nutrients. The ARs for children from USA are often based on ARs for adults that differs from ARs for adults in NNR (2012).

An AR for LC n-3FA and better ARs for children would have improved the semi-quantitative assessment of the fatty acids in all age groups, and all nutrients except vitamin D for children.

13.2.3.2 Contaminants and health outcomes

- There is a general lack of large population studies in which both fish consumption and exposure to contaminants in fish are analysed in relation to health outcomes

Such data could have helped to evaluate the negative effects related to presence of contaminants in fish, and to know whether beneficial effects of fish are attenuated by contaminant exposure.

- There is no available description of dose-response relationships of PCDD/F and DL-PCB exposure and disease (infertility) related to the critical endpoint used as basis for the TWI (reduced sperm concentration)
- There is no available description of dose-response relationships of PFAS exposure and increased infectious disease risk

Such data would have enabled incorporation of exposure to these contaminants into the quantitative benefit-risk assessment.

13.2.4 Data gaps for contaminants that were not included due to lack of data

VKM evaluated several contaminant groups that were not included in the final assessment (see Chapter 2 and Chapter 17, Appendix IV). The most important data gaps related to these contaminants are listed below. Moreover, we highlight the lack of a risk assessments for relevant contaminants, as indicated in Figure 2.3.1-1:

- There is a general lack of studies on epigenomic/transgenerational effects of contaminants on health outcomes
- There is lack of hazard and occurrence data on some forms of organic arsenic, such as arsenolipids and arsenosugars
- There is lack of occurrence data for siloxanes and phthalates in fish
- There is a need for a risk assessment of transformation products of the antioxidants BHA and BHT, which may be present in farmed fish
- There is a need for an updated risk assessment of non-DL PCBs that considers new hazard information
- The risk assessments of the legacy pesticides DDT and chlordane may need to be updated to include new hazard information

New or updated risk assessments may change the outcome of the process of evaluating which contaminants to include in the semi-quantitative risk assessment of contaminants with substantial contribution from fish consumption.

14 Appendix I Risk characterisation of PCDD/Fs alone

The exposure to PCDD/Fs alone (17 congeners) are shown in the Tables 23-1 to 23-3. Since the association for the critical effect used to derive the TWI was only seen for the PCDD/Fs, and the TEF-factor for the most relevant DL-PCB (i.e.PCB-126) may become lower (the TEFs are under revision by WHO, see Chapter 4); exposure to PCDD/Fs alone may become of higher relevance. When considering the PCDD/Fs alone, the proportion of the adult population (74%) and women aged 18-45 years (71%) estimated to be above TWI is lower than for the 29 congeners. The mean exposure to sum PCDD/Fs is 2.6 pg TEQ/kg bw per week in both these age groups. The magnitude of the exceedance of the TWI for PCDD/Fs and DL-PCBs depends on the results of the on-going revision of the TEF factors.

When considering exposures to the 17 congeners of PCDD/Fs only, the mean exposure ranges from 2.6 to 7.2 pg TEQ_{WHO2005}/kg bw/week for adults and women in childbearing age, and children, respectively. Thus the exposure is lower, but still above the TWI.

Table 23-1 Exposure to PCDD/F alone (pg TEQ_{WHO2005}/kg bw/week, upper bound) and proportion exceeding the TWI in all adults and women in childbearing age (Norkost 3) presented as mixed model data from current dietary intake, and with altered fish intake (scenario 1, 2, and 3).

PCDD/Fs (17 congeners)										
	Age	Sex	Mean	SD	P05	P25	Median	P75	P95	>TWI (%)
Current	18+	F/M	2.6	0.9	1.4	2.0	2.5	3.1	4.3	74
Scenario 1	18+	F/M	2.3	0.6	1.5	1.9	2.3	2.7	3.5	67
Scenario 2	18+	F/M	2.9	0.7	2.0	2.4	2.8	3.3	4.1	94
Scenario 3	18+	F/M	3.0	0.7	2.1	2.6	3.0	3.4	4.2	97
Current	18-45	F	2.6	0.9	1.4	1.9	2.4	3.1	4.2	71
Scenario 1	18-45	F	2.5	0.6	1.6	2.0	2.4	2.8	3.6	76
Scenario 2	18-45	F	3.1	0.7	2.2	2.7	3.1	3.5	4.3	98
Scenario 3	18-45	F	3.3	0.7	2.3	2.8	3.2	3.7	4.5	99

Table 23-2 Exposure to PCDD/F alone (pg TEQ_{WHO2005}/kg bw/week, upper bound) among all 13-, 9- and 4-year-olds (Ungkost 3) presented as mixed model data in current situation, scenario 1, scenario 2, and scenario 3.

PCDD/Fs (17 congeners)										
	Age (years)	Sex	Mean	SD	P05	P25	Median	P75	P95	>TWI (%)
Current	13	F/M	3.0	1.2	1.4	2.1	2.8	3.6	5.3	78
Scenario 1	13	F/M	3.1	1.0	1.7	2.4	2.9	3.6	4.9	88
Scenario 2	13	F/M	3.8	1.0	2.4	3.1	3.7	4.4	5.7	99
Scenario 3	13	F/M	3.9	1.0	2.5	3.2	3.8	4.5	58	100
Current	9	F/M	4.2	1.4	2.3	3.3	4.1	5.1	6.7	98
Scenario 1	9	F/M	4.3	1.2	2.6	3.5	4.2	5.1	6.5	99
Scenario 2	9	F/M	5.2	1.2	3.5	4.4	5.1	5.9	7.4	100
Scenario 3	9	F/M	5.4	1.2	3.6	4.5	5.3	6.1	7.6	100
Current	4	F/M	6.7	1.6	4.3	5.5	6.5	7.7	9.5	100
Scenario 1	4	F/M	6.4	1.4	4.3	5.4	6.3	7.3	8.9	100
Scenario 2	4	F/M	7.8	1.4	5.6	6.8	7.7	8.6	10	100
Scenario 3	4	F/M	8.0	1.4	5.9	7.0	7.9	8.9	11	100

Table 23-3 Exposure to PCDD/F alone (pg TEQ_{WHO2005}/kg bw/week, upper bound) among 1- and 2-year-olds (Spedkost 3 and Småbarnskost 3) presented as weighted OIM data in current situation, scenario 1, scenario 2, and scenario 3.

PCDD/Fs (17 congeners)										
	Age	Sex	Mean	SD	P05	P25	Median	P75	P95	>TWI (%)
Current	2	F/M	7.2	3.1	3.5	5.1	6.8	8.7	12	100
Scenario 1	2	F/M	6.9	2.6	3.9	5.2	6.5	8.1	11	100
Scenario 2	2	F/M	8.6	2.7	5.4	6.9	8.2	9.7	13	100
Scenario 3	2	F/M	8.8	2.7	5.6	7.1	8.4	10	13	100
Current	1	F/M	7.1	3.4	2.8	4.7	6.5	8.7	13	98
Scenario 1	1	F/M	6.6	2.5	3.3	4.9	6.2	7.8	11	100
Scenario 2	1	F/M	8.1	2.5	4.8	6.4	7.8	9.5	13	100
Scenario 3	1	F/M	8.4	2.5	5.0	6.6	8.0	9.7	13	100

15 Appendix II: Search strategies

15.1 Preparatory search to identify outcomes and search terms

In Google Scholar we searched for "fish consumption systematic review", and in MEDLINE the following search was set up:

Database: Ovid MEDLINE(R) and In-Process & Other Non-Indexed Citations and Daily
<1946 to November 15, 2019> Search Strategy:

1. fish consumption.mp. (2909)
2. systematic review.mp. or "Systematic Review"/ (156198)
3. meta-analysis.mp. or Meta-Analysis/ (169059)
4. 2 or 3 (254932)
5. 1 and 4 (110)
6. limit 5 to yr="2010 -Current" (88)

15.2 Fish consumption and health outcomes - primary studies)

15.2.1 Original search

Contact person:	Kirsten Eline Rakkestad and Bente Mangschou
Search:	Trude Anine Muggerud and Ragnhild Agathe Ternes
Referee:	Astrid Nøstberg
Comment:	At the request of the client, a search was made for the intake of fish combined with various relevant outcomes and limited to English, Norwegian, Swedish, Danish, German and French. Animal studies are also omitted.
Duplicate check in	Before: 30 558
EndNote:	After: 21 857

Pico:

What is the question that the literature search is meant to answer?	Question in PICO format				Known relevant outcomes
	Population	Intervention	Comparison	Outcome	

What could be the potential health consequences if the Norwegian population maintains, increases, or reduces their consumption of fish		Fish intake		CVD-outcomes Mortality Neurodevelopmental outcomes Birth outcomes Type 2 diabetes Bone health Dental enamel changes Overweight and obesity Immunological diseases Male fertility	
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Database: Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Daily and Versions(R) <1946 to November 22, 2019>

Date: 25.11.2019

Number of hits: 13 379

#	Searches	Results
1	Fishes/	61460
2	("Fishes" or "Fish").tw,kf.	171534
3	exp Trout/ or exp Salmon/ or Flounder/ or Perciformes/ or Gadus Morhua/ or Carps/ or Tuna/ or Perches/ or Esocidae/ or Anguilla/ or Fish products/	47633
4	("Trout?" or "Salmo trutta" or "Oncorhynchus mykiss" or "Salmo mykiss" or "Salmon?" or "salmo salar" or "Oncorhynchus" or "halibut?" or "flounder?" or "European plaice?" or "Hippoglossus hippoglossus" or "Pleuronectes platessa" or "platichthys flesus" or "Mackerel?" or "scomber scombrus" or "Haddock?" or "Melanogrammus aeglefinus" or "Saithe?" or "Pollachius virens" or "Cod?" or "Gadus Morhua" or "Codling?" or "Stockfish*" or "Cliffish*" or "Pollachius pollachius" or "Pollock?" or "Pollack?" or "Carp?" or "cyprinus carpio" or "merluccius merluccius" or "hake?" or "xiphias gladius" or "swordfish*" or "Tuna" or "Katsuwonus pelamis" or "Thunnus thynnus" or "Perch*" or "Perciform*" or "Perca fluviatilis" or "Clupea harengus" or "Herring?" or "Argentina silus" or "Salmo silus" or "Greater argentine" or "smelt?" or "Atlantic argentine" or "Wolffish*" or "Seawolf?" or "Anarhichadidae" or "anarhichas lupus" or "Esocidae" or "Esox lucius" or "Pike?" or "Lophius piscatorius" or "Anglerfish*" or "Monkfish*" or "Anguilla anguilla" or "Eel?" or "Conger conger" or "Sardina pilchardus" or "Sardine?" or "pilchard?" or "Anchov*" or "Engraulis encrasicolus" or "Sprattus sprattus" or "European sprat" or "Brosme brosme" or "Merlangius merlangius" or "Whiting" or "fishproduct?").tw,kf.	169906
5	or/1-4	335346
6	Eating/ or exp Meals/ or Diet/	203519
7	("eat*" or "ate" or "intake?" or "consumption" or "consume?" or "consuming" or "ingestion" or "meal?" or "diet*" or "dine" or "dinner?" or "lunch*" or "breakfast?" or "snack?").tw,kf.	1158373
8	6 or 7	1200355
9	5 and 8	43283
10	Bone density/ or exp Bone Diseases, metabolic/ or exp Fractures, bone/ or Accidental Falls/	280663

#	Searches	Results
11	("Osteoporosis" or "Rickets" or "Osteomalacia" or "vitamin D deficienc*" or (bone adj2 ("disease?" or "density" or "fracture?" or "fragil*" or "broken" or "deminerali#ation?" or "decalcification?")) or "Accidental Fall*" or (("Slip?" or "trip?") adj2 "fall*")).tw,kf.	147845
12	exp Human development/ or Child Development/ or Motor disorders/ or Psychomotor Disorders/ or exp Psychomotor Performance/ or Cognition/ or Cognitive dysfunction/ or exp Neurocognitive disorders/ or Mental health/ or exp Academic performance/ or exp Child behavior/ or Impulsive Behavior/ or "Inhibition (Psychology)"/ or exp Language disorders/ or Mental disorders/ or Behavioral Symptoms/ or Behavior/ or Anxiety disorders/ or exp "Bipolar and related disorders"/ or Anger/ or Affect/ or Depression/ or Mood disorders/ or Aggression/ or exp Schizophrenia/ or exp Neurodevelopmental Disorders/ or exp Autism spectrum disorder/ or Attention deficit disorder with hyperactivity/ or Attention/ or Learning/ or Reading/ or Mathematics/ or Aptitude tests/ or Language tests/ or Communication/ or Language/ or Language development/ or Child language/ or Literacy/ or Intelligence/ or Executive function/ or Social behavior/ or Social adjustment/ or Emotional intelligence/ or Emotions/ or Temperament/ or exp Amnesia/ or Memory Disorders/ or Dementia/ or Alzheimer disease/ or Memory, Short-Term/ or Memory, Long-term/	1489510
13	((("Child*" or "infant*" or "f?etal" or "prenatal" or "pre natal" or "postnatal" or "post natal" or "human" or "antepartum period?" or "ante partum period?") adj3 "development?") or "inhibition" or ("brain" adj2 ("damage?" or "injur*" or "development?" or "disorder?")) or "psychomotor" or "psycho motor" or "motor" or "sensorimotor" or "sensori motor" or "sensorymotor" or "sensory motor" or "cognition" or "cognitive function?" or "Mental health" or "Disorder? of higher cerebral function?" or ("psychological" adj ("well being" or "wellbeing")) or (("neurocognit*" or "neuro cognit*" or "neurological" or "nervous system" or "nervoussystem" or "cognitive" or "development*" or "mental") adj2 ("dysfunction?" or "function?" or "decline?" or "deterioration?" or "Defici*" or "illness*" or "retardation?" or "disturbance?" or "impairment?" or "disorder?" or "impact?" or "disabilit*" or "deviation?" or "development?")) or "neurodevelopment*" or "neuro development*" or "autis*" or "asperger" or "kanner?" or "ASD" or "attention deficit" or "hyperactiv*" or "ADDH" or "ADHD" or "AD/HD" or "ADD" or "minimal brain dysfunction" or "impulsiveness" or "dyslexia" or "dyslexic?" or "dyscalculia" or "dyscalculic?" or "attention" or "learning" or "reading" or "mathematic?" or "math" or "maths" or ("aptitude" adj1 "test?") or (("Education" or "Educational" or "academic" or "school") adj1 ("Status" or "attainment?" or "achievement?" or "performance?" or "underachievement?" or "under achievement?" or "score?" or "success*" or "failure?")) or "executive function?" or "information processing" or "school readiness" or "school ready" or "Emotion*" or "socialemotional" or "social emotional" or "socioemotional" or "socio emotional" or ("social" adj ("development?" or "behavio?r" or "adjustment?")) or ("intel?ectual" adj2 ("development?" or "deficien*" or "disorder?" or "retardation?" or "disabilit*" or "disturbance?" or "impairment?")) or "Communication" or "language?" or "literacy" or "literacies" or "IQ" or "intel?igence" or "Speech disorder?" or "mutism?" or "aphasia" or "stutter*" or "dysphasia" or "alexia" or "anxiet*" or "depression?" or "depressive" or "mood disorder?" or "schizophrenia" or "schizophrenic" or "aggression" or "behavio?r*" or "affect" or "anger" or "bipolar" or "Temperament?" or "personalit*" or "amnesia" or "dementia" or "Alzheimer?" or "Parkinson?" or "huntington?" or ("memory" adj3 ("disorder?" or "impairment?" or "disturbance?" or "deficianc*" or "disabilit*" or "short term" or "shortterm" or "long term" or "longterm" or "verbal recognition?"))).tw,kf.	6453705
14	exp Cardiovascular diseases/ or Cerebrovascular disorders/ or exp Ischemia/ or exp Stroke/	2351074

#	Searches	Results
15	((("Cardiovascular" or "heart" or "cardiac" or "myocardial" or "myo cardial" or "cerebrovascular" or "vascular" or "coronary" or "cerebral" or "peripheral" or "endothelial") adj ("disease?" or "disorder?" or "failure" or "event?" or "health" or "effect?" or "accident?" or "calcification?" or "risk factor?" or "riskfactor?" or "syndrom?" or "syndrome?" or "revasculari#ation?" or "arter*" or "function?" or "dysfunction?" or "attack?" or "arrest" or "apoplex*" or "insufficienc*" or "injur*" or "insult?" or "scleros#s" or "stenos#s" or "restenos#s")) or "cardioprotect*" or "cardio protect*" or "high cardiovascular risk?" or "CVD" or "infarct*" or "reinfarction?" or "aneurysm?" or "angina" or "artherosclero*" or "arthero sclero*" or "arteriosclero*" or "arterio sclero*" or "isch?emi*" or "nonisch?emi*" or "non isch?emic" or "thrombos#s" or "thrombolism?" or "tachycardia*" or "tachyarrhythmia?" or "arrhythmia?" or ((("ventricular" or "arterial") adj ("fibrillation?" or "compliance?" or "stiffness*")) or "sudden cardiac death?" or "stroke?" or "TIA" or ("brain" adj ("h?emorrhage?" or "accident?" or "attack?" or "infarct*" or "insult?")))).tw,kf.	1759689
16	exp Dental Enamel/ or exp Dental Enamel Hypoplasia/ or Tooth Discoloration/	24170
17	((("dental" or "tooth" or "teeth" or "enamel") adj1 ("enamel" or "discolo?ration?" or "malformation?" or "opacit*")) or "hypo?minerali#ation" or ("developmental" adj3 ("dental" or "teeth" or "tooth" or "enamel") adj3 "defect?")).tw,kf.	29565
18	exp Immunity/ or Respiratory Sounds/ or exp Asthma/ or exp Psoriasis/ or exp Eczema/ or Dermatitis/ or exp Arthritis, Rheumatoid/ or Antibodies, antinuclear/ or exp Respiratory Tract Infections/ or exp Multiple sclerosis/ or Lupus Erythematosus, Systemic/ or Scleroderma, Localized/ or Scleroderma, Systemic/	1065950
19	("immunolog*" or "infection resistance" or "immunity" or "autoimmunity" or "auto immunity" or "immunodeficienc*" or "immuno deficienc*" or ("immun*" adj ("system" or "status" or "defense?" or "defence?" or "deficienc*")) or "vaccination response?" or ((("upper" or "lower") adj "respiratory tract infection?" or "respiratory Sound?" or "wheez*" or "asthma*" or "psoriasis" or "eczema*" or "dermatiti*" or "rheumatoid arthritis" or (((("sjogren?" or "sicca") adj "syndrome?" or "syndrom?") or "Antinuclear antibod*" or "Multiple scleros#s" or "Systemic lupus erythematosus" or (("Scleroderma" or "scleros#s") adj1 ("localized" or "systemic")))).tw,kf.	1898586
20	Sperm count/ or Semen/ or exp Infertility, Male/ or exp Spermatozoa/ or Sexual maturation/ or Puberty/	119303
21	("Sperm?" or "semen" or "seminal fluid?" or "ejacul*" or "spermatozo*" or "spermatids" or "spermatocytes" or "spermatogonia" or "Oligospermia" or "Oligozoospermia*" or "Oligoasthenoteratozoospermia" or "asthenozoospermia?" or "asthenospermia?" or "criptozoospermia?" or "azoosperm*" or "globozoospermia?" or "teratospermia?" or "teratozoospermia?" or ((("man" or "male?" or "men") adj1 "infertil*") or "Sex* matur*" or "Pubert*")).tw,kf.	169521
22	Overweight/ or Obesity/ or Obesity, abdominal/ or Obesity, morbid/ or Adiposity/ or Adipocytes/ or Body weight changes/ or Weight gain/ or Pediatric obesity/ or exp Body size/	497001
23	("obesity" or "obesities" or "obese" or "obesitas" or "adipos*" or "fat overload" or "overweight" or "over weight" or "BMI" or "body mass index" or "bodymass index" or "lean body mass" or "lean bodymass" or "fatness" or "adipocyte?" or "lipocyte?" or ((("fat" or "lipid") adj cell?) or ("body" adj ("height?" or "size?" or "weight?")) or ("abdominal" adj ("diameter index" or "height")) or "sagit?al abdominal diameter?" or "height weight ratio?" or "waist circumference?" or "waist height ratio?" or "waist to height ratio?" or ("weight" adj1 ("change*" or "gain*")) or ("excess*" adj2 ("fat" or "weight")))).tw,kf.	754653
24	Birth weight/ or Pregnancy outcome/ or Premature birth/ or Growth/	117455
25	("growth" or ((("premature" or "pre term" or "preterm") adj "birth?") or "SGA" or ((("birth" or "gestational" or "neonatal" or "neo natal" or "newborn" or "new born" or "f?etal" or "f?etus" or "baby" or "babies") adj2 ("weight" or "size?")) or ((("Pregnancy" or "birth" or "obstetric") adj "outcome?")))).tw,kf.	1480176

#	Searches	Results
26	((("allerg*" or "hypersensitivit*" or "hyper sensitivit*" or "sensiti#ation*" or "atopic?" or "atopy" or "atopies") adj5 "prevention").tw,kf.	2899
27	exp Diabetes mellitus/	412389
28	("diabetes" or "sugar sickness" or "hypoglycemia" or "hypo glycemia" or "hyperglycemia" or "hyper glycemia").tw,kf.	542723
29	exp Goiter/	32486
30	("goiter? " or "goitre?").tw,kf.	20598
31	exp Mortality/	368554
32	("mortalit*" or "death rate?" or "deathrate?").tw,kf.	742658
33	or/10-32	13158760
34	9 and 33	23971
35	Animal/ not (animal/ and human/)	4612090
36	34 not 35	13983
37	limit 36 to (danish or english or french or german or multilingual or norwegian or swedish)	13379

Database: Embase 1974 to 2019 November 22

Date: 25.11.2019

Number of hits: 15 922

#	Searches	Results
1	Fishes/	83365
2	("Fishes" or "Fish").tw,kw.	208867
3	exp Trout/ or exp Salmon/ or Flounder/ or Perciformes/ or Gadus Morhua/ or Carps/ or Tuna/ or Perches/ or Esocidae/ or Anguilla/ or Fish products/	23484
4	("Trout?" or "Salmo trutta" or "Oncorhynchus mykiss" or "Salmo mykiss" or "Salmon?" or "salmo salar" or "Oncorhynchus" or "halibut?" or "flounder?" or "European plaice?" or "Hippoglossus hippoglossus" or "Pleuronectes platessa" or "platichthys flesus" or "Mackerel?" or "scomber scombrus" or "Haddock?" or "Melanogrammus aeglefinus" or "Saithe?" or "Pollachius virens" or "Cod?" or "Gadus Morhua" or "Codling?" or "Stockfish*" or "Clipfish*" or "Pollachius pollachius" or "Pollock?" or "Pollack?" or "Carp?" or "cyprinus carpio" or "merluccius merluccius" or "hake?" or "xiphias gladius" or "swordfish*" or "Tuna" or "Katsuwonus pelamis" or "Thunnus thynnus" or "Perch*" or "Perca fluviatilis" or "Perciform*" or "Clupea harengus" or "Herring?" or "Argentina silus" or "Salmo silus" or "Greater argentine" or "smelt?" or "Atlantic argentine" or "Wolfish*" or "Seawolf?" or "Anarhichadidae" or "anarhichas lupus" or "Esocidae" or "Esox lucius" or "Pike?" or "Lophius piscatorius" or "Anglerfish*" or "Monkfish*" or "Anguilla anguilla" or "Eel?" or "Conger conger" or "Sardina pilchardus" or "Sardine?" or "pilchard?" or "Anchov*" or "Engraulis encrasicolus" or "Sprattus sprattus" or "European sprat" or "Brosme brosme" or "Merlangius merlangius" or "Whiting" or "fishproduct?").tw,kw.	206110
5	or/1-4	406916
6	Eating/ or exp Meals/ or Diet/	255553
7	("eat*" or "ate" or "intake?" or "consumption" or "consume?" or "consuming" or "ingestion" or "meal?" or "diet*" or "dine" or "dinner?" or "lunch*" or "breakfast?" or "snack?").tw,kw.	1466052
8	6 or 7	1504332
9	5 and 8	53701
10	Bone density/ or exp Bone Diseases, metabolic/ or exp Fractures, bone/ or Accidental Falls/	445799
11	("Osteoporosis" or "Rickets" or "Osteomalacia" or "vitamin D deficienc*" or (bone adj2 ("disease?" or "density" or "fracture?" or "fragil*" or "broken" or "deminerali#ation?" or "decalciferation?")) or "Accidental Fall*" or (("Slip?" or "trip?") adj2 "fall*")).tw,kw.	209908
12	exp Human development/ or Child Development/ or Motor disorders/ or Psychomotor Disorders/ or exp Psychomotor Performance/ or Cognition/ or Cognitive dysfunction/ or exp Neurocognitive disorders/ or Mental health/ or exp Academic performance/ or exp Child behavior/ or Impulsive Behavior/ or "Inhibition (Psychology)"/ or exp Language disorders/ or Mental disorders/ or Behavioral Symptoms/ or Behavior/ or Anxiety disorders/ or exp "Bipolar and related disorders"/ or Anger/ or Affect/ or Depression/ or Mood disorders/ or Aggression/ or exp Schizophrenia/ or exp Neurodevelopmental Disorders/ or exp Autism spectrum disorder/ or Attention deficit disorder with hyperactivity/ or Attention/ or Learning/ or Reading/ or Mathematics/ or Aptitude tests/ or Language tests/ or Communication/ or Language/ or Language development/ or Child language/ or Literacy/ or Intelligence/ or Executive function/ or Social behavior/ or Social adjustment/ or Emotional intelligence/ or Emotions/ or Temperament/ or exp Amnesia/ or Memory Disorders/ or Dementia/ or Alzheimer disease/ or Memory, Short-Term/ or Memory, Long-term/	3110493

#	Searches	Results
13	(((("Child*" or "infant*" or "f?etal" or "prenatal" or "pre natal" or "postnatal" or "post natal" or "human" or "antepartum period?" or "ante partum period?") adj3 "development?") or "inhibition" or ("brain" adj2 ("damage?" or "injur*" or "development?" or "disorder?")) or "psychomotor" or "psycho motor" or "motor" or "sensorimotor" or "sensori motor" or "sensorymotor" or "sensory motor" or "cognition" or "cognitive function?" or "Mental health" or "Disorder? of higher cerebral function?" or ("psychological" adj ("well being" or "wellbeing")) or (("neurocognit*" or "neuro cognit*" or "neurological" or "nervous system" or "nervoussystem" or "cognitive" or "development*" or "mental") adj2 ("dysfunction?" or "function?" or "decline?" or "deterioration?" or "Defici*" or "illness*" or "retardation?" or "disturbance?" or "impairment?" or "disorder?" or "impact?" or "disabilit*" or "deviation?" or "development?")) or "neurodevelopment*" or "neuro development*" or "autis*" or "asperger" or "kanner?" or "ASD" or "attention deficit" or "hyperactiv*" or "ADDH" or "ADHD" or "AD/HD" or "ADD" or "minimal brain dysfunction" or "impulsiveness" or "dyslexia" or "dyslexic?" or "dyscalculia" or "dyscalculic?" or "attention" or "learning" or "reading" or "mathematic?" or "math" or "maths" or ("aptitude" adj1 "test?") or (("Education" or "Educational" or "academic" or "school") adj1 ("Status" or "attainment?" or "achievement?" or "performance?" or "underachievement?" or "under achievement?" or "score?" or "success*" or "failure?")) or "executive function?" or "information processing" or "school readiness" or "school ready" or "Emotion*" or "socialemotional" or "social emotional" or "socioemotional" or "socio emotional" or ("social" adj ("development?" or "behavio?r" or "adjustment?")) or ("intel?ectual" adj2 ("development?" or "deficien*" or "disorder?" or "retardation?" or "disabilit*" or "disturbance?" or "impairment?")) or "Communication" or "language?" or "literacy" or "literacies" or "IQ" or "intel?igence" or "Speech disorder?" or "mutism?" or "aphasia" or "stutter*" or "dysphasia" or "alexia" or "anxiet*" or "depression?" or "depressive" or "mood disorder?" or "schizophrenia" or "schizophrenic" or "aggression" or "behavio?r*" or "affect" or "anger" or "bipolar" or "Temperament?" or "personalit*" or "amnesia" or "dementia" or "Alzheimer?" or "Parkinson?" or "huntington?" or ("memory" adj3 ("disorder?" or "impairment?" or "disturbance?" or "deficianc*" or "disabilit*" or "short term" or "shortterm" or "long term" or "longterm" or "verbal recognition?"))).tw,kw.	7966130
14	exp Cardiovascular diseases/ or Cerebrovascular disorders/ or exp Ischemia/ or exp Stroke/	3905261
15	(((("Cardiovascular" or "heart" or "cardiac" or "myocardial" or "myo cardial" or "cerebrovascular" or "vascular" or "coronary" or "cerebral" or "peripheral" or "endothelial") adj ("disease?" or "disorder?" or "failure" or "event?" or "health" or "effect?" or "accident?" or "calcification?" or "risk factor?" or "riskfactor?" or "syndrom?" or "syndrome?" or "revasculari#ation?" or "arter*" or "function?" or "dysfunction?" or "attack?" or "arrest" or "apoplex*" or "insufficienc*" or "injur*" or "insult?" or "scleros#s" or "stenos#s" or "restenos#s")) or "cardioprotect*" or "cardio protect*" or "high cardiovascular risk?" or "CVD" or "infarct*" or "reinfarction?" or "aneurysm?" or "angina" or "atherosclero*" or "arthero sclero*" or "arteriosclero*" or "arterio sclero*" or "isch?emi*" or "nonisch?emi*" or "non isch?emic" or "thrombos#s" or "thrombolism?" or "tachycardia*" or "tachyarrhythmia?" or "arrhythmia?" or (("ventricular" or "arterial") adj ("fibrillation?" or "compliance?" or "stiffness*")) or "sudden cardiac death?" or "stroke?" or "TIA" or ("brain" adj ("h?emorrhage?" or "accident?" or "attack?" or "infarct*" or "insult?"))).tw,kw.	2440207
16	exp Dental Enamel/ or exp Dental Enamel Hypoplasia/ or Tooth Discoloration/	24761
17	(((("dental" or "tooth" or "teeth" or "enamel") adj1 ("enamel" or "discolo?ration?" or "malformation?" or "opacit*")) or "hypo?minerali#ation" or ("developmental" adj3 ("dental" or "teeth" or "tooth" or "enamel") adj3 "defect?"))).tw,kw.	28317

#	Searches	Results
18	exp Immunity/ or Respiratory Sounds/ or exp Asthma/ or exp Psoriasis/ or exp Eczema/ or Dermatitis/ or exp Arthritis, Rheumatoid/ or Antibodies, antinuclear/ or exp Respiratory Tract Infections/ or exp Multiple sclerosis/ or Lupus Erythematosus, Systemic/ or Scleroderma, Localized/ or Scleroderma, Systemic/	2377093
19	("immunolog*" or "infection resistance" or "immunity" or "autoimmunity" or "auto immunity" or "immunodeficienc*" or "immuno deficienc*" or ("immun*" adj ("system" or "status" or "defense?" or "defence?" or "deficienc*")) or "vaccination response?" or (("upper" or "lower") adj "respiratory tract infection?" or "respiratory Sound?" or "wheez*" or "asthma*" or "psoriasis" or "eczema*" or "dermatiti*" or "rheumatoid arthritis" or (((("sjogren?" or "sicca") adj "syndrome?") or "syndrom?") or "Antinuclear antibod*" or "Multiple scleros#s" or "Systemic lupus erythematosus" or (("Scleroderma" or "scleros#s") adj1 ("localized" or "systemic")))).tw,kw.	2528520
20	Sperm count/ or Semen/ or exp Infertility, Male/ or exp Spermatozoa/ or Sexual maturation/ or Puberty/	129442
21	("Sperm?" or "semen" or "seminal fluid?" or "ejacul*" or "spermatozo*" or "spermatids" or "spermatocytes" or "spermatogonia" or "Oligospermia" or "Oligozoospermia*" or "Oligoasthenoteratozoospermia" or "asthenozoospermia?" or "asthenospermia?" or "criptozoospermia?" or "azoosperm*" or "globozoospermia?" or "teratospermia?" or "teratozoospermia?" or (("man" or "male?" or "men") adj1 "infertil*" or "Sex* matur*" or "Pubert*")).tw,kw.	203032
22	Obesity/ or Abdominal obesity/ or Morbid obesity/ or Childhood obesity/ or Maternal obesity/ or Adolescent obesity/ or Body weight change/ or Body weight gain/ or Childhood obesity/ or Adipocyte/ or exp Body size/	510381
23	("obesity" or "obesities" or "obese" or "obesitas" or "adipos*" or "fat overload" or "overweight" or "over weight" or "BMI" or "body mass index" or "bodymass index" or "lean body mass" or "lean bodymass" or "fatness" or "adipocyte?" or "lipocyte?" or ("fat" or "lipid") adj cell?) or ("body" adj ("height?" or "size?" or "weight?")) or ("abdominal" adj ("diameter index" or "height?")) or "sagit?al abdominal diameter?" or "height weight ratio?" or "waist circumference?" or "waist height ratio?" or "waist to height ratio?" or ("weight" adj1 ("change*" or "gain*")) or ("excess*" adj2 ("fat" or "weight")))).tw,kw.	1105594
24	Birth weight/ or Pregnancy outcome/ or Premature birth/ or Growth/	189567
25	("growth" or (("premature" or "pre term" or "preterm") adj "birth?") or "SGA" or (("birth" or "gestational" or "neonatal" or "neo natal" or "newborn" or "new born" or "f?etal" or "f?etus" or "baby" or "babies") adj2 ("weight" or "size?")) or (("Pregnancy" or "birth" or "obstetric") adj "outcome?")).tw,kw.	1763942
26	((("allerg*" or "hypersensitivit*" or "hyper sensitivit*" or "sensiti#ation*" or "atopic?" or "atopy" or "atopies") adj5 "prevention").tw,kw.	4175
27	exp Diabetes mellitus/	915202
28	("diabetes" or "sugar sickness" or "hypoglycemia" or "hypo glycemia" or "hyperglycemia" or "hyper glycemia").tw,kw.	814988
29	exp Goiter/	22074
30	("goiter? " or "goitre? ").tw,kw.	20372
31	exp Mortality/	1025200
32	("mortalit*" or "death rate?" or "deathrate?").tw,kw.	1079973
33	or/10-32	17042583
34	9 and 33	31429
35	(animal/ or exp nonhuman/ or Animal experiment/) not ((animal/ or exp nonhuman/ or Animal experiment/) and exp human/)	5867700
36	34 not 35	19268
37	limit 36 to (conference abstracts or embase)	16382
38	limit 37 to (danish or english or french or german or norwegian or swedish)	15922

Database: PsycINFO
Date: 26.11.2019
Number of hits: 1439

#	Searches	Results
1	Fishes/	7256
2	("Fishes" or "Fish").tw.	10342
3	Salmon/ or "Bass (fish)"/	247
4	("Trout?" or "Salmo trutta" or "Oncorhynchus mykiss" or "Salmo mykiss" or "Salmon?" or "salmo salar" or "Oncorhynchus" or "halibut?" or "flounder?" or "European plaice?" or "Hippoglossus hippoglossus" or "Pleuronectes platessa" or "platichthys flesus" or "Mackerel?" or "scomber scombrus" or "Haddock?" or "Melanogrammus aeglefinus" or "Saithe?" or "Pollachius virens" or "Cod?" or "Gadus Morhua" or "Codling?" or "Stockfish*" or "Clipfish*" or "Pollachius pollachius" or "Pollock?" or "Pollack?" or "Carp?" or "cyprinus carpio" or "merluccius merluccius" or "hake?" or "xiphias gladius" or "swordfish*" or "Tuna" or "Katsuwonus pelamis" or "Thunnus thynnus" or "Perch*" or "Perca fluviatilis" or "Perciform*" or "Clupea harengus" or "Herring?" or "Argentina silus" or "Salmo silus" or "Greater argentine" or "smelt?" or "Atlantic argentine" or "Wolffish*" or "Seawolf?" or "Anarhichadidae" or "anarhichas lupus" or "Esocidae" or "Esox lucius" or "Pike?" or "Lophius piscatorius" or "Anglerfish*" or "Monkfish*" or "Anguilla anguilla" or "Eel?" or "Conger conger" or "Sardina pilchardus" or "Sardine?" or "pilchard?" or "Anchov*" or "Engraulis encrasicolus" or "Sprattus sprattus" or "European sprat" or "Brosme brosme" or "Merlangius merlangius" or "Whiting" or "fishproduct?").tw.	25620
5	or/1-4	37596
6	Food intake/ or Ingestion/ or Diets/ or Food intake/	28654
7	("eat*" or "ate" or "intake?" or "consumption" or "consume?" or "consuming" or "ingestion" or "meal?" or "diet*" or "dine" or "dinner?" or "lunch*" or "breakfast?" or "snack?").tw.	218666
8	6 or 7	220834
9	5 and 8	2766
10	Osteoporosis/ or exp Bone disorder/ or Falls/	4134
11	("Osteoporosis" or "Rickets" or "Osteomalacia" or "vitamin D deficienc*" or (bone adj2 ("disease?" or "density" or "fracture?" or "fragil*" or "broken" or "deminerali#ation?" or "decalcification?")) or "Accidental Fall*" or (("Slip?" or "trip?") adj2 "fall*")).tw.	3869
12	exp Human development/ or exp Childhood development/ or exp Prenatal development/ or Postnatal development/ or Nervous system disorders/ or Psychomotor development/ or Motor development/ or Cognition/ or Cognitive impairment/ or Cognitive development/ or exp Neurocognitive disorders/ or Mental health/ or exp Academic achievement/ or Child behavior/ or Behavior problems/ or Impulsiveness/ or "Inhibition (personality)"/ or exp Language disorders/ or Mental disorders/ or Behavior/ or Anxiety disorders/ or exp Bipolar disorder/ or Anger/ or Affection/ or "Depression (Emotion)"/ or Affective disorders/ or Aggressiveness/ or exp Schizophrenia/ or exp Neurodevelopmental Disorders/ or Attention/ or Learning/ or Reading/ or Mathematics/ or Aptitude Measures/ or Communication/ or Language/ or Language development/ or Literacy/ or Intelligence/ or Executive function/ or Social behavior/ or Social adjustment/ or Emotional intelligence/ or Emotions/ or Personality/ or exp Amnesia/ or Memory Disorders/ or Dementia/ or "Alzheimer's disease"/ or Short term memory/ or Long term memory/	1222907

#	Searches	Results
13	((("Child*" or "infant*" or "f?etal" or "prenatal" or "pre natal" or "postnatal" or "post natal" or "human" or "antepartum period?" or "ante partum period?") adj3 "development?") or "inhibition" or ("brain" adj2 ("damage?" or "injur*" or "development?" or "disorder?")) or "psychomotor" or "psycho motor" or "motor" or "sensorimotor" or "sensori motor" or "sensorymotor" or "sensory motor" or "cognition" or "cognitive function?" or "Mental health" or "Disorder? of higher cerebral function?" or ("psychological" adj ("well being" or "wellbeing")) or (("neurocognit*" or "neuro cognit*" or "neurological" or "nervous system" or "nervoussystem" or "cognitive" or "development*" or "mental") adj2 ("dysfunction?" or "function?" or "decline?" or "deterioration?" or "Defici*" or "illness*" or "retardation?" or "disturbance?" or "impairment?" or "disorder?" or "impact?" or "disabilit*" or "deviation?" or "development?")) or "neurodevelopment*" or "neuro development*" or "autis*" or "asperger" or "kanner?" or "ASD" or "attention deficit" or "hyperactiv*" or "ADDH" or "ADHD" or "AD/HD" or "ADD" or "minimal brain dysfunction" or "impulsiveness" or "dyslexia" or "dyslexic?" or "dyscalculia" or "dyscalculic?" or "attention" or "learning" or "reading" or "mathematic?" or "math" or "maths" or ("aptitude" adj1 "test?") or (("Education" or "Educational" or "academic" or "school") adj1 ("Status" or "attainment?" or "achievement?" or "performance?" or "underachievement?" or "under achievement?" or "score?" or "success*" or "failure?")) or "executive function?" or "information processing" or "school readiness" or "school ready" or "Emotion*" or "socialemotional" or "social emotional" or "socioemotional" or "socio emotional" or ("social" adj ("development?" or "behavio?r" or "adjustment?")) or ("intel?ectual" adj2 ("development?" or "deficien*" or "disorder?" or "retardation?" or "disabilit*" or "disturbance?" or "impairment?")) or "Communication" or "language?" or "literacy" or "literacies" or "IQ" or "intel?igence" or "Speech disorder?" or "mutism?" or "aphasia" or "stutter*" or "dysphasia" or "alexia" or "anxiet*" or "depression?" or "depressive" or "mood disorder?" or "schizophrenia" or "schizophrenic" or "aggression" or "behavio?r*" or "affect" or "anger" or "bipolar" or "Temperament?" or "personalit*" or "amnesia" or "dementia" or "Alzheimer?" or "Parkinson?" or "huntington?" or ("memory" adj3 ("disorder?" or "impairment?" or "disturbance?" or "deficianc*" or "disabilit*" or "short term" or "shortterm" or "long term" or "longterm" or "verbal recognition?"))).tw.	3088911
14	exp Cardiovascular disorders/ or Cerebrovascular disorders/ or Cerebrovascular accident/	60364
15	((("Cardiovascular" or "heart" or "cardiac" or "myocardial" or "myo cardial" or "cerebrovascular" or "vascular" or "coronary" or "cerebral" or "peripheral" or "endothelial") adj ("disease?" or "disorder?" or "failure" or "event?" or "health" or "effect?" or "accident?" or "calcification?" or "risk factor?" or "riskfactor?" or "syndrom?" or "syndrome?" or "revasculari#ation?" or "arter*" or "function?" or "dysfunction?" or "attack?" or "arrest" or "apoplex*" or "insufficienc*" or "injur*" or "insult?" or "scleros#s" or "stenos#s" or "restenos#s")) or "cardioprotect*" or "cardio protect*" or "high cardiovascular risk?" or "CVD" or "infarct*" or "reinfarction?" or "aneurysm?" or "angina" or "artherosclero*" or "arthero sclero*" or "arteriosclero*" or "arterio sclero*" or "isch?emi*" or "nonisch?emi*" or "non isch?emic" or "thrombos#s" or "thrombolism?" or "tachycardia*" or "tachyarrhythmia?" or "arrhythmia?" or (("ventricular" or "arterial") adj ("fibrillation?" or "compliance?" or "stiffness*")) or "sudden cardiac death?" or "stroke?" or "TIA" or ("brain" adj ("h?emorrhage?" or "accident?" or "attack?" or "infarct*" or "insult?"))).tw.	91719
16	((("dental" or "tooth" or "teeth" or "enamel") adj1 ("enamel" or "discolo?ration?" or "malformation?" or "opacit*")) or "hypo?minerali#ation" or ("developmental" adj3 ("dental" or "teeth" or "tooth" or "enamel") adj3 "defect?"))).tw.	77
17	exp Respiratory tract disorders/ or Eczema/ or exp Dermatitis/ or Rheumatoid arthritis/ or Asthma/	16589

#	Searches	Results
18	("immunolog*" or "infection resistance" or "immunity" or "autoimmunity" or "auto immunity" or "immunodeficienc*" or "immuno deficienc*" or ("immun*" adj ("system" or "status" or "defense?" or "defence?" or "deficienc*")) or "vaccination response?" or (("upper" or "lower") adj "respiratory tract infection?" or "respiratory Sound?" or "wheez*" or "asthma*" or "psoriasis" or "eczema*" or "dermatiti*" or "rheumatoid arthritis" or (((("sjogren?" or "sicca") adj "syndrome?" or "syndrom?") or "Antinuclear antibod*" or "Multiple scleros#s" or "Systemic lupus erythematosus" or ("Scleroderma" or "scleros#s") adj1 ("localized" or "systemic")))).tw.	139603
19	Sperm/ or Psychosexual development/ or Puberty/	6415
20	("Sperm?" or "semen" or "seminal fluid?" or "ejacul*" or "spermatozo*" or "spermatids" or "spermatocytes" or "spermatogonia" or "Oligospermia" or "Oligozoospermia*" or "Oligoasthenoteratozoospermia" or "asthenozoospermia?" or "asthenospermia?" or "criptozoospermia?" or "azoosperm*" or "globozoospermia?" or "teratospermia?" or "teratozoospermia?" or (("man" or "male?" or "men") adj1 "infertil*") or "Sex* matur*" or "Pubert*").tw.	14887
21	Overweight/ or Obesity/ or Adipocytes/ or Body Mass Index/ or Weight gain/ or exp Body size/	57278
22	("obesity" or "obesities" or "obese" or "obesitas" or "adipos*" or "fat overload" or "overweight" or "over weight" or "BMI" or "body mass index" or "bodymass index" or "lean body mass" or "lean bodymass" or "fatness" or "adipocyte?" or "lipocyte?" or ("fat" or "lipid") adj cell?) or ("body" adj ("height?" or "size?" or "weight?")) or ("abdominal" adj ("diameter index" or "height")) or "sagit?al abdominal diameter?" or "height weight ratio?" or "waist circumference?" or "waist height ratio?" or "waist to height ratio?" or ("weight" adj1 ("change*" or "gain*")) or ("excess*" adj2 ("fat" or "weight")))).tw.	79105
23	Birth weight/ or Pregnancy outcomes/ or Premature Birth/ or Development/	15445
24	("growth" or (("premature" or "pre term" or "preterm") adj "birth?") or "SGA" or (("birth" or "gestational" or "neonatal" or "neo natal" or "newborn" or "new born" or "f?etal" or "f?etus" or "baby" or "babies") adj2 ("weight" or "size?")) or (("Pregnancy" or "birth" or "obstetric") adj "outcome?"))).tw.	106935
25	((("allerg*" or "hypersensitivit*" or "hyper sensitivit*" or "sensiti#ation*" or "atopic?" or "atopyor atopies") adj5 "prevention").tw.	42
26	exp Diabetes mellitus/	8074
27	("diabetes" or "sugar sickness" or "hypoglycemia" or "hypo glycemia" or "hyperglycemia" or "hyper glycemia").tw.	30009
28	exp Goiters/	44
29	("goiter? " or "goitre? ").tw.	226
30	"Death and Dying"/	29933
31	("mortalit*" or "death rate?" or "deathrate?").tw.	40312
32	or/10-31	3351801
33	9 and 32	2225
34	(animal not (animal and human)).po.	355388
35	33 not 34	1489
36	limit 35 to (danish or english or french or german or norwegian or swedish)	1439

15.2.2 Updated search

Contact person: Kirsten Eline Rakkestad

Search: Trude Anine Muggerud

Referee: Ragnhild Agathe Ternes

Comment: Update of the fish intake search from november 2019.

Duplicate check in Before duplicate check: 5744

EndNote: After duplicate check: 4527

What is the question that the literature search is meant to answer?	Question in PICO format				Known relevant outcomes
	Population	Intervention	Comparison	Outcome	
What could be the potential health consequences if the Norwegian population maintains, increases, or reduces their consumption of fish		Fish intake		CVD-outcomes Mortality Neurodevelopmental outcomes Birth outcomes Type 2 diabetes Bone health Dental enamel changes Overweight and obesity Immunological diseases Male fertility	

Database: Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Daily and Versions(R) <1946 to October 07, 2021>

Date: 08.10.2021

Number of hits: 3275

#	Searches	
1	Fishes/	65803

#	Searches	
2	("Fishes" or "Fish").tw,kf.	192727
3	exp Trout/ or exp Salmon/ or Flounder/ or Perciformes/ or Gadus Morhua/ or Carps/ or Tuna/ or Perches/ or Esocidae/ or Anguilla/ or Fish products/	52047
4	("Trout?" or "Salmo trutta" or "Oncorhynchus mykiss" or "Salmo mykiss" or "Salmon?" or "salmo salar" or "Oncorhynchus" or "halibut?" or "flounder?" or "European plaice?" or "Hippoglossus hippoglossus" or "Pleuronectes platessa" or "platichthys flesus" or "Mackerel?" or "scomber scombrus" or "Haddock?" or "Melanogrammus aeglefinus" or "Saithe?" or "Pollachius virens" or "Cod?" or "Gadus Morhua" or "Codling?" or "Stockfish*" or "Clipfish*" or "Pollachius pollachius" or "Pollock?" or "Pollack?" or "Carp?" or "cyprinus carpio" or "merluccius merluccius" or "hake?" or "xiphias gladius" or "swordfish*" or "Tuna" or "Katsuwonus pelamis" or "Thunnus thynnus" or "Perch*" or "Perciform*" or "Perca fluviatilis" or "Clupea harengus" or "Herring?" or "Argentina silus" or "Salmo silus" or "Greater argentine" or "smelt?" or "Atlantic argentine" or "Wolffish*" or "Seawolf?" or "Anarhichadidae" or "anarhichas lupus" or "Esocidae" or "Esox lucius" or "Pike?" or "Lophius piscatorius" or "Anglerfish*" or "Monkfish*" or "Anguilla anguilla" or "Eel?" or "Conger conger" or "Sardina pilchardus" or "Sardine?" or "pilchard?" or "Anchov*" or "Engraulis encrasicolus" or "Sprattus sprattus" or "European sprat" or "Brosme brosme" or "Merlangius merlangius" or "Whiting" or "fishproduct?").tw,kf.	192791
5	or/1-4	376485
6	Eating/ or exp Meals/ or Diet/	224948
7	("eat*" or "ate" or "intake?" or "consumption" or "consume?" or "consuming" or "ingestion" or "meal?" or "diet*" or "dine" or "dinner?" or "lunch*" or "breakfast?" or "snack?").tw,kf.	1313605
8	6 or 7	1357035
9	5 and 8	49680
10	Bone density/ or exp Bone Diseases, metabolic/ or exp Fractures, bone/ or Accidental Falls/	305007
11	("Osteoporosis" or "Rickets" or "Osteomalacia" or "vitamin D deficienc*" or (bone adj2 ("disease?" or "density" or "fracture?" or "fragil*" or "broken" or "deminerali#ation?" or "decalcification?")) or "Accidental Fall*" or ((("Slip?" or "trip?") adj2 "fall*")).tw,kf.	165652
12	exp Human development/ or Child Development/ or Motor disorders/ or Psychomotor Disorders/ or exp Psychomotor Performance/ or Cognition/ or Cognitive dysfunction/ or exp Neurocognitive disorders/ or Mental health/ or exp Academic performance/ or exp Child behavior/ or Impulsive Behavior/ or exp Inhibition, Psychological/ or exp Language disorders/ or Mental disorders/ or Behavioral Symptoms/ or Behavior/ or Anxiety disorders/ or exp "Bipolar and related disorders"/ or Anger/ or Affect/ or Depression/ or Mood disorders/ or Aggression/ or exp Schizophrenia/ or exp Neurodevelopmental Disorders/ or exp Autism spectrum disorder/ or Attention deficit disorder with hyperactivity/ or Attention/ or Learning/ or Reading/ or Mathematics/ or Aptitude tests/ or Language tests/ or Communication/ or Language/ or Language development/ or Child language/ or Literacy/ or Intelligence/ or Executive function/ or Social behavior/ or Social adjustment/ or Emotional intelligence/ or Emotions/ or Temperament/ or exp Amnesia/ or Memory Disorders/ or Dementia/ or Alzheimer disease/ or Memory, Short-Term/ or Memory, Long-term/	1649580

#	Searches	
13	((("Child*" or "infant*" or "f?etal" or "prenatal" or "pre natal" or "postnatal" or "post natal" or "human" or "antepartum period?" or "ante partum period?") adj3 "development?") or "inhibition" or ("brain" adj2 ("damage?" or "injur*" or "development?" or "disorder?")) or "psychomotor" or "psycho motor" or "motor" or "sensorimotor" or "sensori motor" or "sensorymotor" or "sensory motor" or "cognition" or "cognitive function?" or "Mental health" or "Disorder? of higher cerebral function?" or ("psychological" adj ("well being" or "wellbeing")) or (("neurocognit*" or "neuro cognit*" or "neurological" or "nervous system" or "nervoussystem" or "cognitive" or "development*" or "mental") adj2 ("dysfunction?" or "function?" or "decline?" or "deterioration?" or "Defici*" or "illness*" or "retardation?" or "disturbance?" or "impairment?" or "disorder?" or "impact?" or "disabilit*" or "deviation?" or "development?")) or "neurodevelopment*" or "neuro development*" or "autis*" or "asperger" or "kanner?" or "ASD" or "attention deficit" or "hyperactiv*" or "ADDH" or "ADHD" or "AD/HD" or "ADD" or "minimal brain dysfunction" or "impulsiveness" or "dyslexia" or "dyslexic?" or "dyscalculia" or "dyscalculic?" or "attention" or "learning" or "reading" or "mathematic?" or "math" or "maths" or ("aptitude" adj1 "test?") or ("Education" or "Educational" or "academic" or "school") adj1 ("Status" or "attainment?" or "achievement?" or "performance?" or "underachievement?" or "under achievement?" or "score?" or "success*" or "failure?")) or "executive function?" or "information processing" or "school readiness" or "school ready" or "Emotion*" or "socialemotional" or "social emotional" or "socioemotional" or "socio emotional" or ("social" adj ("development?" or "behavio?r" or "adjustment?")) or ("intel?ectual" adj2 ("development?" or "deficien*" or "disorder?" or "retardation?" or "disabilit*" or "disturbance?" or "impairment?")) or "Communication" or "language?" or "literacy" or "literacies" or "IQ" or "intel?igence" or "Speech disorder?" or "mutism?" or "aphasia" or "stutter*" or "dysphasia" or "alexia" or "anxiet*" or "depression?" or "depressive" or "mood disorder?" or "schizophrenia" or "schizophrenic" or "aggression" or "behavio?r*" or "affect" or "anger" or "bipolar" or "Temperament?" or "personalit*" or "amnesia" or "dementia" or "Alzheimer?" or "Parkinson?" or "huntington?" or ("memory" adj3 ("disorder?" or "impairment?" or "disturbance?" or "deficianc*" or "disabilit*" or "short term" or "shortterm" or "long term" or "longterm" or "verbal recognition?"))).tw,kf.	7377550
14	exp Cardiovascular diseases/ or Cerebrovascular disorders/ or exp Ischemia/ or exp Stroke/	2565569
15	((("Cardiovascular" or "heart" or "cardiac" or "myocardial" or "myo cardial" or "cerebrovascular" or "vascular" or "coronary" or "cerebral" or "peripheral" or "endothelial") adj ("disease?" or "disorder?" or "failure" or "event?" or "health" or "effect?" or "accident?" or "calcification?" or "risk factor?" or "riskfactor?" or "syndrom?" or "syndrome?" or "revasculari#ation?" or "arter*" or "function?" or "dysfunction?" or "attack?" or "arrest" or "apoplex*" or "insufficienc*" or "injur*" or "insult?" or "scleros#s" or "stenos#s" or "restenos#s")) or "cardioprotect*" or "cardio protect*" or "high cardiovascular risk?" or "CVD" or "infarct*" or "reinfarction?" or "aneurysm?" or "angina" or "artherosclero*" or "arthero sclero*" or "arteriosclero*" or "arterio sclero*" or "isch?emi*" or "nonisch?emi*" or "non isch?emic" or "thrombos#s" or "thrombolism?" or "tachycardia*" or "tachyarrhythmia?" or "arrhythmia?" or (("ventricular" or "arterial") adj ("fibrillation?" or "compliance?" or "stiffness*")) or "sudden cardiac death?" or "stroke?" or "TIA" or ("brain" adj ("hemorrhage?" or "accident?" or "attack?" or "infarct*" or "insult?"))).tw,kf.	1957389
16	exp Dental Enamel/ or exp Dental Enamel Hypoplasia/ or Tooth Discoloration/	25464
17	((("dental" or "tooth" or "teeth" or "enamel") adj1 ("enamel" or "discolo?ration?" or "malformation?" or "opacit*")) or "hypo?minerali#ation" or ("developmental" adj3 ("dental" or "teeth" or "tooth" or "enamel") adj3 "defect?"))).tw,kf.	32018
18	exp Immunity/ or Respiratory Sounds/ or exp Asthma/ or exp Psoriasis/ or exp Eczema/ or Dermatitis/ or exp Arthritis, Rheumatoid/ or Antibodies, antinuclear/ or exp Respiratory Tract Infections/ or exp Multiple sclerosis/ or Lupus Erythematosus, Systemic/ or Scleroderma, Localized/ or Scleroderma, Systemic/	1290879

#	Searches	
19	("immunolog*" or "infection resistance" or "immunity" or "autoimmunity" or "auto immunity" or "immunodeficienc*" or "immuno deficienc*" or ("immun*" adj ("system" or "status" or "defense?" or "defence?" or "deficienc*")) or "vaccination response?" or (("upper" or "lower") adj "respiratory tract infection?") or "respiratory Sound?" or "wheez*" or "asthma*" or "psoriasis" or "eczema*" or "dermatiti*" or "rheumatoid arthritis" or (("sjogren?" or "sicca") adj ("syndrome?" or "syndrom?")) or "Antinuclear antibod*" or "Multiple scleros#s" or "Systemic lupus erythematosus" or ("Scleroderma" or "scleros#s") adj1 ("localized" or "systemic")).tw,kf.	1227293
20	Sperm count/ or Semen/ or exp Infertility, Male/ or exp Spermatozoa/ or Sexual maturation/ or Puberty/	126591
21	("Sperm?" or "semen" or "seminal fluid?" or "ejacul*" or "spermatozo*" or "spermatids" or "spermatocytes" or "spermatogonia" or "Oligospermia" or "Oligozoospermia*" or "Oligoasthenoteratozoospermia" or "asthenozoospermia?" or "asthenospermia?" or "criptozoospermia?" or "azoosperm*" or "globozoospermia?" or "teratospermia?" or "teratozoospermia?" or (("man" or "male?" or "men") adj1 "infertil*") or "Sex* matur*" or "Pubert*").tw,kf.	184344
22	Overweight/ or Obesity/ or Obesity, abdominal/ or Obesity, morbid/ or Adiposity/ or Adipocytes/ or Body weight changes/ or Weight gain/ or Pediatric obesity/ or exp Body size/	543913
23	("obesity" or "obesities" or "obese" or "obesitas" or "adipos*" or "fat overload" or "overweight" or "over weight" or "BMI" or "body mass index" or "bodymass index" or "lean body mass" or "lean bodymass" or "fatness" or "adipocyte?" or "lipocyte?" or (("fat" or "lipid") adj cell?) or ("body" adj ("height?" or "size?" or "weight?")) or ("abdominal" adj ("diameter index" or "height")) or "sagit?al abdominal diameter?" or "height weight ratio?" or "waist circumference?" or "waist height ratio?" or "waist to height ratio?" or ("weight" adj1 ("change*" or "gain*")) or ("excess*" adj2 ("fat" or "weight"))).tw,kf.	864410
24	Birth weight/ or Pregnancy outcome/ or Premature birth/ or Growth/	127408
25	("growth" or (("premature" or "pre term" or "preterm") adj "birth?") or "SGA" or (("birth" or "gestational" or "neonatal" or "neo natal" or "newborn" or "new born" or "f?etal" or "f?etus" or "baby" or "babies") adj2 ("weight" or "size?")) or (("Pregnancy" or "birth" or "obstetric") adj "outcome?").tw,kf.	1657056
26	((("allerg*" or "hypersensitivit*" or "hyper sensitivit*" or "sensiti#ation*" or "atopic?" or "atopy" or "atopies") adj5 "prevention").tw,kf.	3205
27	exp Diabetes mellitus/	456517
28	("diabetes" or "sugar sickness" or "hypoglycemia" or "hypo glycemia" or "hyperglycemia" or "hyper glycemia" or diabetic?).tw,kf.	723772
29	exp Goiter/	33668
30	("goiter? " or "goitre?").tw,kf.	21428
31	exp Mortality/	407355
32	("mortalit*" or "death rate?" or "deathrate?").tw,kf.	874327
33	or/10-32	14433603
34	9 and 33	27599
35	Animals/ not (animals/ and humans/)	4862083
36	34 not 35	15902
37	limit 36 to (danish or english or french or german or multilingual or norwegian or swedish)	15280
38	(2020* or 2021*).ed,ep,yr,dp,dt.	3452076
39	(201911* or 201912*).ep,ed,dt.	400988
40	38 or 39	3654104
41	37 and 40	3275

Database: Embase 1974 to 2021 October 07

Date: 08.10.2021

Number of hits: 2469

#	Searches	
1	Fish/	99246
2	("Fishes" or "Fish").tw,kw.	229739
3	exp Salmonine/ or exp Flatfish/ or exp Gadiformes/ or Tuna/ or exp Perch/ or exp Herring/ or exp Esocidae/ or exp "Anguilla (fish)"/ or Sardine/ or Anchovy/ or Fish product/ or Fish meat/ or Fish roe/	21186
4	("Trout?" or "Salmo trutta" or "Oncorhynchus mykiss" or "Salmo mykiss" or "Salmon?" or "salmo salar" or "Oncorhynchus" or "halibut?" or "flounder?" or "European plaice?" or "Hippoglossus hippoglossus" or "Pleuronectes platessa" or "platichthys flesus" or "Mackerel?" or "scomber scombrus" or "Haddock?" or "Melanogrammus aeglefinus" or "Saithe?" or "Pollachius virens" or "Cod?" or "Gadus Morhua" or "Codling?" or "Stockfish*" or "Clipfish*" or "Pollachius pollachius" or "Pollock?" or "Pollack?" or "Carp?" or "cyprinus carpio" or "merluccius merluccius" or "hake?" or "xiphias gladius" or "swordfish*" or "Tuna" or "Katsuwonus pelamis" or "Thunnus thynnus" or "Perch*" or "Perca fluviatilis" or "Perciform*" or "Clupea harengus" or "Herring?" or "Argentina silus" or "Salmo silus" or "Greater argentine" or "smelt?" or "Atlantic argentine" or "Wolffish*" or "Seawolf?" or "Anarhichadidae" or "anarhichas lupus" or "Esocidae" or "Esox lucius" or "Pike?" or "Lophius piscatorius" or "Anglerfish*" or "Monkfish*" or "Anguilla anguilla" or "Eel?" or "Conger conger" or "Sardina pilchardus" or "Sardine?" or "pilchard?" or "Anchov*" or "Engraulis encrasicolus" or "Sprattus sprattus" or "European sprat" or "Brosme brosme" or "Merlangius merlangius" or "Whiting" or "fishproduct?").tw,kw.	233345
5	or/1-4	454665
6	Eating/ or Meal/ or Ingestion/ or Diet/ or Food intake/	418239
7	("eat*" or "ate" or "intake?" or "consumption" or "consume?" or "consuming" or "ingestion" or "meal?" or "diet*" or "dine" or "dinner?" or "lunch*" or "breakfast?" or "snack?").tw,kw.	1652076
8	6 or 7	1720381
9	5 and 8	61718
10	Bone density/ or exp Bone disease/ or exp Fracture/ or Falling/	1242554
11	("Osteoporosis" or "Rickets" or "Osteomalacia" or "vitamin D deficienc*" or (bone adj2 ("disease?" or "density" or "fracture?" or "fragil*" or "broken" or "deminerali#ation?" or "decalcification?")) or "Accidental Fall*" or ((("Slip?" or "trip?") adj2 "fall*")).tw,kw.	234851
12	exp Human development/ or exp Postnatal development/ or exp prenatal development/ or Motor dysfunction/ or Psychomotor disorder/ or Hyperactivity/ or exp Psychomotor performance/ or Psychomotor development/ or Motor development/ or Cognition/ or Cognitive defect/ or Cognitive development/ or exp Disorders of higher cerebral function/ or exp mental health/ or exp academic achievement/ or exp child behavior/ or Problem behavior/ or Impulsiveness/ or exp Language disability/ or Mental disease/ or Behavior/ or Anxiety disorder/ or exp Bipolar disorder/ or Anger/ or Affect/ or Depression/ or Mood disorder/ or Aggression/ or exp Schizophrenia/ or Attention deficit disorder/ or Attention/ or Learning/ or Reading/ or Mathematics/ or Aptitude test/ or Language test/ or Interpersonal communication/ or Language/ or Language development/ or Literacy/ or Intelligence/ or Executive function/ or Social status/ or Social behavior/ or Social adaption/ or Emotional intelligence/ or Emotion/ or Temperament/ or exp Amnesia/ or Memory disorder/ or Dementia/ or Alzheimer disease/ or Short term memory/ or Long term memory/	3247568

#	Searches	
13	((("Child*" or "infant*" or "f?etal" or "prenatal" or "pre natal" or "postnatal" or "post natal" or "human" or "antepartum period?" or "ante partum period?") adj3 "development?") or "inhibition" or ("brain" adj2 ("damage?" or "injur*" or "development?" or "disorder?")) or "psychomotor" or "psycho motor" or "motor" or "sensorimotor" or "sensori motor" or "sensorymotor" or "sensory motor" or "cognition" or "cognitive function?" or "Mental health" or "Disorder? of higher cerebral function?" or ("psychological" adj ("well being" or "wellbeing")) or (("neurocognit*" or "neuro cognit*" or "neurological" or "nervous system" or "nervoussystem" or "cognitive" or "development*" or "mental") adj2 ("dysfunction?" or "function?" or "decline?" or "deterioration?" or "Defici*" or "illness*" or "retardation?" or "disturbance?" or "impairment?" or "disorder?" or "impact?" or "disabilit*" or "deviation?" or "development?")) or "neurodevelopment*" or "neuro development*" or "autis*" or "asperger" or "kanner?" or "ASD" or "attention deficit" or "hyperactiv*" or "ADDH" or "ADHD" or "AD/HD" or "ADD" or "minimal brain dysfunction" or "impulsiveness" or "dyslexia" or "dyslexic?" or "dyscalculia" or "dyscalculic?" or "attention" or "learning" or "reading" or "mathematic?" or "math" or "maths" or ("aptitude" adj1 "test?") or ("Education" or "Educational" or "academic" or "school") adj1 ("Status" or "attainment?" or "achievement?" or "performance?" or "underachievement?" or "under achievement?" or "score?" or "success*" or "failure?")) or "executive function?" or "information processing" or "school readiness" or "school ready" or "Emotion*" or "socialemotional" or "social emotional" or "socioemotional" or "socio emotional" or ("social" adj ("development?" or "behavio?r" or "adjustment?")) or ("intel?ectual" adj2 ("development?" or "deficien*" or "disorder?" or "retardation?" or "disabilit*" or "disturbance?" or "impairment?")) or "Communication" or "language?" or "literacy" or "literacies" or "IQ" or "intel?igence" or "Speech disorder?" or "mutism?" or "aphasia" or "stutter*" or "dysphasia" or "alexia" or "anxiet*" or "depression?" or "depressive" or "mood disorder?" or "schizophrenia" or "schizophrenic" or "aggression" or "behavio?r*" or "affect" or "anger" or "bipolar" or "Temperament?" or "personalit*" or "amnesia" or "dementia" or "Alzheimer?" or "Parkinson?" or "huntington?" or ("memory" adj3 ("disorder?" or "impairment?" or "disturbance?" or "deficianc*" or "disabilit*" or "short term" or "shortterm" or "long term" or "longterm" or "verbal recognition?"))).tw,kw.	9028003
14	exp Cardiovascular disease/ or Cerebrovascular disease/ or Cerebrovascular accident/ or exp Ischemia/	4357727
15	((("Cardiovascular" or "heart" or "cardiac" or "myocardial" or "myo cardial" or "cerebrovascular" or "vascular" or "coronary" or "cerebral" or "peripheral" or "endothelial") adj ("disease?" or "disorder?" or "failure" or "event?" or "health" or "effect?" or "accident?" or "calcification?" or "risk factor?" or "riskfactor?" or "syndrom?" or "syndrome?" or "revasculari#ation?" or "arter*" or "function?" or "dysfunction?" or "attack?" or "arrest" or "apoplex*" or "insufficienc*" or "injur*" or "insult?" or "scleros#s" or "stenos#s" or "restenos#s")) or "cardioprotect*" or "cardio protect*" or "high cardiovascular risk?" or "CVD" or "infarct*" or "reinfarction?" or "aneurysm?" or "angina" or "artherosclero*" or "arthero sclero*" or "arteriosclero*" or "arterio sclero*" or "isch?emi*" or "nonisch?emi*" or "non isch?emic" or "thrombos#s" or "thrombolism?" or "tachycardia*" or "tachyarrhythmia?" or "arrhythmia?" or (("ventricular" or "arterial") adj ("fibrillation?" or "compliance?" or "stiffness*")) or "sudden cardiac death?" or "stroke?" or "TIA" or ("brain" adj ("h?emorrhage?" or "accident?" or "attack?" or "infarct*" or "insult?"))).tw,kw.	2686876
16	Enamel/ or Enamel hypoplasia/ or Tooth discoloration/	26744
17	((("dental" or "tooth" or "teeth" or "enamel") adj1 ("enamel" or "discolo?ration?" or "malformation?" or "opacit*")) or "hypo?minerali#ation" or ("developmental" adj3 ("dental" or "teeth" or "tooth" or "enamel") adj3 "defect?"))).tw,kw.	30725
18	exp Immunity/ or Immune deficiency/ or exp Respiratory tract infection/ or Abnormal respiratory sound/ or Wheezing/ or exp Psoriasis/ or exp Eczema/ or exp Dermatitis/ or exp Rheumatoid arthritis/ or exp Asthma/ or Antinuclear antibody/ or Multiple sclerosis/ or Systemic lupus erythematosus/ or exp Scleroderma/	2824602

#	Searches	
19	("immunolog*" or "infection resistance" or "immunity" or "autoimmunity" or "auto immunity" or "immunodeficienc*" or "immuno deficienc*" or ("immun*" adj ("system" or "status" or "defense?" or "defence?" or "deficienc*")) or "vaccination response?" or (("upper" or "lower") adj "respiratory tract infection?") or "respiratory Sound?" or "wheez*" or "asthma*" or "psoriasis" or "eczema*" or "dermatiti*" or "rheumatoid arthritis" or (("sjogren?" or "sicca") adj ("syndrome?" or "syndrom?")) or "Antinuclear antibod*" or "Multiple scleros#s" or "Systemic lupus erythematosus" or ("Scleroderma" or "scleros#s") adj1 ("localized" or "systemic")).tw,kw.	1614871
20	Sperm count/ or exp Sperm/ or exp Male infertility/ or exp Spermatozoon/ or Sexual maturation/ or Puberty/	141627
21	("Sperm?" or "semen" or "seminal fluid?" or "ejacul*" or "spermatozo*" or "spermatids" or "spermatocytes" or "spermatogonia" or "Oligospermia" or "Oligozoospermia*" or "Oligoasthenoteratozoospermia" or "asthenozoospermia?" or "asthenospermia?" or "criptozoospermia?" or "azoosperm*" or "globozoospermia?" or "teratospermia?" or "teratozoospermia?" or (("man" or "male?" or "men") adj1 "infertil*") or "Sex* matur*" or "Pubert*").tw,kw.	220902
22	Obesity/ or Abdominal obesity/ or morbid obesity/ or childhood obesity/ or maternal obesity/ or adolescent obesity/ or Adipocyte/ or Body weight change/ or Body weight gain/ or Body size/ or Body height/ or Body weight/ or Sagittal abdominal diameter/ or Weight height ratio/ or Waist circumference/ or Waist to height ratio/	904742
23	("obesity" or "obesities" or "obese" or "obesitas" or "adipos*" or "fat overload" or "overweight" or "over weight" or "BMI" or "body mass index" or "bodymass index" or "lean body mass" or "lean bodymass" or "fatness" or "adipocyte?" or "lipocyte?" or (("fat" or "lipid") adj cell?) or ("body" adj ("height?" or "size?" or "weight?")) or ("abdominal" adj ("diameter index" or "height")) or "sagit?al abdominal diameter?" or "height weight ratio?" or "waist circumference?" or "waist height ratio?" or "waist to height ratio?" or ("weight" adj1 ("change*" or "gain*")) or ("excess*" adj2 ("fat" or "weight"))).tw,kw.	1265629
24	exp Birth weight/ or Growth/ or Body growth/ or Prematurity/ or Pregnancy outcome/	293692
25	("growth" or (("premature" or "pre term" or "preterm") adj "birth?") or "SGA" or (("birth" or "gestational" or "neonatal" or "neo natal" or "newborn" or "new born" or "f?etal" or "f?etus" or "baby" or "babies") adj2 ("weight" or "size?")) or (("Pregnancy" or "birth" or "obstetric") adj "outcome?").tw,kw.	1949577
26	((("allerg*" or "hypersensitivit*" or "hyper sensitivit*" or "sensiti#ation*" or "atopic?" or "atopy" or "atopies") adj5 "prevention").tw,kw.	4740
27	exp Diabetes mellitus/	1049028
28	("diabetes" or "sugar sickness" or "hypoglycemia" or "hypo glycemia" or "hyperglycemia" or "hyper glycemia" or diabetic?).tw,kw.	1067462
29	exp Goiter/	23679
30	("goiter? " or "goitre? ").tw,kw.	21348
31	exp Mortality/	1188926
32	("mortalit*" or "death rate?" or "deathrate?").tw,kw.	1272912
33	or/10-32	19082957
34	9 and 33	36598
35	(animal/ or exp nonhuman/ or Animal experiment/) not ((animal/ or exp nonhuman/ or Animal experiment/) and exp human/)	6293086
36	34 not 35	22181
37	limit 36 to (conference abstracts or embase)	18863
38	limit 37 to (danish or english or french or german or norwegian or swedish)	18368
39	(2020* or 2021*).yr,dd,dp,dc.	3767517
40	(201911* or 201912*).dd,dc.	320397
41	39 or 40	4054681
42	38 and 41	2469

15.3 Fish consumption and health outcomes – systematic reviews and meta-analyses

15.3.1 Original search

Fish intake

Contact person: Kirsten Rakkestad

Search: Ragnhild Agathe Ternes

Comment: Har gjort eit search for perioden 2015-2019 frå before. Men dette searchet er for perioden 2016-2020.

Duplicate check in EndNote: Before duplicate check: 715
After duplicate check: 488

Database: Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Daily and Versions(R) <1946 to December 14, 2020>

Date: 15.12.20

Number of hits: 334

#	Searches	
1	Fishes/	63810
2	("fishes" or "fish").tw,kf.	183532
3	exp Trout/ or exp Salmon/ or Flounder/ or Perciformes/ or Gadus Morhua/ or Carps/ or Tuna/ or Perches/ or Esocidae/ or Anguilla/ or Fish products/	49905

#	Searches	
4	("Trout?" or "Salmo trutta" or "Oncorhynchus mykiss" or "Salmo mykiss" or "Salmon?" or "salmo salar" or "Oncorhynchus" or "halibut?" or "flounder?" or "European plaice?" or "Hippoglossus hippoglossus" or "Pleuronectes platessa" or "platichthys flesus" or "Mackerel?" or "scomber scombrus" or "Haddock?" or "Melanogrammus aeglefinus" or "Saithe?" or "Pollachius virens" or "Cod?" or "Gadus Morhua" or "Codling?" or "Stockfish*" or "Clipfish*" or "Pollachius pollachius" or "Pollock?" or "Pollack?" or "Carp?" or "cyprinus carpio" or "merluccius merluccius" or "hake?" or "xiphias gladius" or "swordfish*" or "Tuna" or "Katsuwonus pelamis" or "Thunnus thynnus" or "Perch*" or "Perca fluviatilis" or "Clupea harengus" or "Herring?" or "Argentina silus" or "Salmo silus" or "Greater argentine" or "smelt?" or "Atlantic argentine" or "Wolffish*" or "Seawolf?" or "Anarhichadidae" or "anarhichas lupus" or "Esocidae" or "Esox lucius" or "Pike?" or "Lophius piscatorius" or "Anglerfish*" or "Monkfish*" or "Anguilla anguilla" or "Eel?" or "Conger conger" or "Sardina pilchardus" or "Sardine?" or "pilchard?" or "Anchov*" or "Engraulis encrasicolus" or "Sprattus sprattus" or "European sprat" or "Brosme brosme" or "Merlangius merlangius" or "Whiting" or "fishproduct?").tw,kf.	181022
5	or/1-4	358104
6	Eating/ or exp Meals/ or Diet/	213366
7	("eat*" or "ate" or "intake?" or "consumption" or "consume?" or "consuming" or "ingestion" or "meal?" or "diet*" or "dine" or "dinner?" or "lunch*" or "breakfast?" or "snack?").tw,kf.	1243511
8	6 or 7	1286256
9	5 and 8	46833
10	Animals/ not (animals/ and humans/)	4734099
11	9 not 10	27737
12	limit 11 to "reviews (maximizes specificity)"	487
13	Meta-Analysis/ or ((systematic* adj2 review*) or metaanal* or "meta anal*" or (review and ((structured or database* or systematic*) adj2 search*)) or "integrative review*" or (evidence adj2 review*)).tw,kf,bt.	363766
14	12 or (11 and 13)	713
15	limit 14 to yr="2016 -Current"	334

Database: Embase <1974 to 2020 December 14>

Date: 15.12.20

Number of hits: 381

#	Searches	
1	Fish/	96775
2	("fishes" or "fish").tw,kw.	223318
3	exp Salmonine/ or exp Salmon/ or exp Flatfish/ or exp Gadiformes/ or Tuna/ or exp Perch/ or exp Herring/ or exp Esocidae/ or exp "Anguilla (fish)"/ or Sardine/ or Anchovy/ or Fish product/ or Fish meat/ or Fish roe/	19376

#	Searches	
4	("Trout?" or "Salmo trutta" or "Oncorhynchus mykiss" or "Salmo mykiss" or "Salmon?" or "salmo salar" or "Oncorhynchus" or "halibut?" or "flounder?" or "European plaice?" or "Hippoglossus hippoglossus" or "Pleuronectes platessa" or "platichthys flesus" or "Mackerel?" or "scomber scombrus" or "Haddock?" or "Melanogrammus aeglefinus" or "Saithe?" or "Pollachius virens" or "Cod?" or "Gadus Morhua" or "Codling?" or "Stockfish*" or "Cliffish*" or "Pollachius pollachius" or "Pollock?" or "Pollack?" or "Carp?" or "cyprinus carpio" or "merluccius merluccius" or "hake?" or "xiphias gladius" or "swordfish*" or "Tuna" or "Katsuwonus pelamis" or "Thunnus thynnus" or "Perch*" or "Perca fluviatilis" or "Clupea harengus" or "Herring?" or "Argentina silus" or "Salmo silus" or "Greater argentine" or "smelt?" or "Atlantic argentine" or "Wolffish*" or "Seawolf?" or "Anarhichadidae" or "anarhichas lupus" or "Esocidae" or "Esox lucius" or "Pike?" or "Lophius piscatorius" or "Anglerfish*" or "Monkfish*" or "Anguilla anguilla" or "Eel?" or "Conger conger" or "Sardina pilchardus" or "Sardine?" or "pilchard?" or "Anchov*" or "Engraulis encrasicolus" or "Sprattus sprattus" or "European sprat" or "Brosme brosme" or "Merlangius merlangius" or "Whiting" or "fishproduct?").tw,kw.	221509
5	or/1-4	437467
6	Food intake/ or Eating/ or Meal/ or Diet/	379490
7	("eat*" or "ate" or "intake?" or "consumption" or "consume?" or "consuming" or "ingestion" or "meal?" or "diet*" or "dine" or "dinner?" or "lunch*" or "breakfast?" or "snack?").tw,kw.	1579185
8	6 or 7	1642449
9	5 and 8	59166
10	(animal/ or exp nonhuman/ or Animal experiment/) not ((animal/ or exp nonhuman/ or Animal experiment/) and exp human/)	6140622
11	9 not 10	35115
12	limit 11 to (conference abstracts or embase)	28795
13	limit 12 to "reviews (maximizes specificity)"	528
14	Meta-Analysis/ or "systematic review"/ or ((systematic* adj2 review*) or metaanal* or "meta anal*" or (review and ((structured or database* or systematic*) adj2 search*)) or "integrative review*" or (evidence adj2 review*)).tw,kw.	527915
15	13 or (12 and 14)	879
16	limit 15 to yr="2016 -Current"	381

15.3.2 Updated search

Contact person: Kirsten Eline Rakkestad

Search: Trude Anine Muggerud

Comments: Update of the fish intake search from november 2019, limited to systematic reviews

Duplicate check in Before duplicate check: 417
EndNote: After duplicate check: 310

Database: Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Daily and Versions(R) <1946 to October 04, 2021>

Date: 05.10.21

Number of hits: 217

#	Searches	
1	Fishes/	65739
2	("Fishes" or "Fish").tw,kf.	192497
3	exp Trout/ or exp Salmon/ or Flounder/ or Perciformes/ or Gadus Morhua/ or Carps/ or Tuna/ or Perches/ or Esocidae/ or Anguilla/ or Fish products/	51983
4	("Trout?" or "Salmo trutta" or "Oncorhynchus mykiss" or "Salmo mykiss" or "Salmon?" or "salmo salar" or "Oncorhynchus" or "halibut?" or "flounder?" or "European plaice?" or "Hippoglossus hippoglossus" or "Pleuronectes platessa" or "platichthys flesus" or "Mackerel?" or "scomber scombrus" or "Haddock?" or "Melanogrammus aeglefinus" or "Saithe?" or "Pollachius virens" or "Cod?" or "Gadus Morhua" or "Codling?" or "Stockfish*" or "Clipfish*" or "Pollachius pollachius" or "Pollock?" or "Pollack?" or "Carp?" or "cyprinus carpio" or "merluccius merluccius" or "hake?" or "xiphias gladius" or "swordfish*" or "Tuna" or "Katsuwonus pelamis" or "Thunnus thynnus" or "Perch*" or "Perca fluviatilis" or "Clupea harengus" or "Herring?" or "Argentina silus" or "Salmo silus" or "Greater argentine" or "smelt?" or "Atlantic argentine" or "Wolffish*" or "Seawolf?" or "Anarhichadidae" or "anarhichas lupus" or "Esocidae" or "Esox lucius" or "Pike?" or "Lophius piscatorius" or "Anglerfish*" or "Monkfish*" or "Anguilla anguilla" or "Eel?" or "Conger conger" or "Sardina pilchardus" or "Sardine?" or "pilchard?" or "Anchov*" or "Engraulis encrasicolus" or "Sprattus sprattus" or "European sprat" or "Brosme brosme" or "Merlangius merlangius" or "Whiting" or "fishproduct?").tw,kf.	190943
5	or/1-4	375883
6	Eating/ or exp Meals/ or Diet/	224607
7	("eat*" or "ate" or "intake?" or "consumption" or "consume?" or "consuming" or "ingestion" or "meal?" or "diet*" or "dine" or "dinner?" or "lunch*" or "breakfast?" or "snack?").tw,kw.	1304375
8	6 or 7	1349472
9	5 and 8	49456
10	Animals/ not (animals/ and humans/)	4858504
11	9 not 10	29147
12	limit 11 to "reviews (maximizes specificity)"	529
13	Meta-Analysis/ or Network Meta-Analysis/ or ((systematic* adj2 review*) or metaanal* or "meta anal*" or (review and ((structured or database* or systematic*) adj2 search*)) or "integrative review*" or (evidence adj2 review*)).tw,kf,bt.	407248
14	12 or (11 and 13)	776
15	(2020* or 2021*).ed,ep,yr,dp,dt.	3420043
16	(201911* or 201912*).ep,ed,dt.	400976
17	15 or 16	3622155
18	14 and 17	217

Database: **Embase 1974 to 2021 October 04**

Date: 05.10.21

Number of hits: 200

#	Searches	
1	Fish/	99137
2	("Fishes" or "Fish").tw,kw.	229417
3	exp Salmonine/ or exp Flatfish/ or exp Gadiformes/ or Tuna/ or exp Perch/ or exp Herring/ or exp Esocidae/ or exp "Anguilla (fish)"/ or Sardine/ or Anchovy/ or Fish product/ or Fish meat/ or Fish roe/	21143
4	("Trout?" or "Salmo trutta" or "Oncorhynchus mykiss" or "Salmo mykiss" or "Salmon?" or "salmo salar" or "Oncorhynchus" or "halibut?" or "flounder?" or "European plaice?" or "Hippoglossus hippoglossus" or "Pleuronectes platessa" or "platichthys flesus" or "Mackerel?" or "scomber scombrus" or "Haddock?" or "Melanogrammus aeglefinus" or "Saithe?" or "Pollachius virens" or "Cod?" or "Gadus Morhua" or "Codling?" or "Stockfish*" or "Clipfish*" or "Pollachius pollachius" or "Pollock?" or "Pollack?" or "Carp?" or "cyprinus carpio" or "merluccius merluccius" or "hake?" or "xiphias gladius" or "swordfish*" or "Tuna" or "Katsuwonus pelamis" or "Thunnus thynnus" or "Perch*" or "Perca fluviatilis" or "Perciform*" or "Clupea harengus" or "Herring?" or "Argentina silus" or "Salmo silus" or "Greater argentine" or "smelt?" or "Atlantic argentine" or "Wolffish*" or "Seawolf?" or "Anarhichadidae" or "anarhichas lupus" or "Esocidae" or "Esox lucius" or "Pike?" or "Lophius piscatorius" or "Anglerfish*" or "Monkfish*" or "Anguilla anguilla" or "Eel?" or "Conger conger" or "Sardina pilchardus" or "Sardine?" or "pilchard?" or "Anchov*" or "Engraulis encrasicolus" or "Sprattus sprattus" or "European sprat" or "Brosme brosme" or "Merlangius merlangius" or "Whiting" or "fishproduct?").tw,kw.	233011
5	or/1-4	454042
6	Eating/ or Meal/ or Ingestion/ or Diet/ or Food intake/	417650
7	("eat*" or "ate" or "intake?" or "consumption" or "consume?" or "consuming" or "ingestion" or "meal?" or "diet*" or "dine" or "dinner?" or "lunch*" or "breakfast?" or "snack?").tw,kw.	1649307
8	6 or 7	1717523
9	5 and 8	61601
10	(animal/ or exp nonhuman/ or Animal experiment/) not ((animal/ or exp nonhuman/ or Animal experiment/) and exp human/)	6287677
11	9 not 10	36495
12	limit 11 to (conference abstracts or embase)	29987
13	limit 12 to "reviews (maximizes specificity)"	567
14	exp Meta-Analysis/ or "systematic review"/ or ((systematic* adj2 review*) or metaanal* or "meta anal*" or (review and ((structured or database* or systematic*) adj2 search*)) or "integrative review*" or (evidence adj2 review*)).tw,kw.	586989
15	13 or (12 and 14)	941
16	(2020* or 2021*).yr,dd,dp,dc.	3726257
17	(201911* or 201912*).dd,dc.	320927
18	16 or 17	4013914
19	15 and 18	200

15.4 Nutrients and health outcomes – systematic reviews and meta-analyses

15.4.1 All nutrients and sperm quality, fertility

Contact person: Kirsten Eline Rakkestad
Search: Trude Anine Muggerud
Comment: Related to the update of the search on fish intake. Only systematic reviews
Duplicate check i Before Duplicate check: 194
EndNote: After Duplicate check: 140

Database: Ovid MEDLINE(R) and Epub Ahead of Print, In-Process, In-Data-Review & Other Non-Indexed Citations, Daily and Versions(R) <1946 to October 22, 2021>
Date: 25.10.21

Number of hits: 63 systematic reviews

#	Searches	
1	Sperm count/ or Semen/ or exp Infertility, Male/ or exp Spermatozoa/ or Sexual maturation/ or Puberty/	126685
2	("Sperm?" or "semen" or "seminal fluid?" or "ejacul*" or "spermatozo*" or "spermatids" or "spermatocytes" or "spermatogonia" or "Oligospermia" or "Oligozoospermia*" or "Oligoasthenoteratozoospermia" or "asthenozoospermia?" or "asthenospermia?" or "criptozoospermia?" or "azoosperm*" or "globozoospermia?" or "teratospermia?" or "teratozoospermia?" or (("man" or "male?" or "men") adj1 "infertil*") or "Sex* matur*" or "Pubert*").tw,kf.	184475
3	1 or 2	214364
4	exp Fatty Acids, Omega-3/ or Fatty Acids, Unsaturated/ or Fish oils/ or Cod liver oil/	49077
5	((("omega 3" adj ("fatty acid?" or "carboxylic acid?")) or (("n 3" or n3) adj ("fatty acid?" or oil? or pufa)) or ((eicosapentaenoic or eicosapentenoic or eicosapentanoic or timnodonic) adj acid?) or eicosapentaenoate or "1553-41-9" or ("5,8,11,14,17" adj ("icosapentaenoic acid?" or icosapentaenoic acid? or "pentaene carboxylic acid?" or "pentaenoic acid?")) or icosapentor or aan7qov9ea or icosapentaenoate or icosapent or "docosahexaenoic acid?" or dhasco or docosahexaenoate or "121191-40-0" or "122045-76-5" or "123301-30-4" or "123301-31-5" or "124020-09-3" or "2091-24-9" or "25167-62-8" or "32839-18-2" or "6217-54-5" or "6610-27-1" or "89022-31-1" or "91403-70-2" or "long chain fatty acid?" or "long chain polyunsaturated fatty acid?" or "LC PUFA" or "LC fatty acid?" or ((polyunsaturated or "poly unsaturated" or polyunsaturation or unsaturated) adj ("fatty acid?" or fat or lipid?)) or "alkenyl fatty acid?" or "docosapentaoenic acid?" or "fish liver oil?" or "fish oil?" or "tuna oil?" or "8001-69-2" or "cod liver oil?" or "codfish liver oil?" or "codliver oil?").tw,kf.	73917
6	exp Vitamin D/	63238

#	Searches	
7	("vitamin d" or "1406-16-2" or cholecalciferol? or hydroxycholecalciferol? or "hydroxy cholecalciferol?" or calcifediol? or dihydroxycholecalciferol? or "dihydro cholecalciferol?" or calcitriol? or "24,25-dihydroxyvitamin D" or ergocalciferol? or dihydrotachysterol? or "25-hydroxyvitamin D" or lunacalcipol or doxercalciferol or paricalcitol).tw,kf.	76632
8	Iodine/	25807
9	("iodine" or "iodide").tw,kf.	90114
10	Exp Vitamin B12/	22760
11	((((vitamin? or acravite or apavite or delagrangine or flavin or galto or horfervit or mille or monovit or pierrel or siegfried or vicotrat or weber or bagovit or bentavit or betamine or vitapur or crodabion or crystal or godabione or davitamon or douzoral or dumovit or eritrovit or hemosalus or hypovitaminosis or ido or osfavite or lagavit or lifaton or pharmitovite or ucemine or parentosol) adj1 (b12 or "b 12")) or ((betalily or betalin or betaline or betetapion or clarentin or clarentine or viemin or "vita no." or vitabee or erftamin or creliverol or erftamine or heptenyl or la or norivite) adj "12") or "12 oral" or "5,6 dimethylbenzimidazole b12 coenzyme" or almeret or "alpha(5,6 dimethylbenzimidazolyl)cobamylcyanide" or anacobin? or antipernicin? or "aquocobinamide cyanide" or arcored or "b docin" or bedoc or bedoce or bedodec or bedodeka or bedoxyl or bedoz or bedozane or bedumil or behepan or behepane or beniform or benol or berubi or berubigen or berubigene or berubin or berubine or betolvex or bevatine or bevidoral or bevidox or bevite or bex or bexii or bexitab or bimil or biocres or biopar or bitevan or byladoce or "cabadon m" or calomist or catavin or catavine or cn or cobalamine or "cobadoce forte" or "cobal-1000" or cobalamide or cobali? or coballamine or cobalmed or cobaltron? or cobamin? or cobastab or cobavite or cobemine or cobione or "cobolin-m" or cobrumin or cobruline or cohemine or coheminecompensal 25,000 or covit or cresiro or cresiol or crystamin or crystamine or crystimin 1000 or crystwel or cyanocobalamin or cyanaton? or cyanocobalamin or cobalamin or "cyano 5,6 dimethylbenzimidazolylcobamide" or "cyano cobalamin?" or cyanobalamin or cyanocobal* or cycobemin* or cyclolamin? or cycoplex or cyomin or cyredin or cytacone or cytagon or cytamen or cytamene or cytatone or cytatone or cytobex or cytobion or cytobione or depo-cobolin or dicibin or distividdobetin or dobetine or "doce oral" or docecrisina or docemine or doceoral or docibin? or docigram or docivit or dodecabee or dodecavite or dodevitina or dodex or dozevoll or ducobee or ducobee depot or embiol or emobione or endoglobin or eritrone or eritrosir or eruhaemon or erycytol or erythrotin or erythrotine or examen? or "extrinsic factor" or fermin or griseovit or grisevit or grisovit or "hematolaminhemo b doze" or hemoergene or hemomin or hemomine or hepagon or hepagone or hepavit or hepcovite or intrinase or intrindon or intrinolone or "lactobacillus lactis dornier factor" or "livonal schering" or "lfd factor" or megabione or megalovel or mepharnbin or mepharrubine or milbedocemillevit or navagron? or neurobaltin? or "neuroforte-r" or normocytin or palvite or pernaevit or pernical or pernicipur or pernpuvon or pernorol or pinkamin? or plecyamin? or poyamin? or rametine or rectocenga or recytomin? or redamin? or redisol or reticulogen? or rhodacystrobeltvit or rojamin or rotamin? or rubavit or rubentin? or rubesol or rubion? or rubivitan or rubivite or rubramin or rubranova or rubrine or rubripca or rubrocitol or rubrovit or rubyvan or rubyvit or ruvite or "cyclolamins.p. cyclolamine" or sytobex or transcyanocobalamin or "twel be" or twelbe or tweltone or "twelve oral" or twelveoral or "vi-twel" or vibalt or vibicon? or vibicon? or vibisone or "vicapan n" or vitarubin or virubra or vitadom).tw,kf.	55517
12	Selenium/	21961
13	Selenium.tw,kf	31322
14	or/4-13	372968
15	3 and 14	3153
16	limit 15 to (danish or english or french or german or interlingua or multilingual or norwegian or swedish)	3006
17	Animals/ not (animals/ and humans/)	4864995
18	16 not 17	1713

#	Searches	
19	limit 18 to "reviews (maximizes specificity)"	53
20	Meta-Analysis/ or Network Meta-Analysis/ or ((systematic* adj2 review*) or metaanal* or "meta anal*" or (review and ((structured or database* or systematic*) adj2 search*)) or "integrative review*" or (evidence adj2 review*)).tw,kf,bt.	409593
21	19 or (18 and 20)	63

Database: Embase <1974 to 2021 October 22>

Date: 25.10.21

Number of hits: 107 systematic reviews

#	Searches	
1	Sperm count/ or exp Sperm/ or exp Male infertility/ or exp Spermatozoon/ or Sexual maturation/ or Puberty/	141758
2	("Sperm?" or "semen" or "seminal fluid?" or "ejacul*" or "spermatozo*" or "spermatids" or "spermatocytes" or "spermatogonia" or "Oligospermia" or "Oligozoospermia*" or "Oligoasthenoteratozoospermia" or "asthenozoospermia?" or "asthenospermia?" or "criptozoospermia?" or "azoosperm*" or "globozoospermia?" or "teratospermia?" or "teratozoospermia?" or (("man" or "male?" or "men") adj1 "infertil*") or "Sex* matur*" or "Pubert*").tw,kf.	222408
3	1 or 2	248810
4	omega 3 fatty acid/ or Icosapentaenoic Acid/ or Docosahexaenoic Acid/ or Long chain fatty acid/ or unsaturated fatty acid/ or polyunsaturated fatty acid/ or docosapentaenoic acid/ or Fish oil/ or Cod liver oil/	94446
5	((("omega 3" adj ("fatty acid?" or "carboxylic acid?")) or (("n 3" or n3) adj ("fatty acid?" or oil? or pufa)) or ((eicosapentaenoic or eicosapentenoic or eicosapentanoic or timnodonic) adj acid?) or eicosapentaenoate or "1553-41-9" or ("5,8,11,14,17" adj ("icosapentaenoic acid?" or icosapentaenoic acid? or "pentaene carboxylic acid?" or "pentaenoic acid?")) or icosapentor or aan7qov9ea or icosapentaenoate or icosapent or "docosahexaenoic acid?" or dhasco or docosahexaenoate or "121191-40-0" or "122045-76-5" or "123301-30-4" or "123301-31-5" or "124020-09-3" or "2091-24-9" or "25167-62-8" or "32839-18-2" or "6217-54-5" or "6610-27-1" or "89022-31-1" or "91403-70-2" or "long chain fatty acid?" or "long chain polyunsaturated fatty acid?" or "LC PUFA" or "LC fatty acid?" or ((polyunsaturated or "poly unsaturated" or polyunsaturation or unsaturated) adj ("fatty acid?" or fat or lipid?)) or "alkenyl fatty acid?" or "docosapentaoenic acid?" or "fish liver oil?" or "fish oil?" or "tuna oil?" or "8001-69-2" or "cod liver oil?" or "codfish liver oil?" or "codliver oil?").tw,kf.	89686
6	exp Vitamin D/	154855
7	("vitamin d" or "1406-16-2" or cholecalciferol? or hydroxycholecalciferol? or "hydroxy cholecalciferol?" or calcifediol? or dihydroxycholecalciferol? or "dihydro cholecalciferol?" or calcitriol? or "24,25-dihydroxyvitamin D" or ergocalciferol? or dihydrotachysterol? or "25-hydroxyvitamin D" or lunacalcipol or doxercalciferol or paricalcitol).tw,kf.	111737
8	Iodine/	27515
9	("iodine" or "iodide").tw,kf.	101917
10	Cobalamin/ or Cyanocobalamin/	41961

#	Searches	
11	((vitamin? or acravit or apavit or delagrange or flavin or galto or horfervit or mille or monovit or pierrel or siegfried or vicotrat or weber or bagovit or bentavit or betamine or vitapur or crodabion or crystal or godabione or davitamon or douzoral or dumovit or eritrovit or hemosalus or hypovitaminosis or ido or osfavit or lagavit or lifaton or pharमतovit or ucemine or parentosol) adj1 (b12 or "b 12")) or ((betalily or betalin or betaline or betetapion or clarentin or clarentine or viemin or "vita no." or vitabee or erftamin or creliverol or erftamine or heptenyl or la or norivite) adj "12") or "12 oral" or "5,6 dimethylbenzimidazole b12 coenzyme" or almeret or "alpha(5,6 dimethylbenzimidazolyl)cobamycyanid" or anacobin? or antipernicin? or "aquocobinamide cyanide" or arcored or "b docin" or bedoc or bedoce or bedodec or bedodeka or bedoxyl or bedoz or bedozane or bedumil or behepan or behepane or beniform or benol or berubi or berubigen or berubigene or berubin or berubine or betolvex or bevatine or bevidoral or bevidox or bevitec or bex or bexii or bexitab or bimil or biocres or biopar or bitevan or byladoce or "cabadon m" or calomist or catavin or catavine or cn or cobalamine or "cobadoce forte" or "cobal-1000" or cobalamide or cobali? or coballamine or cobalmed or cobaltron? or cobamin? or cobastab or cobavite or cobeminum or cobione or "cobolin-m" or cobrumin or cobrumine or cohemin or cohemincompensal 25,000 or covit or cresiro or cresirol or crystamin or crystamine or crystimin 1000 or crystwel or cyanocobalamin or cyanaton? or cyanocobalamin or cobalamin or "cyano 5,6 dimethylbenzimidazolylcobamide" or "cyano cobalamin?" or cyanobalamin or cyanocobal* or cycobemin* or cycolamin? or cycoplex or cyomin or cyredin or cytacone or cytagon or cytamen or cytamene or cytatton or cytatone or cytobex or cytobion or cytobione or depo-cobolin or dicibin or distitdobetn or dobetine or "doce oral" or docecrisina or docemine or doceoral or docibin? or docigram or docivit or dodecabee or dodecavite or dodevitina or dodex or dozefful or ducobee or ducobee depot or embiol or emobione or endoglobin or eritrone or eritrosir or eruhaemon or erycytol or erythrotin or erythrotine or examen? or "extrinsic factor" or fermin or griseovit or grisevit or grisovit or "hematolaminhemo b doze" or hemoergene or hemomin or hemomine or hepagon or hepagone or hepavit or hepcovite or intrinase or intrindon or intrinolone or "lactobacillus lactis dörner factor" or "livonal schering" or "ltd factor" or megabione or megalovel or mepharnbin or mepharrubine or milbedocemillevit or navagron? or neurobaltin? or "neuroforte-r" or normocytin or palvite or pernaevit or pernical or pernicipur or pernicipuvon or pernoral or pinkamin? or plecyamin? or poyamin? or rametine or rectocenga or recytomin? or redamin? or redisol or reticulogen? or rhodacrystrobeltvit or rojamin or rotamin? or rubavit or rubentin? or rubesol or rubion? or rubivitan or rubivite or rubramin or rubranova or rubrine or rubripca or rubrocitol or rubrovit or rubyvan or rubyvit or ruvite or "cycolamins.p. cycolamine" or sytobex or transcyanocobalamin or "twel be" or twelbe or tweltone or "twelve oral" or twelveoral or "vi-twel" or vialt or vibecon? or vibicon? or vibisone or "vicapan n" or vitarubin or virubra or vitadom).tw,kf.	59267
12	Selenium/ or Selenium intake/	41316
13	Selenium.tw,kf.	37427
14	or/4-13	513414
15	3 and 14	5116
16	limit 15 to embase	3294
17	limit 16 to (danish or english or french or german or norwegian or polyglot or swedish)	3193
18	(animal/ or exp nonhuman/ or Animal experiment/) not ((animal/ or exp nonhuman/ or Animal experiment/) and exp human/)	6297136
19	17 not 18	2218
20	limit 19 to "reviews (maximizes specificity)"	53
21	exp Meta-Analysis/ or "systematic review"/ or ((systematic* adj2 review*) or metaanal* or "meta anal*" or (review and ((structured or database* or systematic*) adj2 search*)) or "integrative review*" or (evidence adj2 review*)).tw,kf.	591992
22	20 or (19 and 21)	107

Database: Cochrane Database of Systematic Reviews, Issue 10 of 12, October 2021

Date: 25.10.21

Number of hits: 8 systematic reviews

#	Searches	
#1	[mh ^"Sperm count"]	340
#2	[mh ^"Semen"]	316
#3	[mh "Infertility, Male"]	774
#4	[mh "Spermatozoa"]	462
#5	[mh ^"Sexual maturation"]	42
#6	[mh ^"Puberty"]	303
#7	("Sperm?" or "semen" or (seminal NEXT fluid?) or "ejacul*" or "spermatozo*" or "spermatids" or "spermatocytes" or "spermatogonia" or "Oligospermia" or "Oligozoospermia*" or "Oligoasthenoteratozoospermia" or "asthenozoospermia?" or "asthenospermia?" or "criptozoospermia?" or "azoosperm*" or "globozoospermia?" or "teratospermia?" or "teratozoospermia?" or (("man" or "male?" or "men") NEAR/1 "infertil*") or (Sex* NEXT matur*) or "Pubert*"):ti,ab	5101
#8	{OR #1-#7}	5612
#9	[mh "Fatty acids, Omega-3"]	3265
#10	[mh ^"Fatty acids, Unsaturated"]	745
#11	[mh ^"Fish oils"]	1068
#12	[mh ^"Cod liver oil"]	39
#13	((("omega 3" NEXT (fatty or carboxylic) NEXT acid?) or (("n 3" or n3) NEXT ("fatty acid" or "fatty acids" or oil? or pufa)) or ((eicosapentaenoic or eicosapentenoic or eicosapentanoic or timnodonic or docosahexaenoic or "long chain fatty" or "long chain polyunsaturated fatty" or "LC fatty" or "alkenyl fatty" or "docosapentaoenic") NEXT acid?) or eicosapentaenoate or "1553-41-9" or ("5,8,11,14,17" NEXT ("icosapentaenoic acid" or "icosapentaenoic acids" or "pentaene carboxylic acid" or "pentaene carboxylic acids" or "pentaenoic acid" or "pentaenoic acids")) or icosapentor or aan7qov9ea or icosapentaenoate or icosapent or dhasco or docosahexaenoate or "121191-40-0" or "122045-76-5" or "123301-30-4" or "123301-31-5" or "124020-09-3" or "2091-24-9" or "25167-62-8" or "32839-18-2" or "6217-54-5" or "6610-27-1" or "89022-31-1" or "91403-70-2" or "LC PUFA" or ((polyunsaturated or "poly unsaturated" or polyunsaturation or unsaturated) NEXT ("fatty acid" or "fatty acids" or fat or lipid?)) or (("fish liver" or "fish" or "tuna" or "cod liver" or "codfish liver" or codliver) NEXT oil?) or "8001-69-2"):ti,ab	10319
#14	[mh "Vitamin D"]	5769
#15	("vitamin d" or "1406-16-2" or cholecalciferol? or hydroxycholecalciferol? or (hydroxyl NEXT cholecalciferol?) or calcifediol? or dihydroxycholecalciferol? or (dihydro NEXT cholecalciferol?) or calcitriol? or "24,25-dihydroxyvitamin D" or ergocalciferol? or dihydrotachysterol? or "25-hydroxyvitamin D" or lunacalcipol or doxercalciferol or paricalcitol):ti,ab	13301
#16	[mh ^"Iodine"]	612
#17	(Iodine or iodide):ti,ab	4253
#18	[mh "Vitamin B12"]	930

#	Searches	
#19	((vitamin? or acravit or apavit or delagrange or flavin or galto or horfervit or mille or monovit or pierrel or siegfried or vicotrat or weber or bagovit or bentavit or betamine or vitapur or crodabion or crystal or godabione or davitamon or douzoral or dumovit or eritrovit or hemosalus or hypovitaminosis or ido or osfavit or lagavit or lifaton or pharmatovit or ucemine or parentosol) NEAR/1 (b12 or "b 12")) or ((betalily or betalin or betaline or betetapion or clarentin or clarentine or viemin or "vita no." or vitabee or erftamin or creliverol or erftamine or heptenyl or la or norivite) NEXT "12") or "12 oral" or "5,6 dimethylbenzimidazole b12 coenzyme" or almeret or "alpha(5,6 dimethylbenzimidazolyl)cobamycyanid" or anacobin? or antipernicin? or "aquocobinamide cyanide" or arcored or "b docin" or bedoc or bedoce or bedodec or bedodeka or bedoxyl or bedoz or bedozane or bedumil or behepan or behepane or beniform or benol or berubi or berubigen or berubigene or berubin or berubine or betolvex or bevatine or bevidoral or bevidox or bevitec or bex or bexii or bexitab or bimil or biocres or biopar or bitevan or byladoce or "cabadon m" or calomist or catavin or catavine or cn or cobalamine or "cobadoce forte" or "cobal-1000" or cobalamide or cobali? or coballamine or cobalmed or cobaltron? or cobamin? or cobastab or cobavite or cobeminum or cobione or "cobolin-m" or cobrumin or cobrumine or cohemin or "coheminecompensal 25,000" or covit or cresiro or cresirol or crystamin or crystamine or "crystimin 1000" or crystwel or cyanocobalamin or cyanaton? or cyanocobalamin or cobalamin or "cyano 5,6 dimethylbenzimidazolylcobamide" or "cyano cobalamin?" or cyanobalamin or cyanocobal* or cycobemin* or cycolamin? or cycoplex or cyomin or cyredin or cytacone or cytagon or cytamen or cytamene or cytaton or cytatone or cytobex or cytobion or cytobione or "depo cobolin" or dicibin or distivitdobetin or dobetine or "doce oral" or docecrisina or docemine or doceoral or docibin? or docigram or docivit or dodecabee or dodecavite or dodevitina or dodex or dozefull or ducobee or ducobee depot or embiol or emobione or endoglobin or eritrone or eritrosir or eruhaemon or erycytol or erythrotin or erythrotine or examen? or "extrinsic factor" or fermin or griseovit or grisevit or grisovit or "hematolaminhemo b doze" or hemoergene or hemomin or hemomine or hepagon or hepagone or hepavit or hepcovite or intrinase or intrindon or intrinolone or "lactobacillus lactis dornier factor" or "livonal schering" or "ltd factor" or megabione or megalovel or mepharnbin or mepharnubine or milbedocemillevit or navagron? or neurobaltin? or "neuroforte-r" or normocytin or palvite or pernaevit or pernical or pernicipur or pernicipuvon or pernorat or pinkamin? or plecyamin? or poyamin? or rametine or rectocenga or recytomin? or redamin? or redisol or reticulogen? or rhodacystrobeltvit or rojamin or rotamin? or rubavit or rubentin? or rubesol or rubion? or rubivitan or rubivite or rubramin or rubranova or rubrine or rubripca or rubrocitol or rubrovit or rubyvan or rubyvit or ruvite or "cycolamins.p. cycolamine" or sytobex or transcyanocobalamin or "twel be" or twelbe or tweltone or "twelve oral" or twelveoral or "vi twel" or vibalt or vibicon? or vibicon? or vibisone or "vicapan n" or vitarubin or virubra or vitadom):ti,ab	4528
#20	[mh ^"Selenium"]	738
#21	(Selenium):ti,ab	1995
#22	{OR #9-#21}	35170
#23	#8 AND #22	199
#24	#8 AND #22 in Cochrane Reviews	8

Database: Epistemonikos

Date: 25.10.21

Number of hits: 16 systematic reviews without internal duplicates.

((("Sperm*" or "semen" or "seminal fluid*" or "ejacul*" or "spermatozo*" or "spermatids" or "spermatocytes" or "spermatogonia" or "Oligospermia" or "Oligozoospermia*" or

"Oligoasthenoteratozoospermia" or "asthenozoospermia*" or "asthenospermia*" or "criptozoospermia*" or "azoosperm*" or "globozoospermia*" or "teratospermia*" or "teratozoospermia*" or "male infertility" or "infertile men" or "Sexual matur*" or "Pubert*") AND ("omega 3 fatty acid" OR "n 3 fatty acid" OR "eicosapentaenoic acid" OR "eicosapentaenoate" OR icosapent OR "docosahexaenoic acid" OR "long chain fatty acids" OR "long chain polyunsaturated fatty acids" OR "LC PUFA" OR "polyunsaturated fatty acid" OR "fish oil" OR "cod liver oil")) = 2 systematic reviews

((("Sperm*" or "semen" or "seminal fluid*" or "ejacul*" or "spermatozo*" or "spermatids" or "spermatocytes" or "spermatogonia" or "Oligospermia" or "Oligozoospermia*" or "Oligoasthenoteratozoospermia" or "asthenozoospermia*" or "asthenospermia*" or "criptozoospermia*" or "azoosperm*" or "globozoospermia*" or "teratospermia*" or "teratozoospermia*" or "male infertility" or "infertile men" or "Sexual matur*" or "Pubert*") AND ("vitamin d" or "1406-16-2" or cholecalciferol* or hydroxycholecalciferol* or "hydroxy cholecalciferol*" or calcifediol* or dihydroxycholecalciferol* or "dihydro cholecalciferol*" or calcitriol* or "24,25-dihydroxyvitamin D" or ergocalciferol* or dihydrotachysterol* or "25-hydroxyvitamin D" or lunacalcipol or doxercalciferol or paricalcitol)) = 5 systematic reviews

((("Sperm*" or "semen" or "seminal fluid*" or "ejacul*" or "spermatozo*" or "spermatids" or "spermatocytes" or "spermatogonia" or "Oligospermia" or "Oligozoospermia*" or "Oligoasthenoteratozoospermia" or "asthenozoospermia*" or "asthenospermia*" or "criptozoospermia*" or "azoosperm*" or "globozoospermia*" or "teratospermia*" or "teratozoospermia*" or "male infertility" or "infertile men" or "Sexual matur*" or "Pubert*") AND (iodine or iodide)) = 1 systematic review

((("Sperm*" or "semen" or "seminal fluid*" or "ejacul*" or "spermatozo*" or "spermatids" or "spermatocytes" or "spermatogonia" or "Oligospermia" or "Oligozoospermia*" or "Oligoasthenoteratozoospermia" or "asthenozoospermia*" or "asthenospermia*" or "criptozoospermia*" or "azoosperm*" or "globozoospermia*" or "teratospermia*" or "teratozoospermia*" or "male infertility" or "infertile men" or "Sexual matur*" or "Pubert*") AND ("vitamin b12" or "vitamin b 12" or cyanocobalamin or Cobalamin or cyanocobal*)) = 1 systematic review

((("Sperm*" or "semen" or "seminal fluid*" or "ejacul*" or "spermatozo*" or "spermatids" or "spermatocytes" or "spermatogonia" or "Oligospermia" or "Oligozoospermia*" or "Oligoasthenoteratozoospermia" or "asthenozoospermia*" or "asthenospermia*" or "criptozoospermia*" or "azoosperm*" or "globozoospermia*" or "teratospermia*" or "teratozoospermia*" or "male infertility" or "infertile men" or "Sexual matur*" or "Pubert*") AND (Selenium))= 9 systematic reviews

15.4.2 LC n-3 fatty acids

15.4.2.1 LCN-3FA and neurodevelopment, cognitive function/disorders, diabetes, mortality, birth outcomes, prevention RA/MS

Database: Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Daily and Versions(R) <1946 to May 06, 2020>

Date: 14.05.2020

Number of hits: 873

#	Searches	
1	exp Fatty Acids, Omega-3/ or Fatty Acids, Unsaturated/ or Fish oils/ or Cod liver oils/	45471
2	((("omega 3" adj ("fatty acid?" or "carboxylic acid?")) or (("n 3" or n3) adj ("fatty acid?" or oil? or pufa)) or ((eicosapentaenoic or eicosapentenoic or eicosapentanoic or timnodonic) adj acid?) or eicosapentaenoate or "1553-41-9" or ("5,8,11,14,17" adj ("icosapentaenoic acid?" or icosapentaenoic acid? or "pentaene carboxylic acid?" or "pentaenoic acid?")) or icosapentor or aan7qov9ea or icosapentaenoate or icosapent or "docosahexaenoic acid?" or dhasco or docosahexaenoate or "121191-40-0" or "122045-76-5" or "123301-30-4" or "123301-31-5" or "124020-09-3" or "2091-24-9" or "25167-62-8" or "32839-18-2" or "6217-54-5" or "6610-27-1" or "89022-31-1" or "91403-70-2" or "long chain fatty acid?" or "long chain polyunsaturated fatty acid?" or "LC PUFA" or "LC fatty acid?" or ((polyunsaturated or "poly unsaturated" or polyunsaturation or unsaturated) adj ("fatty acid?" or fat or lipid?)) or "alkenyl fatty acid?" or "docosapentaoenic acid?" or "fish liver oil?" or "fish oil?" or "tuna oil?" or "8001-69-2" or "cod liver oil?" or "codfish liver oil?" or "codliver oil?").tw,kf.	67701
3	1 or 2	81069
4	exp Human development/ or Child Development/ or Motor disorders/ or Psychomotor Disorders/ or exp Psychomotor Performance/ or Cognition/ or Cognitive dysfunction/ or exp Neurocognitive disorders/ or Mental health/ or exp Academic performance/ or exp Child behavior/ or Impulsive Behavior/ or "Inhibition (Psychology)" or exp Language disorders/ or Mental disorders/ or Behavioral Symptoms/ or Behavior/ or Anxiety disorders/ or exp "Bipolar and related disorders"/ or Anger/ or Affect/ or Depression/ or Mood disorders/ or Aggression/ or exp Schizophrenia/ or exp Neurodevelopmental Disorders/ or exp Autism spectrum disorder/ or Attention deficit disorder with hyperactivity/ or Attention/ or Learning/ or Reading/ or Mathematics/ or Aptitude tests/ or Language tests/ or Communication/ or Language/ or Language development/ or Child language/ or Literacy/ or Intelligence/ or Executive function/ or Social behavior/ or Social adjustment/ or Emotional intelligence/ or Emotions/ or Temperament/ or exp Amnesia/ or Memory Disorders/ or Dementia/ or Alzheimer disease/ or Memory, Short-Term/ or Memory, Long-term/	1521961

#	Searches	
5	((("Child*" or "infant*" or "f?etal" or "prenatal" or "pre natal" or "postnatal" or "post natal" or "human" or "antepartum period?" or "ante partum period?") adj3 "development?") or "inhibition" or ("brain" adj2 ("damage?" or "injur*" or "development?" or "disorder?")) or "psychomotor" or "psycho motor" or "motor" or "sensorimotor" or "sensori motor" or "sensorymotor" or "sensory motor" or "cognition" or "cognitive function?" or "Mental health" or "Disorder? of higher cerebral function?" or ("psychological" adj ("well being" or "wellbeing"))) or (("neurocognit*" or "neuro cognit*" or "neurological" or "nervous system" or "nervoussystem" or "cognitive" or "development*" or "mental") adj2 ("dysfunction?" or "function?" or "decline?" or "deterioration?" or "Defici*" or "illness*" or "retardation?" or "disturbance?" or "impairment?" or "disorder?" or "impact?" or "disabilit*" or "deviation?" or "development?")) or "neurodevelopment*" or "neuro development*" or "autis*" or "asperger" or "kanner?" or "ASD" or "attention deficit" or "hyperactiv*" or "ADDH" or "ADHD" or "AD/HD" or "ADD" or "minimal brain dysfunction" or "impulsiveness" or "dyslexia" or "dyslexic?" or "dyscalculia" or "dyscalculic?" or "attention" or "learning" or "reading" or "mathematic?" or "math" or "maths" or ("aptitude" adj1 "test?") or (("Education" or "Educational" or "academic" or "school") adj1 ("Status" or "attainment?" or "achievement?" or "performance?" or "underachievement?" or "under achievement?" or "score?" or "success*" or "failure?")) or "executive function?" or "information processing" or "school readiness" or "school ready" or "Emotion*" or "socialemotional" or "social emotional" or "socioemotional" or "socio emotional" or ("social" adj ("development?" or "behavio?r" or "adjustment?")) or ("intel?ectual" adj2 ("development?" or "deficien*" or "disorder?" or "retardation?" or "disabilit*" or "disturbance?" or "impairment?")) or "Communication" or "language?" or "literacy" or "literacies" or "IQ" or "intel?igence" or "Speech disorder?" or "mutism?" or "aphasia" or "stutter*" or "dysphasia" or "alexia" or "anxiet*" or "depression?" or "depressive" or "mood disorder?" or "schizophrenia" or "schizophrenic" or "aggression" or "behavio?r*" or "affect" or "anger" or "bipolar" or "Temperament?" or "personalit*" or "amnesia?" or "dementia?" or "Alzheimer?" or "Parkinson?" or "huntington?" or ("memory" adj3 ("disorder?" or "impairment?" or "disturbance?" or "deficianc*" or "disabilit*" or "short term" or "shortterm" or "long term" or "longterm" or "verbal recognition?"))).tw,kf.	6651738
6	4 or 5	7076711
7	Birth weight/ or Pregnancy outcome/ or Premature birth/ or Growth/	119676
8	("growth" or (("premature" or "pre term" or "preterm") adj "birth?") or "SGA" or (("birth" or "gestational" or "neonatal" or "neo natal" or "newborn" or "new born" or "f?etal" or "f?etus" or "baby" or "babies") adj2 ("weight" or "size?")) or (("Pregnancy" or "birth" or "obstetric") adj "outcome?"))).tw,kf.	1520469
9	7 or 8	1574202
10	exp Arthritis, Rheumatoid/ or exp Multiple sclerosis/	169615
11	("rheumatoid arthritis" or "Multiple scleros#s").tw,kf.	177507
12	10 or 11	226430
13	exp Diabetes mellitus/	420863
14	("diabetes" or "sugar sickness" or "hypoglycemia" or "hypo glycemia" or "hyperglycemia" or "hyper glycemia").tw,kf.	558807
15	13 or 14	673662
16	exp Mortality/	377696
17	("mortalit*" or "death rate?" or "deathrate?").tw,kf.	768289
18	16 or 17	1003208
19	6 or 9 or 12 or 15 or 18	9404423
20	3 and 19	33201
21	Animal/ not (animal/ and human/)	4663303
22	20 not 21	23240
23	limit 22 to (danish or english or french or german or norwegian or swedish)	22441
24	limit 23 to "reviews (maximizes specificity)"	799

#	Searches	
25	Meta-Analysis/ or ((systematic* adj2 review*) or metaanal* or "meta anal*" or (review and ((structured or database* or systematic*) adj2 search*)) or "integrative review*" or (evidence adj2 review*)).tw,kf,bt.	331133
26	24 or (23 and 25)	1160
27	limit 26 to yr="2010 -Current"	873

Database: Embase <1974 to 2020 May 06>

Date: 14.05.2020

Number of hits: 1318

#	Searches	
1	omega 3 fatty acid/ or Icosapentaenoic Acid/ or Docosahexaenoic Acid/ or Long chain fatty acid/ or unsaturated fatty acid/ or polyunsaturated fatty acid/ or docosapentaenoic acid/ or Fish oil/ or Cod liver oil/	86695
2	((("omega 3" adj ("fatty acid?" or "carboxylic acid?")) or (("n 3" or n3) adj ("fatty acid?" or oil? or pufa)) or ((eicosapentaenoic or eicosapentenoic or eicosapentanoic or timnodonic) adj acid?) or eicosapentaenoate or "1553-41-9" or ("5,8,11,14,17" adj ("icosapentaenoic acid?" or icosapentaenoic acid? or "pentaene carboxylic acid?" or "pentaenoic acid?")) or icosapentor or aan7qov9ea or icosapentaenoate or icosapent or "docosahexaenoic acid?" or dhasco or docosahexaenoate or "121191-40-0" or "122045-76-5" or "123301-30-4" or "123301-31-5" or "124020-09-3" or "2091-24-9" or "25167-62-8" or "32839-18-2" or "6217-54-5" or "6610-27-1" or "89022-31-1" or "91403-70-2" or "long chain fatty acid?" or "long chain polyunsaturated fatty acid?" or "LC PUFA" or "LC fatty acid?" or ((polyunsaturated or "poly unsaturated" or polyunsaturation or unsaturated) adj ("fatty acid?" or fat or lipid?)) or "alkenyl fatty acid?" or "docosapentaoenic acid?" or "fish liver oil?" or "fish oil?" or "tuna oil?" or "8001-69-2" or "cod liver oil?" or "codfish liver oil?" or "codliver oil?").tw,kw.	83078
3	1 or 2	110131
4	exp Human development/ or exp postnatal development/ or exp prenatal development/ or Motor disorders/ or Psychomotor Disorder/ or Hyperactivity/ or exp Psychomotor Performance/ or Psychomotor development/ or Motor development/ or Cognition/ or Cognitive dysfunction/ or Cognitive development/ or exp Disorders of higher cerebral function/ or exp Mental health/ or exp Academic achievement/ or exp Child behavior/ or Problem behavior/ or Impulsiveness/ or "Inhibition (Psychology)" or exp Language disability/ or Mental disease/ or Behavior/ or Anxiety disorder/ or exp Bipolar disorder/ or Anger/ or Affect/ or Depression/ or Mood disorder/ or Aggression/ or exp Schizophrenia/ or exp Mental disease/ or Attention deficit disorder/ or Attention/ or Learning/ or Reading/ or Mathematics/ or Aptitude test/ or Language test/ or Interpersonal communication/ or Language/ or Language development/ or Literacy/ or Intelligence/ or Executive function/ or Social behavior/ or Social adaption/ or Emotional intelligence/ or Emotion/ or Temperament/ or exp Amnesia/ or Memory Disorder/ or Dementia/ or Alzheimer disease/ or Short term memory/ or Long term memory/	3458647

#	Searches	
5	((("Child*" or "infant*" or "f?etal" or "prenatal" or "pre natal" or "postnatal" or "post natal" or "human" or "antepartum period?" or "ante partum period?") adj3 "development?") or "inhibition" or ("brain" adj2 ("damage?" or "injur*" or "development?" or "disorder?")) or "psychomotor" or "psycho motor" or "motor" or "sensorimotor" or "sensori motor" or "sensorymotor" or "sensory motor" or "cognition" or "cognitive function?" or "Mental health" or "Disorder? of higher cerebral function?" or ("psychological" adj ("well being" or "wellbeing")) or (("neurocognit*" or "neuro cognit*" or "neurological" or "nervous system" or "nervoussystem" or "cognitive" or "development*" or "mental") adj2 ("dysfunction?" or "function?" or "decline?" or "deterioration?" or "Defici*" or "illness*" or "retardation?" or "disturbance?" or "impairment?" or "disorder?" or "impact?" or "disabilit*" or "deviation?" or "development?")) or "neurodevelopment*" or "neuro development*" or "autis*" or "asperger" or "kanner?" or "ASD" or "attention deficit" or "hyperactiv*" or "ADDH" or "ADHD" or "AD/HD" or "ADD" or "minimal brain dysfunction" or "impulsiveness" or "dyslexia" or "dyslexic?" or "dyscalculia" or "dyscalculic?" or "attention" or "learning" or "reading" or "mathematic?" or "math" or "maths" or ("aptitude" adj1 "test?") or ("Education" or "Educational" or "academic" or "school") adj1 ("Status" or "attainment?" or "achievement?" or "performance?" or "underachievement?" or "under achievement?" or "score?" or "success*" or "failure?")) or "executive function?" or "information processing" or "school readiness" or "school ready" or "Emotion*" or "socialemotional" or "social emotional" or "socioemotional" or "socio emotional" or ("social" adj ("development?" or "behavio?r" or "adjustment?")) or ("intel?ectual" adj2 ("development?" or "deficien*" or "disorder?" or "retardation?" or "disabilit*" or "disturbance?" or "impairment?")) or "Communication" or "language?" or "literacy" or "literacies" or "IQ" or "intel?igence" or "Speech disorder?" or "mutism?" or "aphasia" or "stutter*" or "dysphasia" or "alexia" or "anxiet*" or "depression?" or "depressive" or "mood disorder?" or "schizophrenia" or "schizophrenic" or "aggression" or "behavio?r*" or "affect" or "anger" or "bipolar" or "Temperament?" or "personalit*" or "amnesia?" or "dementia?" or "Alzheimer?" or "Parkinson?" or "huntington?" or ("memory" adj3 ("disorder?" or "impairment?" or "disturbance?" or "deficianc*" or "disabilit*" or "short term" or "shortterm" or "long term" or "longterm" or "verbal recognition?"))).tw,kw.	8226565
6	4 or 5	9320257
7	exp Birth weight/ or Pregnancy outcome/ or Prematurity/ or Growth/	261860
8	("growth" or (("premature" or "pre term" or "preterm") adj "birth?") or "SGA" or (("birth" or "gestational" or "neonatal" or "neo natal" or "newborn" or "new born" or "f?etal" or "f?etus" or "baby" or "babies") adj2 ("weight" or "size?")) or (("Pregnancy" or "birth" or "obstetric") adj "outcome?")).tw,kw.	1815975
9	7 or 8	1934009
10	exp Rheumatoid arthritis/ or Multiple sclerosis/	318280
11	("rheumatoid arthritis" or "Multiple scleros#s").tw,kw.	267344
12	10 or 11	350253
13	exp Diabetes mellitus/	941136
14	("diabetes" or "sugar sickness" or "hypoglycemia" or "hypo glycemia" or "hyperglycemia" or "hyper glycemia").tw,kw.	839774
15	13 or 14	1116607
16	exp Mortality/	1057714
17	("mortalit*" or "death rate?" or "deathrate?").tw,kw.	1120148
18	16 or 17	1457778
19	6 or 9 or 12 or 15 or 18	12408339
20	3 and 19	52282
21	limit 20 to embase	38552
22	(animal/ or exp nonhuman/ or Animal experiment/) not ((animal/ or exp nonhuman/ or Animal experiment/) and exp human/)	5992536
23	21 not 22	27236

#	Searches	
24	limit 23 to (danish or english or french or german or norwegian or swedish)	26462
25	limit 24 to "reviews (maximizes specificity)"	862
26	Meta-Analysis/ or "systematic review"/ or ((systematic* adj2 review*) or metaanal* or "meta anal*" or (review and ((structured or database* or systematic*) adj2 search*)) or "integrative review*" or (evidence adj2 review*)).tw,kw.	480496
27	25 or (24 and 26)	1897
28	limit 27 to yr="2010 -Current"	1318

Database: Cochrane Database of Systematic Reviews Issue 5 of 12, May 2020

Date: 14.05.2020

Number of hits: 51 systematic reviews

#	Searches	
#1	[mh "Fatty acids, Omega-3"]	2966
#2	[mh ^"Fatty acids, Unsaturated"]	697
#3	[mh ^"Fish oils"]	1003
#4	[mh ^"Cod liver oils"]	0
#5	((("omega 3" NEXT (fatty or carboxylic) NEXT acid?) or (("n 3" or n3) NEXT ("fatty acid" or "fatty acids" or oil? or pufa)) or ((eicosapentaenoic or eicosapentenoic or eicosapentanoic or timnodonic or docosahexaenoic or "long chain fatty" or "long chain polyunsaturated fatty" or "LC fatty" or "alkenyl fatty" or "docosapentaoenic") NEXT acid?) or eicosapentaenoate or "1553-41-9" or ("5,8,11,14,17" NEXT ("icosapentaenoic acid" or "icosapentaenoic acids" or "pentaene carboxylic acid" or "pentaene carboxylic acids" or "pentaenoic acid" or "pentaenoic acids"))) or icosapentor or aan7qov9ea or icosapentaenoate or icosapent or dhasco or docosahexaenoate or "121191-40-0" or "122045-76-5" or "123301-30-4" or "123301-31-5" or "124020-09-3" or "2091-24-9" or "25167-62-8" or "32839-18-2" or "6217-54-5" or "6610-27-1" or "89022-31-1" or "91403-70-2" or "LC PUFA" or ((polyunsaturated or "poly unsaturated" or polyunsaturation or unsaturated) NEXT ("fatty acid" or "fatty acids" or fat or lipid?)) or (("fish liver" or "fish" or "tuna" or "cod liver" or "codfish liver" or codliver) NEXT oil?) or "8001-69-2"):ti,ab	7224
#6	{OR #1-#5}	8172
#7	[mh "Human development"]	2499
#8	[mh ^"Child Development"]	1825
#9	[mh ^"Motor disorders"]	27
#10	[mh ^"Psychomotor Disorders"]	187
#11	[mh "Psychomotor Performance"]	8685
#12	[mh ^"Cognition"]	7236
#13	[mh ^"Cognitive dysfunction"]	1305
#14	[mh "Neurocognitive disorders"]	10689
#15	[mh "Academic performance"]	47
#16	[mh ^"Mental health"]	1407
#17	[mh "Child behavior"]	2043
#18	[mh ^"Impulsive Behavior"]	452
#19	[mh ^"Inhibition (Psychology)"]	578
#20	[mh "Language disorders"]	1316
#21	[mh ^"Mental disorders"]	3581
#22	[mh ^"Behavioral Symptoms"]	178
#23	[mh ^"Behavior"]	849

#	Searches	
#24	[mh ^"Anxiety disorders"]	3622
#25	[mh "Bipolar and related disorders"]	2637
#26	[mh ^"Anger"]	424
#27	[mh ^"Affect"]	4312
#28	[mh ^"Depression"]	11792
#29	[mh ^"Mood disorders"]	802
#30	[mh ^"Aggression"]	1168
#31	[mh "Schizophrenia"]	7397
#32	[mh "Neurodevelopmental Disorders"]	7475
#33	[mh "Autism spectrum disorder"]	1392
#34	[mh ^"Attention deficit disorder with hyperactivity"]	2685
#35	[mh ^"Attention"]	5106
#36	[mh ^"Learning"]	2131
#37	[mh ^"Reading"]	834
#38	[mh ^"Mathematics"]	396
#39	[mh ^"Aptitude tests"]	29
#40	[mh ^"Language tests"]	236
#41	[mh ^"Communication"]	2148
#42	[mh ^"Language"]	651
#43	[mh ^"Language development"]	262
#44	[mh ^"Child language"]	131
#45	[mh ^"Literacy"]	26
#46	[mh ^"Intelligence"]	577
#47	[mh ^"Executive function"]	938
#48	[mh ^"Social behavior"]	1581
#49	[mh ^"Social adjustment"]	911
#50	[mh ^"Emotional intelligence"]	78
#51	[mh ^"Emotions"]	3143
#52	[mh ^"Temperament"]	117
#53	[mh "Amnesia"]	285
#54	[mh ^"Memory Disorders"]	881
#55	[mh ^"Dementia"]	2289
#56	[mh ^"Alzheimer disease"]	3307
#57	[mh ^"Memory, Short-Term"]	1453
#58	[mh ^"Memory, Long-term"]	46

#	Searches	
#59	(Child* or infant* or fetal or foetal or faetal or prenatal or "pre natal" or postnatal or "post natal" or human or (("antepartum period" or "ante partum period") NEAR/3 development?) or inhibition or (brain NEAR/2 (damage? or injur* or development? or disorder?)) or psychomotor or "psycho motor" or motor or sensorimotor or "sensori motor" or sensorymotor or "sensory motor" or cognition or (cognitive NEXT function?) or "Mental health" or (("Disorder of higher cerebral") NEXT function?) or (psychological NEXT ("well being" or wellbeing)) or ((neurocognitive or "neuro cognitive" or neurological or "nervous system" or nervoussystem or cognitive or development or developmental or mental) NEAR/2 (dysfunction? or function? or decline? or deterioration? or Defici* or illness* or retardation? or disturbance? or impairment? or disorder? or impact? or disabilit* or deviation? or development?)) or neurodevelopment* or (neuro NEXT development*) or autis* or Asperger or kanner? or ASD or "attention deficit" or hyperactiv* or ADDH or ADHD or "AD/HD" or ADD or "minimal brain dysfunction" or impulsiveness or dyslexia or dyslexic? or dyscalculia or dyscalculic? or attention or learning or reading or mathematic? or math or maths or (aptitude NEAR/1 test?) or ((Education or Educational or academic or school) NEAR/1 (Status or attainment? or achievement? or performance? or underachievement? or "under achievement" or "under achievements" or score? or success* or failure?)) or (executive NEXT function?) or "information processing" or "school readiness" or "school ready" or Emotion* or socialemotional or "social emotional" or socioemotional or "socio emotional" or (social NEXT (development? or behavior or behaviour or adjustment?)) or ((intellectual or intellectual) NEAR/2 (development? or deficien* or disorder? or retardation? or disabilit* or disturbance? or impairment?)) or Communication or language? or literacy or literacies or IQ or intelligence or intelligence or "Speech disorder?" or mutism? or aphasia or stutter* or dysphasia or alexia or anxiet* or depression? or depressive or (mood NEXT disorder?) or schizophrenia or schizophrenic or aggression or behavior* or behavior* or affect or anger or bipolar or Temperament? or personalit* or amnesia? or dementia? or Alzheimer? or Parkinson? or huntington? or (memory NEAR/3 (disorder? or impairment? or disturbance? or deficianc* or disabilit* or "short term" or shortterm or "long term" or longterm)) or (verbal NEXT recognition?):ti,ab	497158
#60	{OR #7-#59}	510587
#61	[mh ^"Birth weight"]	1629
#62	[mh ^"Pregnancy outcome"]	2942
#63	[mh ^"Premature birth"]	1413
#64	[mh ^"Growth"]	717
#65	(growth or ((premature or "pre term" or preterm) NEXT birth?) or SGA or ((birth or gestational or neonatal or "neo natal" or newborn or "new born" or fetal or foetal or faetal or fetus or foetus or faetus or baby or babies) NEAR/2 (weight or size?)) or ((Pregnancy or birth or obstetric) NEXT outcome?):ti,ab	46175
#66	{OR #61-#65}	49334
#67	[mh "Arthritis, Rheumatoid"]	6011
#68	[mh "Multiple sclerosis"]	3352
#69	("rheumatoid arthritis" or "Multiple sclerosis" or "Multiple skleroses"):ti,ab	13582
#70	{OR #67-#69}	17831
#71	[mh "Diabetes mellitus"]	30486
#72	(diabetes or "sugar sickness" or hypoglycemia or "hypo glycemia" or hyperglycemia or "hyper glycemia"):ti,ab	71928
#73	{OR #71-#72}	77846
#74	[mh "Mortality"]	12784
#75	(mortalit* or (death NEXT rate?) or deathrate?):ti,ab	680549
#76	{OR #74-#75}	13645
#77	#60 OR #66 or #70 or #73 or #76	606236
#78	#6 and #77 in Cochrane Reviews	62

#	Searches	
#79	#6 and #77 with Cochrane Library publication date Between Jan 2010 and Jun 2020, in Cochrane Reviews	51

Database: Epistemonikos

Date: 14.05.2020

Number of hits: 237 systematic reviews without internal duplicates.

("omega 3 fatty acid" OR "n 3 fatty acid" OR "eicosapentaenoic acid" OR "eicosapentaenoate" OR icosapent OR "docosahexaenoic acid" OR "long chain fatty acids" OR "long chain polyunsaturated fatty acids" OR "LC PUFA" OR "polyunsaturated fatty acid" OR "fish oil" OR "cod liver oil") AND ("Child development" OR "infant development" OR "fetal development" OR "prenatal development" OR "postnatal development" OR "human development" OR "inhibition" OR "brain damage" OR "brain injury" OR "brain development" OR "psychomotor" OR "motor" OR "sensorimotor" OR "cognition" OR "cognitive function" OR "Mental health" OR "psychological wellbeing" OR "neurological" OR "nervous system" OR "cognitive dysfunction" OR "cognitive function" OR "cognitive development" OR "development*" OR "mental illness" OR neurodevelopment* OR "neuro development" OR "neuro developmental" OR autis* OR asperger OR kanner* OR "ASD" OR "attention deficit" OR hyperactiv* OR "ADDH" OR "ADHD" OR "AD/HD" OR "ADD" OR "impulsiveness") = 125 systematic reviews

("omega 3 fatty acid" OR "n 3 fatty acid" OR "eicosapentaenoic acid" OR "eicosapentaenoate" OR icosapent OR "docosahexaenoic acid" OR "long chain fatty acids" OR "long chain polyunsaturated fatty acids" OR "LC PUFA" OR "polyunsaturated fatty acid" OR "fish oil" OR "cod liver oil") AND ("dyslexia" OR dyslexic* OR "dyscalculia" OR dyscalculic* OR "attention" OR "learning" OR "reading" OR mathematic* OR math* OR "Education" OR "Educational status" OR "academic achievement" OR "academic failure" OR "school performance" OR "executive function*" OR "information processing" OR "school readiness" OR Emotion* OR "socialemotional" OR "socioemotional" OR "social development" OR "intellectual development" OR "intellectual disability" OR "intellectual disabilities" OR "intellectual disorder") = 37 systematic reviews

("omega 3 fatty acid" OR "n 3 fatty acid" OR "eicosapentaenoic acid" OR "eicosapentaenoate" OR icosapent OR "docosahexaenoic acid" OR "long chain fatty acids" OR "long chain polyunsaturated fatty acids" OR "LC PUFA" OR "polyunsaturated fatty acid" OR "fish oil" OR "cod liver oil") AND ("Communication" OR "language" OR "literacy" OR "IQ" OR "intelligence" OR "Speech disorder" OR "mutism" OR "anxiety" OR "anxieties" OR "depression" OR "depressive" OR "mood disorder" OR "schizophrenia" OR "schizophrenic" OR "aggression" OR "behavior" OR "affect" OR "anger" OR "bipolar" OR "Temperament" OR personalit* OR amnesia? OR dementia? OR Alzheimer* OR Parkinson* OR huntington* OR

"memory disorder" OR "memory impairment" OR "short term memory" OR "long term memory" OR "verbal recognition") = 136 systematic reviews

("omega 3 fatty acid" OR "n 3 fatty acid" OR "eicosapentaenoic acid" OR "eicosapentaenoate" OR icosapent OR "docosahexaenoic acid" OR "long chain fatty acids" OR "long chain polyunsaturated fatty acids" OR "LC PUFA" OR "polyunsaturated fatty acid" OR "fish oil" OR "cod liver oil") AND ("birth weight" OR "birth size" OR "gestational weight" OR "gestational size" OR "neonatal size" OR "neonatal weight" OR "newborn weight" OR "newborn size" OR "fetal weight" OR "fetal size" OR "fetus size" OR "fetus weight" OR "pregnancy outcome" OR "birth outcome" OR "obstetric outcome" OR mortality OR "death rate" OR "diabetes mellitus" OR "rheumatoid arthritis" OR "Multiple sclerosis") = 131 systematic reviews

15.4.2.2 *LCn-3FA and CVD*

Contact person: Bente Mangschou

Search: Trude Anine Muggerud

Referee: Astrid Merethe Nøstberg

Comment: Searchterms from previous search for fish intake. Limit to systematic reviews/meta-analyses, 2016-current, language, animal studies excluded.

Duplicate check in Before Duplicate check: 848

EndNote: After Duplicate check: 564

Database: Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Daily and Versions(R) <1946 to June 22, 2021>

Date: 23.06.2021

Number of hits: 251 systematic reviews

#	Searches	
1	exp Fatty Acids, Omega-3/ or Fatty Acids, Unsaturated/ or Fish oils/ or Cod liver oil/	48296
2	((("omega 3" adj ("fatty acid?" or "carboxylic acid?")) or (("n 3" or n3) adj ("fatty acid?" or oil? or pufa)) or ((eicosapentaenoic or eicosapentenoic or eicosapentanoic or timnodonic) adj acid?) or eicosapentaenoate or "1553-41-9" or ("5,8,11,14,17" adj ("icosapentaenoic acid?" or "pentaene carboxylic acid?" or "pentaenoic acid?")) or icosapentor or aan7qov9ea or icosapentaenoate or icosapent or "docosahexaenoic acid?" or dhasco or docosahexaenoate or "121191-40-0" or "122045-76-5" or "123301-30-4" or "123301-31-5" or "124020-09-3" or "2091-24-9" or "25167-62-8" or "32839-18-2" or "6217-54-5" or "6610-27-1" or "89022-31-1" or "91403-70-2" or "long chain fatty acid?" or "long chain polyunsaturated fatty acid?" or "LC PUFA" or "LC fatty acid?" or ((polyunsaturated or "poly unsaturated" or polyunsaturation or unsaturated) adj ("fatty acid?" or fat or lipid?)) or "alkenyl fatty acid?" or "docosapentaenoic acid?" or "fish liver oil?" or "fish oil?" or "tuna oil?" or "8001-69-2" or "cod liver oil?" or "codfish liver oil?" or "codliver oil?").tw,kf.	72623
3	1 or 2	86484
4	exp Cardiovascular diseases/ or Cerebrovascular disorders/ or exp Ischemia/ or exp Stroke/	2527981
5	((("Cardiovascular" or "heart" or "cardiac" or "myocardial" or "myo cardial" or "cerebrovascular" or "vascular" or "coronary" or "cerebral" or "peripheral" or "endothelial") adj ("disease?" or "disorder?" or "failure" or "event?" or "health" or "effect?" or "accident?" or "calcification?" or "risk factor?" or "riskfactor?" or "syndrom?" or "syndrome?" or "revasculari#ation?" or "arter*" or "function?" or "dysfunction?" or "attack?" or "arrest" or "apoplex*" or "insufficienc*" or "injur*" or "insult?" or "scleros#s" or "stenos#s" or "restenos#s")) or "cardioprotect*" or "cardio protect*" or "high cardiovascular risk?" or "CVD" or "infarct*" or "reinfarction?" or "aneurysm?" or "angina" or "artherosclero*" or "arthero sclero*" or "arteriosclero*" or "arterio sclero*" or "isch?emi*" or "nonisch?emi*" or "non isch?emic" or "thrombos#s" or "thrombolism?" or "tachycardia*" or "tachyarrhythmia?" or "arrhythmia?" or (("ventricular" or "arterial") adj ("fibrillation?" or "compliance?" or "stiffness*")) or "sudden cardiac death?" or "stroke?" or "TIA" or ("brain" adj ("h?emorrhage?" or "accident?" or "attack?" or "infarct*" or "insult?"))).tw,kf.	1924288
6	4 or 5	3149781
7	3 and 6	12974
8	limit 7 to yr="2016 -Current"	3042
9	limit 8 to "reviews (maximizes specificity)"	147
10	Meta-Analysis/ or ((systematic* adj2 review*) or metaanal* or "meta anal*" or (review and ((structured or database* or systematic*) adj2 search*)) or "integrative review*" or (evidence adj2 review*)).tw,kf,bt.	391393
11	9 or (8 and 10)	255
12	Animals/ not (animals/ and humans/)	4816074
13	11 not 12	253
14	limit 13 to (danish or english or french or german or interlingua or multilingual or norwegian or swedish)	251

Database: Embase 1974 to 2021 June 22

Date: 23.06.21

Number of hits: 523 systematic reviews

#	Searches	
1	omega 3 fatty acid/ or Icosapentaenoic Acid/ or Docosahexaenoic Acid/ or Long chain fatty acid/ or unsaturated fatty acid/ or polyunsaturated fatty acid/ or docosapentaenoic acid/ or Fish oil/ or Cod liver oil/	92909

#	Searches	
2	((("omega 3" adj ("fatty acid?" or "carboxylic acid?")) or (("n 3" or n3) adj ("fatty acid?" or oil? or pufa)) or ((eicosapentaenoic or eicosapentenoic or eicosapentanoic or timnodonic) adj acid?) or eicosapentaenoate or "1553-41-9" or ("5,8,11,14,17" adj ("icosapentaenoic acid?" or "pentaene carboxylic acid?" or "pentaenoic acid?")) or icosapentor or aan7qov9ea or icosapentaenoate or icosapent or "docosahexaenoic acid?" or dhasco or docosahexaenoate or "121191-40-0" or "122045-76-5" or "123301-30-4" or "123301-31-5" or "124020-09-3" or "2091-24-9" or "25167-62-8" or "32839-18-2" or "6217-54-5" or "6610-27-1" or "89022-31-1" or "91403-70-2" or "long chain fatty acid?" or "long chain polyunsaturated fatty acid?" or "LC PUFA" or "LC fatty acid?" or ((polyunsaturated or "poly unsaturated" or polyunsaturation or unsaturated) adj ("fatty acid?" or fat or lipid?)) or "alkenyl fatty acid?" or "docosapentaoenic acid?" or "fish liver oil?" or "fish oil?" or "tuna oil?" or "8001-69-2" or "cod liver oil?" or "codfish liver oil?" or "codliver oil?").tw,kw.	88604
3	1 or 2	117988
4	exp Cardiovascular disease/ or Cerebrovascular disease/ or exp Ischemia/ or exp Cerebrovascular accident/	4290831
5	((("Cardiovascular" or "heart" or "cardiac" or "myocardial" or "myo cardial" or "cerebrovascular" or "vascular" or "coronary" or "cerebral" or "peripheral" or "endothelial") adj ("disease?" or "disorder?" or "failure" or "event?" or "health" or "effect?" or "accident?" or "calcification?" or "risk factor?" or "riskfactor?" or "syndrom?" or "syndrome?" or "revasculari#ation?" or "arter*" or "function?" or "dysfunction?" or "attack?" or "arrest" or "apoplex*" or "insufficienc*" or "injur*" or "insult?" or "scleros#s" or "stenos#s" or "restenos#s")) or "cardioprotect*" or "cardio protect*" or "high cardiovascular risk?" or "CVD" or "infarct*" or "reinfarction?" or "aneurysm?" or "angina" or "artherosclero*" or "arthero sclero*" or "arteriosclero*" or "arterio sclero*" or "isch?emi*" or "nonisch?emi*" or "non isch?emic" or "thrombos#s" or "thrombolism?" or "tachycardia*" or "tachyarrhythmia?" or "arrhythmia?" or (("ventricular" or "arterial") adj ("fibrillation?" or "compliance?" or "stiffness*")) or "sudden cardiac death?" or "stroke?" or "TIA" or ("brain" adj ("h?emorrhage?" or "accident?" or "attack?" or "infarct*" or "insult?"))).tw,kw.	2694844
6	4 or 5	4783212
7	3 and 6	26784
8	limit 7 to yr="2016 -Current"	7046
9	limit 8 to (conference abstracts or embase)	6572
10	limit 9 to "reviews (maximizes specificity)"	262
11	Meta-Analysis/ or "systematic review"/ or ((systematic* adj2 review*) or metaanal* or "meta anal*" or (review and ((structured or database* or systematic*) adj2 search*)) or "integrative review*" or (evidence adj2 review*)).tw,kw.	568622
12	10 or (9 and 11)	531
13	(animal/ or exp nonhuman/ or Animal experiment/) not ((animal/ or exp nonhuman/ or Animal experiment/) and exp human/)	6238318
14	12 not 13	525
15	limit 14 to (danish or english or french or german or norwegian or polyglot or swedish)	523

Database: Cochrane Database of Systematic Reviews: Issue 6 of 12, June 2021

Date: 23.06.2021

Number of hits: 9 Cochrane reviews

#	Searches	
#1	[mh "Fatty acids, Omega-3"]	3169
#2	[mh ^"Fatty acids, Unsaturated"]	728
#3	[mh ^"Fish oils"]	1042
#4	[mh ^"Cod liver oils"]	0
#5	((("omega 3" NEXT (fatty or carboxylic) NEXT acid?) or (("n 3" or n3) NEXT ("fatty acid" or "fatty acids" or oil? or pufa)) or ((eicosapentaenoic or eicosapentenoic or eicosapentanoic or timnodonic or docosahexaenoic or "long chain fatty" or "long chain polyunsaturated fatty" or "LC fatty" or "alkenyl fatty" or "docosapentaoenic") NEXT acid?) or eicosapentaenoate or "1553-41-9" or ("5,8,11,14,17" NEXT ("icosapentaenoic acid" or "icosapentaenoic acids" or "pentaene carboxylic acid" or "pentaene carboxylic acids" or "pentaenoic acid" or "pentaenoic acids"))) or icosapentor or aan7qov9ea or icosapentaenoate or icosapent or dhasco or docosahexaenoate or "121191-40-0" or "122045-76-5" or "123301-30-4" or "123301-31-5" or "124020-09-3" or "2091-24-9" or "25167-62-8" or "32839-18-2" or "6217-54-5" or "6610-27-1" or "89022-31-1" or "91403-70-2" or "LC PUFA" or ((polyunsaturated or "poly unsaturated" or polyunsaturation or unsaturated) NEXT ("fatty acid" or "fatty acids" or fat or lipid?)) or (("fish liver" or fish or tuna or "cod liver" or "codfish liver" or codliver) NEXT oil?) or "8001-69-2"):ti,ab	10134
#6	#1 or #2 or #3 or #4 or #5	10630
#7	[mh "Cardiovascular diseases"]	110764
#8	[mh ^"Cerebrovascular disorders"]	1442
#9	[mh "Ischemia"]	14347
#10	[mh "Stroke"]	10342
#11	((((Cardiovascular or heart or cardiac or myocardial or "myo cardial" or cerebrovascular or vascular or coronary or cerebral or peripheral or endothelial) NEXT (disease? or disorder? or failure or event? or health or effect? or accident? or calcification? or (risk NEXT factor?) or riskfactor? or syndrom? or syndrome? or revascularisation? or revascularization? or arter* or function? or dysfunction? or attack? or arrest or apoplex* or insufficienc* or injur* or insult? or sclerosis or scleroses or stenosis or stenoses or restenosis or restenoses)) or cardioprotect* or (cardio NEXT protect*) or ("high cardiovascular" NEXT risk?) or CVD or infarct* or reinfarction? or aneurysm? or angina or arterosclero* or (arthero NEXT sclero*) or arteriosclero* or (arterio NEXT sclero*) or isch?emi* or nonisch?emi* or "non isch?emic" or thrombosis or thromboses or thrombolism? or tachycardia* or tachyarrhythmia? or arrhythmia? or ((ventricular or arterial) NEXT (fibrillation? or compliance? or stiffness*)) or ("sudden cardiac" NEXT death?) or stroke? or TIA or (brain NEXT (h?emorrhage? or accident? or attack? or infarct* or insult?))):ti,ab	212887
#12	#7 or #8 or #9 or #10 or #11	253470
#13	#6 and #12	2834
#14	#6 and #12 with Cochrane Library publication date Between Jan 2016 and Jun 2021	1501
#15	#6 and #12 with Cochrane Library publication date Between Jan 2016 and Jun 2021, in Cochrane Reviews	9

Database: Epistemonikos

Date: 23.06.2021

Comment: Limited to last 5 years, and systematic reviews, structured reviews and broad synthesis.

Number of hits: 65 hits without internal duplicates

("omega 3" OR "n 3 fatty acid" OR "eicosapentaenoic acid" OR "eicosapentaenoate" OR icosapent OR "docosahexaenoic acid" OR "long chain fatty acids" OR "long chain polyunsaturated fatty acids" OR "LC PUFA" OR "polyunsaturated fatty acid" OR "fish oil" OR "cod liver oil") AND ("Cardiovascular disease" OR "Cardiovascular diseases" OR "Cardiovascular disorders" OR "Cardiovascular failure" OR "Cardiovascular event" OR "Cardiovascular events" OR "Cardiovascular health" OR "Cardiovascular effects" OR "Cardiovascular accidents" OR "Cardiovascular risk factors" OR "Cardiovascular function" OR "Cardiovascular dysfunction") = 44 hits

("omega 3" OR "n 3 fatty acid" OR "eicosapentaenoic acid" OR "eicosapentaenoate" OR icosapent OR "docosahexaenoic acid" OR "long chain fatty acids" OR "long chain polyunsaturated fatty acids" OR "LC PUFA" OR "polyunsaturated fatty acid" OR "fish oil" OR "cod liver oil") AND ("Cardiac disease" OR "Cardiac diseases" OR "Cardiac disorders" OR "Cardiac failure" OR "Cardiac events" OR "Cardiac health" OR "Cardiac effects" OR "Cardiac risk factors" OR "Cardiac syndrome" OR "Cardiac function" OR "Cardiac dysfunction" OR "Cardiac arrest" OR "Cardiac insufficiency" OR "Cardiac injury" OR "Cardiac injuries" OR "cardioprotection") = 2 hits

("omega 3" OR "n 3 fatty acid" OR "eicosapentaenoic acid" OR "eicosapentaenoate" OR icosapent OR "docosahexaenoic acid" OR "long chain fatty acids" OR "long chain polyunsaturated fatty acids" OR "LC PUFA" OR "polyunsaturated fatty acid" OR "fish oil" OR "cod liver oil") AND ("high cardiovascular risk" OR "CVD" OR "infarct" OR "infarcts" OR "infarction" OR "reinfarction" OR "Aneurysm" OR "aneurysms" OR "Angina" OR "arteriosclerosis" OR "ischemia" OR "ischaemia" OR "thrombosis" OR "thromboses" OR "thrombolism" OR "tachycardia" OR "tachyarrhythmia" OR "tachyarrhythmias" OR "arrhythmia" OR "arrhythmias" OR "sudden cardiac death" OR "stroke" OR "strokes" OR "TIA" OR "ventricular fibrillation" OR "arterial compliance" OR "arterial stiffness" OR "brain hemorrhage" OR "brain haemorrhage" OR "brain infarct" OR "brain infarcts") = 35 hits

("omega 3" OR "n 3 fatty acid" OR "eicosapentaenoic acid" OR "eicosapentaenoate" OR icosapent OR "docosahexaenoic acid" OR "long chain fatty acids" OR "long chain polyunsaturated fatty acids" OR "LC PUFA" OR "polyunsaturated fatty acid" OR "fish oil" OR "cod liver oil") AND ("heart disease" OR "heart diseases" OR "heart disorder" OR "heart disorders" OR "heart failure" OR "heart dysfunction" OR "heart dysfunctions" OR "heart attack" OR "heart attacks" OR "heart health" OR "heart syndrome" OR "heart injury" OR "heart injuries") = 17 hits

("omega 3" OR "n 3 fatty acid" OR "eicosapentaenoic acid" OR "eicosapentaenoate" OR icosapent OR "docosahexaenoic acid" OR "long chain fatty acids" OR "long chain polyunsaturated fatty acids" OR "LC PUFA" OR "polyunsaturated fatty acid" OR "fish oil" OR "cod liver oil") AND ("vascular disease" OR "vascular diseases" OR "vascular disorder" OR "vascular disorders" OR "vascular event" OR "vascular events" OR "vascular health" OR "vascular effects" OR "vascular accident" OR "vascular calcification" OR "vascular risk

factors" OR "vascular function" OR "vascular dysfunction" OR "vascular insufficiency" OR "vascular injury" OR "vascular injuries" OR "vascular stenosis" OR "cerebrovascular disease" OR "cerebrovascular diseases" OR "cerebrovascular disorder" OR "cerebrovascular disorders" OR "cerebrovascular event" OR "cerebrovascular events" OR "cerebrovascular accident" OR "cerebrovascular accidents" OR "cerebrovascular risk factors" OR "cerebrovascular function" OR "cerebrovascular injury" OR "cerebrovascular injuries" OR "cerebrovascular attack" OR "cerebrovascular insufficiency") = 5 hits

("omega 3" OR "n 3 fatty acid" OR "eicosapentaenoic acid" OR "eicosapentaenoate" OR icosapent OR "docosahexaenoic acid" OR "long chain fatty acids" OR "long chain polyunsaturated fatty acids" OR "LC PUFA" OR "polyunsaturated fatty acid" OR "fish oil" OR "cod liver oil") AND ("myocardial disease" OR "myocardial disorder" OR "myocardial failure" OR "myocardial effects" OR "myocardial insult" OR "coronary disease" OR "coronary disorders" OR "coronary health" OR "coronary event" OR "coronary events" OR "coronary risk factors" OR "coronary syndrome" OR "coronary syndromes" OR "coronary artery" OR "coronary arteries" OR "coronary vascularization" OR "coronary vascularisation" OR "coronary stenosis" OR "coronary stenoses" OR "coronary restenosis" OR "cerebral disease" OR "cerebral events" OR "cerebral injury" OR "cerebral vascularization" OR "cerebral function" OR "cerebral dysfunction" OR "peripheral artery" OR "peripheral arteries" OR "peripheral arterial" OR "endothelial function" OR "endothelial dysfunction") = 6 hits

15.4.3 Vitamin D

15.4.3.1 *Vitamin D and birth weight, respiratory tract infections*

Database: Embase <1974 to 2020 April 27>

Date: 29.04.20

Number of hits: 317

#	Searches	
1	exp Vitamin D/	139619
2	("vitamin d" or "1406-16-2" or cholecalciferol? or hydroxycholecalciferol? or "hydroxy cholecalciferol?" or calcifediol? or dihydroxycholecalciferol? or "dihydro cholecalciferol?" or calcitriol? or "24,25-dihydroxyvitamin D" or ergocalciferol? or dihydrotachysterol? or "25-hydroxyvitamin D" or lunacalcipol or doxercalciferol or paricalcitol).tw,kw.	100428
3	1 or 2	155131
4	exp Respiratory Tract Infection/	387907
5	"respiratory tract infection?".tw,kw.	32533
6	Birth weight/ or Pregnancy outcome/ or Prematurity/ or Growth/ or Body growth/	238102
7	("growth" or (("premature" or "pre term" or "preterm") adj "birth?") or "SGA" or (("birth" or "gestational" or "neonatal" or "neo natal" or "newborn" or "new born" or "f?etal" or "f?etus" or "baby" or "babies") adj2 ("weight" or "size?")) or (("pregnancy" or "birth" or "obstetric") adj "outcome?")).tw,kw.	1812796
8	or/4-7	2302334
9	3 and 8	17862

#	Searches	
10	limit 9 to embase	13360
11	(animal/ or exp nonhuman/ or Animal experiment/) not ((animal/ or exp nonhuman/ or Animal experiment/) and exp human/)	5985714
12	10 not 11	11377
13	limit 12 to (danish or english or french or german or norwegian or swedish)	11041
14	limit 13 to "reviews (maximizes specificity)"	193
15	Meta-Analysis/ or "systematic review"/ or ((systematic* adj2 review*) or metaanal* or "meta anal*" or (review and ((structured or database* or systematic*) adj2 search*)) or "integrative review*" or (evidence adj2 review*)).tw,kw.	478715
16	14 or (15 and 13)	418
17	limit 16 to yr="2011 -Current"	317

Database: Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Daily and Versions(R) <1946 to April 24, 2020>

Date: 28.04.20

Number of hits: 199

#	Searches	
1	exp Vitamin D/	58417
2	("vitamin d" or "1406-16-2" or cholecalciferol? or hydroxycholecalciferol? or "hydroxy cholecalciferol?" or calcifediol? or dihydroxycholecalciferol? or "dihydro cholecalciferol?" or calcitriol? or "24,25-dihydroxyvitamin D" or ergocalciferol? or dihydrotachysterol? or "25-hydroxyvitamin D" or lunacalcipol or doxercalciferol or paricalcitol).tw,kf.	69341
3	1 or 2	85744
4	exp Respiratory Tract Infections/	354559
5	"respiratory tract infection?".tw,kf.	22859
6	Birth weight/ or Pregnancy outcome/ or Premature birth/ or Growth/	119478
7	("growth" or (("premature" or "pre term" or "preterm") adj "birth?") or "SGA" or (("birth" or "gestational" or "neonatal" or "neo natal" or "newborn" or "new born" or "f?etal" or "f?etus" or "baby" or "babies") adj2 ("weight" or "size?")) or (("pregnancy" or "birth" or "obstetric") adj "outcome?")).tw,kf.	1518234
8	or/4-7	1929284
9	3 and 8	9592
10	Animal/ not (animal/ and human/)	4659965
11	9 not 10	7818
12	limit 11 to (danish or english or french or german or multilingual or norwegian or swedish)	7507
13	limit 12 to "reviews (maximizes specificity)"	171
14	Meta-Analysis/ or ((systematic* adj2 review*) or metaanal* or "meta anal*" or (review and ((structured or database* or systematic*) adj2 search*)) or "integrative review*" or (evidence adj2 review*)).tw,kf,bt.	329978
15	13 or (12 and 14)	243
16	limit 15 to yr="2011 -Current"	199

Database: Cochrane Database of Systematic Reviews Issue 4 of 12, April 2020

Date: 28.04.20

Number of hits: 16

#	Searches	
#1	[mh "Vitamin D"]	5190
#2	("vitamin d" or "1406-16-2" or cholecalciferol? or hydroxycholecalciferol? or (hydroxyl NEXT cholecalciferol?) or calcifediol? or dihydroxycholecalciferol? or (dihydro NEXT cholecalciferol?) or calcitriol? or "24,25-dihydroxyvitamin D" or ergocalciferol? or dihydrotachysterol? or "25-hydroxyvitamin D" or lunacalcipol or doxercalciferol or paricalcitol):ti,ab	11251
#3	#1 or #2	12221
#4	[mh "Respiratory Tract Infections"]	14230
#5	("respiratory tract infection?"):ti,ab	2212
#6	[mh ^"Birth weight"]	1626
#7	[mh ^"Pregnancy outcome"]	2932
#8	[mh ^"Premature birth"]	1406
#9	[mh "Growth"]	19535
#10	("growth" or (("premature" or "pre term" or "preterm") NEXT "birth?") or "SGA" or (("birth" or "gestational" or "neonatal" or "neo natal" or "newborn" or "new born" or "f?etal" or "f?etus" or "baby" or "babies") NEAR/2 ("weight" or "size?")) or (("pregnancy" or "birth" or "obstetric") NEXT "outcome?")):ti,ab	46033
#11	{or #4-#10}	80178
#12	#3 and #11	1207
#13	#3 and #11 with Cochrane Library publication date Between Jan 2011 and Apr 2020, in Cochrane Reviews	16

Database: Epistemonikos

Date: 28.04.20

Number of hits: 24

("vitamin d" or "1406-16-2" or cholecalciferol* or hydroxycholecalciferol* or "hydroxy cholecalciferol*" or calcifediol* or dihydroxycholecalciferol* or "dihydro cholecalciferol*" or calcitriol* or "24,25-dihydroxyvitamin D" or ergocalciferol* or dihydrotachysterol* or "25-hydroxyvitamin D" or lunacalcipol or doxercalciferol or paricalcitol) AND ("respiratory tract infection*" or "birth weight" or "birth size*" or "gestational weight" or "gestational size*" or "neonatal size*" or "neonatal weight" or "neo natal size*" or "neo natal weight" or "new born weight" or "newborn weight" or "new born size*" or "newborn size*" or "foetal weight" or "foetal size*" or "faetal weight" or "faetal size*" or "fetal weight" or "fetal size*" or "foetus size*" or "foetus weight" or "faetus size*" or "faetus weight" or "fetus size*" or "fetus weight" or "baby size*" or "baby weight" or "pregnancy outcome*" or "birth outcome*" or "obstetric outcome*")

Publication year 2011-2020 and Publication type Systematic Review

15.5 Methylmercury – systematic reviews, meta-analyses and Norwegian publications

15.5.1 Original search

Contact person: Kirsten Eline Rakkestad

Search: Ragnhild Agathe Ternes

Referee: Bente Foss

Comment: Search for systematic reviews and norwegian publications.

Duplicate check in EndNote: Before Duplicate check: 168 systematic reviews, 257 norwegian publications
After Duplicate check: 88 systematic reviews, 140 norwegian publications

Database: Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Daily and Versions(R) <1946 to January 08, 2021>

Date: 11.01.21

Number of hits: 38 systematic reviews, 96 norwegian publications

#	Searches	
1	Methylmercury Compounds/	6124
2	("methyl mercury" or methylmercury or MeHg).tw,kf.	7415
3	1 or 2	8343
4	limit 3 to yr="2012 -Current"	3065
5	limit 4 to "reviews (maximizes specificity)"	26
6	Meta-Analysis/ or ((systematic* adj2 review*) or metaanal* or "meta anal*" or (review and ((structured or database* or systematic*) adj2 search*)) or "integrative review*" or (evidence adj2 review*)).tw,kf,bt.	367506
7	5 or (4 and 6)	38
8	exp Norway/	39609
9	(norway or norwegian? or norge).tw,cp,in,lg,kf.	204821

#	Searches	
10	(sykehus* or sjukehus* or ((universitet* or University or univ) and (haukeland or nordnorge or norge* or bergen or stavanger or tromsø or tromsø or trondheim or levanger or gjovik or gjoevik or harstad or lillehammer or narvik or nesna or stord or haugesund or voldal or aalesund or alesund or nord)) or sentralsjukehus* or sentralsykehus* or Finnmarkssykehuset or Helgelandssykehuset or Nordlandssykehuset or innlandet or "Olav? Hospital?" or revmatismesykehus or lungesykehus or "Hospitalet Betanien" or Kysthospitalet or Aleris or Feiringklinikken or Glittreklinikken or "Hjertesenteret i Oslo" or "Medi 3" or "Volvat Medisinske Senter" or "Helse Vest" or "Helse Stavanger" or "Helse fonna" or "helse bergen" or "helse forde" or "helse foerde" or sjukehusapotek* or sykehusapotek* or "helse midt norge" or "helse midtnorge" or "Ambulanse Midtnorge" or "Ambulanse Midt norge" or "helse nord" or "Helse Sorost" or "Helse Sor ost" or "Helse Soeroest" or "Helse Soer oest" or sunnaas or sunnas or sorlandet or soerlandet).cp,in,tw,kf.	71408
11	(Akershus or Viken or Austagder or Agder or Buskerud or Finnmark or Hedmark or Hordaland or Romsdal or Nordland or Nordtrondelag or Trondelag or Nordtroendelag or Troendelag or Oppland or Oslo or Rogaland or Fjordane or Sortrondelag or Soertroendelag or Telemark or Troms or Vestagder or Vestfold or Ostfold or Oestfold or Longyearbyen or innlandet or vestland).cp,in,tw,kf.	81241
12	tidsskrift for den norske laegeforening.jn.	33304
13	or/8-12	220420
14	4 and 13	96

Database: **Embase <1974 to 2020 January 08>**

Date: 11.01.20

Number of hits: 42 systematic reviews, 98 norwegian publications

#	Searches	
1	Methylmercury/	7027
2	("methyl mercury" or methylmercury or MeHg).tw,kw.	8940
3	1 or 2	10167
4	limit 3 to yr="2012 -Current"	3596
5	limit 4 to (conference abstracts or embase)	3017
6	limit 5 to "reviews (maximizes specificity)"	28
7	Meta-Analysis/ or "systematic review"/ or ((systematic* adj2 review*) or metaanal* or "meta anal*" or (review and ((structured or database* or systematic*) adj2 search*)) or "integrative review*" or (evidence adj2 review*)).tw,kw.	533792
8	6 or (5 and 7)	42
9	Norway/ or "Svalbard and Jan Mayen"/	43542
10	(norway or norwegian? or norge).cp,in,ad,tw,lg,kw.	302926

#	Searches	
11	(sykehus* or sjukehus* or ((universitet* or University or univ) and (haukeland or nordnorge or norge* or bergen or stavanger or tromso or tromsoe or trondheim or levanger or gjovik or gjoevik or harstad or lillehammer or narvik or nesna or stord or haugesund or voldal or aalesund or alesund or nord)) or sentralsjukehus* or sentralsykehus* or Finnmarkssykehuset or Helgelandssykehuset or Nordlandssykehuset or innlandet or "Olav? Hospital?" or revmatismesykehus or lungesykehus or "Hospitalet Betanien" or Kysthospitalet or Aleris or Feiringklinikken or Glittreklinikken or "Hjertesenteret i Oslo" or "Medi 3" or "Volvat Medisinske Senter" or "Helse Vest" or "Helse Stavanger" or "Helse fonna" or "helse bergen" or "helse forde" or "helse foerde" or sjukehusapotek* or sykehusapotek* or "helse midt norge" or "helse midtnorge" or "Ambulanse Midtnorge" or "Ambulanse Midt norge" or "helse nord" or "Helse Sorost" or "Helse Sor ost" or "Helse Soeroest" or "Helse Soer oest" or sunnaas or sunnas or sorlandet or soerlandet).cp,in,ad,ti,ab,kw.	115141
12	(Akershus or Viken or Austagder or Agder or Buskerud or Finnmark or Hedmark or Hordaland or Romsdal or Nordland or Nordtrondelag or Trondelag or Nordtroendelag or Troendelag or Oppland or Oslo or Rogaland or Fjordane or Sortrondelag or Soertroendelag or Telemark or Troms or Vestagder or Vestfold or Ostfold or Oestfold or Longyearbyen or innlandet or vestland).cp,in,ad,ti,ab,kw.	130022
13	(oslonorway or bergennorway or sandnesnorway or stavangernorway or trondheimnorway or tromsonorway or tromsoenorway or Akershusnorway or Vikennorway or Austagdernorway or Agdernorway or Buskerudnorway or Finnmarknorway or Hedmarknorway or Hordalandnorway or Romsdalnorway or Nordlandnorway or Nordtrondelagnorway or Nordtroendelagnorway or Trondelagnorway or Troendelagnorway or Opplandnorway or Rogalandnorway or Fjordanenorway or Sortrondelagnorway or Sortroendelagnorway or Telemarknorway or Tromsnorway or Vestagdernorway or Vestfoldnorway or Ostfoldnorway or Oestfoldnorway or innlandetnorway or vestlandnorway).cp,in,ad,ti,ab,kw.	577
14	(tidsskrift for den norske laegeforening or tidsskrift for den norske laegeforening tidsskrift for praktisk or tidsskrift for den norske laegeforening tidsskrift for praktisk medicin ny raeke).jn.	28598
15	or/9-14	330876
16	5 and 15	98

Database: **APA PsycInfo <1806 to December Week 4 2020>**

Date: 05.01.20

Number of hits: 4 systematic reviews, 1 norweian publication

#	Searches	
1	("methyl mercury" or methylmercury or MeHg).tw.	310
2	limit 1 to yr="2012 -Current"	96
3	limit 2 to "reviews (maximizes specificity)"	2
4	(meta analysis or "systematic review").md. or meta analysis/ or ((systematic* adj2 review*) or metaanal* or "meta anal*" or (review and ((structured or database* or systematic*) adj2 search*)) or "integrative review*" or (evidence adj2 review*)).tw.	84652
5	3 or (2 and 4)	4
6	(norway or norwegian? or norge).in,cq,lo,tw,lg,ca.	39963

#	Searches	
7	(sykehus* or sjukehus* or ((universitet* or University or univ) and (haukeland or nordnorge or norge* or bergen or stavanger or tromsø or tromsø or trondheim or levanger or gjovik or gjoevik or harstad or lillehammer or narvik or nesna or stord or haugesund or voldal or aalesund or alesund or nord)) or sentralsjukehus* or sentralsykehus* or Finnmarkssykehuset or Helgelandssykehuset or Nordlandssykehuset or innlandet or "Olav? Hospital?" or revmatismesykehuset or lungesykehuset or "Hospitalet Betanien" or Kysthospitalet or Aleris or Feiringklinikken or Glittreklinikken or "Hjertesenteret i Oslo" or "Medi 3" or "Volvat Medisinske Senter" or "Helse Vest" or "Helse Stavanger" or "Helse fonna" or "helse bergen" or "helse forde" or "helse foerde" or sjukehusapotek* or sykehusapotek* or "helse midt norge" or "helse midtnorge" or "Ambulanse Midtnorge" or "Ambulanse Midt norge" or "helse nord" or "Helse Sorost" or "Helse Sor ost" or "Helse Soeroest" or "Helse Soer oest" or sunnaas or sunnas or sorlandet or soerlandet).cq,in,tw,ca.	14891
8	(Akershus or Viken or Austagder or Agder or Buskerud or Finnmark or Hedmark or Hordaland or Romsdal or Nordland or Nordtrondelag or Trondelag or Nordtroendelag or Troendelag or Oppland or Oslo or Rogaland or Fjordane or Sortrondelag or Soertroendelag or Telemark or Troms or Vestagder or Vestfold or Ostfold or Oestfold or Longyearbyen or innlandet or vestland).cq,in,tw,ca.	19266
9	(tidsskrift for den norske laegeforening or tidsskrift for norsk psykologforening).jn.	1451
10	or/6-9	42179
11	2 and 10	1

Database: Web of Science

Date: 06.01.20

Number of hits: 62 systematic reviews, 58 norwegian publications

#		Searches
# 6	58	#2 and #5 Timespan=2012-2021
# 5	43,808	TS=("norway" or "norwegian\$" or "norge") Timespan=2012-2021
# 4	62	#2 and #3 Timespan=2012-2021
# 3	302,303	TS=((("systematic*" NEAR/1 "review*") or ("review" and (("structured" or "database*" or "systematic*") NEAR/1 "search*"))) or "integrative review*" or ("evidence" NEAR/1 "review*")) OR TI=("metaanal*" or "meta anal*") OR AB=("metaanal*" or "meta anal*") Timespan=2012-2021
# 2	5,688	#1 Timespan=2012-2021
# 1	12,645	TS=("methyl mercury" or "methylmercury" or "MeHg")

Database: Epistemonikos

Date: 08.01.21

Comment: Two separate searches

Number of hits: 22 systematic reviews, 1 norwegian publication

("methyl mercury" or "methylmercury" or "MeHg")

Publication year: 2012-2021

((("methyl mercury" or "methylmercury" or "MeHg") AND ("norway" or "norwegian*" or "norge"))

Publication year: 2012-2021

Database: Cochrane Central Register of Controlled Trials

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Cochrane Database of Systematic Reviews

Issue 1 of 12, January 2021

Date: 08.01.21

Number of hits: 0 systematic reviews, 3 norwegian publications

#	Searches	
#1	[mh "Methylmercury Compounds"]	0
#2	("methyl mercury" or methylmercury or MeHg):ti,ab	20
#3	#1 or #2	20
#4	#3 with Cochrane Library publication date Between Jan 2012 and Jan 2021, in Cochrane Reviews	0
#5	#3	20
#6	[mh "Norway"]	1044
#7	("norway" or "norwegian?" or "norge")	9408
#8	#6 or #7	9408
#9	#3 and #8	3
#10	#9 with Publication Year from 2012 to 2021, with Cochrane Library publication date Between Jan 2012 and Jan 2021, in Trials	3

15.5.2 Updated search

Contact person: Kirsten Eline Rakkestad

Search: Trude Anine Muggerud

Comment: Update of search from January 2021. Search for systematic reviews and norwegian publications separately

Duplicate check in EndNote:

Before Duplicate check: 33 systematic reviews, 30 norwegian publications

After Duplicate check: 18 systematic reviews, 18 norwegian publications

Database: Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Daily and Versions(R) <1946 to October 01, 2021>

Date: 04.10.21

Number of hits: 8 systematic reviews, 12 norwegian publications

- 1 Methylmercury Compounds/ 6297
- 2 ("methyl mercury" or methylmercury or MeHg).tw,kf. 7630
- 3 1 or 2 8565
- 4 2021*.ed,ep,yr,dp,dt.1955922
- 5 3 and 4 395
- 6 limit 5 to "reviews (maximizes specificity)" 5
- 7 Meta-Analysis/ or Network Meta-Analysis/ or ((systematic* adj2 review*) or metaanal* or "meta anal*" or (review and ((structured or database* or systematic*) adj2 search*)) or "integrative review*" or (evidence adj2 review*)).tw,kf,bt. 407444
- 8 6 or (5 and 7) 8
- 9 exp Norway/ 41139
- 10 (norway or norwegian? or norge).tw,cp,in,lg,kf. 214496
- 11 (sykehus* or sjukehus* or ((universitet* or University or univ) and (haukeland or nordnorge or norge* or bergen or stavanger or tromso or tromsoe or trondheim or levanger or gjovik or gjoevik or harstad or lillehammer or narvik or nesna or stord or haugesund or volda or aalesund or alesund or nord)) or sentralsjukehus* or sentralsykehus* or Finnmarkssykehuset or Helgelandssykehuset or Nordlandssykehuset or innlandet or "Olav?

Hospital?" or revmatismesykehus or lungesykehus or "Hospitalet Betanien" or Kysthospitalet or Aleris or Feiringklinikken or Glittreklinikken or "Hjertesenteret i Oslo" or "Medi 3" or "Volvat Medisinske Senter" or "Helse Vest" or "Helse Stavanger" or "Helse fonna" or "helse bergen" or "helse forde" or "helse foerde" or sjukehusapotek* or sykehusapotek* or "helse midt norge" or "helse midtnorge" or "Ambulanse Midtnorge" or "Ambulanse Midt norge" or "helse nord" or "Helse Sorost" or "Helse Sor ost" or "Helse Soeroest" or "Helse Soer oest" or sunnaas or sunnas or sorlandet or soerlandet).cp,in,tw,kf. 77402

12 (Akershus or Viken or Austagder or Agder or Buskerud or Finnmark or Hedmark or Hordaland or Romsdal or Nordland or Nordtrondelag or Trondelag or Nordtroendelag or Troendelag or Oppland or Oslo or Rogaland or Fjordane or Sortrondelag or Soertroendelag or Telemark or Troms or Vestagder or Vestfold or Ostfold or Oestfold or Longyearbyen or innlandet or vestland).cp,in,tw,kf. 86228

13 tidsskrift for den norske laegeforening.jn. 33536

14 9 or 10 or 11 or 12 or 13 231746

15 5 and 14 12

Database: Embase 1974 to 2021 October 01

Date: 04.10.21

Number of hits: 10 systematic reviews, 13 norwegian publications

1 Methylmercury/ 7173

2 ("methyl mercury" or methylmercury or MeHg).tw,kw. 9090

3 1 or 2 10340

4 2021*.yr,dd,dp,dc. 1965697

5 3 and 4 406

6 limit 5 to (conference abstracts or embase) 338

7 limit 6 to "reviews (maximizes specificity)" 8

- 8 Meta-Analysis/ or "systematic review"/ or ((systematic* adj2 review*) or metaanal* or "meta anal*" or (review and ((structured or database* or systematic*) adj2 search*)) or "integrative review*" or (evidence adj2 review*)).tw,kw. 587632
- 9 7 or (6 and 8) 10
- 10 Norway/ or "Svalbard and Jan Mayen"/ 44915
- 11 (norway or norwegian? or norge).cp,in,ad,tw,lg,kw. 311322
- 12 (sykehus* or sjukehus* or ((universitet* or University or univ) and (haukeland or nordnorge or norge* or bergen or stavanger or tromso or tromsoe or trondheim or levanger or gjovik or gjoevik or harstad or lillehammer or narvik or nesna or stord or haugesund or voldal or aalesund or alesund or nord)) or sentralsjukehus* or sentralsykehus* or Finnmarkssykehuset or Helgelandssykehuset or Nordlandssykehuset or innlandet or "Olav? Hospital?" or revmatismesykehus or lungesykehus or "Hospitalet Betanien" or Kysthospitalet or Aleris or Feiringklinikken or Glittreklinikken or "Hjertesenteret i Oslo" or "Medi 3" or "Volvat Medisinske Senter" or "Helse Vest" or "Helse Stavanger" or "Helse fonna" or "helse bergen" or "helse forde" or "helse foerde" or sjukehusapotek* or sykehusapotek* or "helse midt norge" or "helse midtnorge" or "Ambulanse Midtnorge" or "Ambulanse Midt norge" or "helse nord" or "Helse Sorost" or "Helse Sor ost" or "Helse Soeroest" or "Helse Soer oest" or sunnaas or sunnas or sorlandet or soerlandet).cp,in,ad,ti,ab,kw. 120958
- 13 (Akershus or Viken or Austagder or Agder or Buskerud or Finnmark or Hedmark or Hordaland or Romsdal or Nordland or Nordtrondelag or Trondelag or Nordtroendelag or Troendelag or Oppland or Oslo or Rogaland or Fjordane or Sortrondelag or Soertroendelag or Telemark or Troms or Vestagder or Vestfold or Ostfold or Oestfold or Longyearbyen or innlandet or vestland).cp,in,ad,ti,ab,kw. 135083
- 14 (oslonorway or bergennorway or sandnesnorway or stavangernorway or trondheimnorway or tromsonorway or tromsoenorway or Akershusnorway or Vikennorway or Austagdernorway or Agdernorway or Buskerudnorway or Finnmarknorway or Hedmarknorway or Hordalandnorway or Romsdalnorway or Nordlandnorway or Nordtrondelagnorway or Nordtroendelagnorway or Trondelagnorway or Troendelagnorway or Opplandnorway or Rogalandnorway or Fjordanenorway or Sortrondelagnorway or Sortroendelagnorway or Telemarknorway or Tromsnorway or Vestagdernorway or Vestfoldnorway or Ostfoldnorway or Oestfoldnorway or innlandetnorway or vestlandnorway).cp,in,ad,ti,ab,kw. 603
- 15 (tidsskrift for den norske laegeforening or tidsskrift for den norske laegeforening tidsskrift for praktisk or tidsskrift for den norske laegeforening tidsskrift for praktisk medicin ny raekke).jn. 28757
- 16 10 or 11 or 12 or 13 or 14 or 15 341026
- 17 6 and 16 13

Database: APA PsycInfo <1806 to October Week 1 2021>

Date: 04.10.21

Number of hits: 0

1 ("methyl mercury" or methylmercury or MeHg).tw. 313

2 2021*.yr,dp,up. 356724

3 1 and 2 5

4 limit 3 to "reviews (maximizes specificity)" 0

5 (meta analysis or "systematic review").md. or meta analysis/ or ((systematic* adj2 review*) or metaanal* or "meta anal*" or (review and ((structured or database* or systematic*) adj2 search*)) or "integrative review*" or (evidence adj2 review*)).tw. 92219

6 4 or (3 and 5) 0

7 (norway or norwegian? or norge).in,cq,lo,tw,lg,ca. 41940

8 (sykehus* or sjukehus* or ((universitet* or University or univ) and (haukeland or nordnorge or norge* or bergen or stavanger or tromso or tromsoe or trondheim or levanger or gjovik or gjoevik or harstad or lillehammer or narvik or nesna or stord or haugesund or voldal or aalesund or alesund or nord)) or sentralsjukehus* or sentralsykehus* or Finnmarkssykehuset or Helgelandssykehuset or Nordlandssykehuset or innlandet or "Olav? Hospital?" or revmatismesykehus or lungesykehus or "Hospitalet Betanien" or Kysthospitalet or Aleris or Feiringklinikken or Glittreklinikken or "Hjertesenteret i Oslo" or "Medi 3" or "Volvat Medisinske Senter" or "Helse Vest" or "Helse Stavanger" or "Helse fonna" or "helse bergen" or "helse forde" or "helse foerde" or sjukehusapotek* or sykehusapotek* or "helse midt norge" or "helse midtnorge" or "Ambulanse Midtnorge" or "Ambulanse Midt norge" or "helse nord" or "Helse Sorost" or "Helse Sor ost" or "Helse Soeroest" or "Helse Soer oest" or sunnaas or sunnas or sorlandet or soerlandet).cq,in,tw,ca.15781

9 (Akershus or Viken or Austagder or Agder or Buskerud or Finnmark or Hedmark or Hordaland or Romsdal or Nordland or Nordtrondelag or Trondelag or Nordtroendelag or Troendelag or Oppland or Oslo or Rogaland or Fjordane or Sortrondelag or Soertroendelag

or Telemark or Troms or Vestagder or Vestfold or Ostfold or Oestfold or Longyearbyen or innlandet or vestland).cq,in,tw,ca. 20216

10 (tidsskrift for den norske laegeforening or tidsskrift for norsk psykologforening).jn. 1451

11 7 or 8 or 9 or 10 44280

12 3 and 11 0

Database: Web of Science

Date: 04.10.21

Number of hits: 11 systematic reviews, 5 norwegian publications

#6 #2 and #5 11

#5 TS=("norway" or "norwegian\$" or "norge") and 2021 or 2020 (Publication Years) 10,434

#4 #2 and #3 20

#3 TS=(("systematic*" NEAR/1 "review*") or ("review" and (("structured" or "database*" or "systematic*") NEAR/1 "search*")) or "integrative review*" or ("evidence" NEAR/1 "review*")) OR TI=("metaanal*" or "meta anal*") OR AB=("metaanal*" or "meta anal*") and 2021 or 2020 (Publication Years) 105,206

#2

TS=("methyl mercury" or "methylmercury" or "MeHg") and 2021 or 2020 (Publication Years) 1,191

#1 TS=("methyl mercury" or "methylmercury" or "MeHg") 13,158

Database: Epistemonikos

Date: 04.10.21

Comment: Two separate searches

Number of hits: 3 hits

("methyl mercury" or "methylmercury" or "MeHg")

Publication year: 2021-2021 = 3 hits

((("methyl mercury" or "methylmercury" or "MeHg") AND ("norway" or "norwegian*" or "norge"))

Publication year: 2021-2021 = 0 hits

Database: Cochrane Central Register of Controlled Trials

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Cochrane Database of Systematic Reviews

Issue 10 of 12, October 2021

Dato: 04.10.21

Number of hits: 1 original study, 0 norwegian publications

#1 [mh " Methylmercury Compounds"] 0

#2 ("methyl mercury" or methylmercury or MeHg):ti,ab 21

#3 #1 or #2 21

#4 #3 with Cochrane Library publication date Between Jan 2021 and Oct 202 1

#5 [mh "Norway"] 1125

#6 ("norway" or "norwegian?" or "norge") 9840

#7 #5 or #6 9840

#8 #3 and #7 3

#9 #3 and #7 with Cochrane Library publication date Between Jan 2021 and Oct 2021

16 Appendix III: Quality assessment tools

16.1 Quality assessment of primary studies (NNR)

16.1.1 Quality assessment tool for clinical trials

Authors: _____

Year: _____

Main outcome: _____

1. General questions and study design					Requires yes for level		
					A	B	C
a) Research question/hypothesis clearly formulated?	Yes	No	Can't tell	NA	X	X	
b) Was the study design suited to test the research hypothesis?	Yes	No	Can't tell	NA	X	X	
c) Was the duration of the study suited to test the research hypothesis?	Yes	No	Can't tell	NA	X		

2. Participation and compliance						
a) Population (target group) well described?	Yes	No	Can't tell	NA	X	X
b) Sample (possible participants) recruited in an acceptable way?	Yes	No	Can't tell	NA	X	X
c) Criteria for inclusion/exclusion clearly formulated and acceptable?	Yes	No	Can't tell	NA	X	
d) Actual participants comparable with the relevant (target) population?	Yes	No	Can't tell	NA	X	
e) Method of randomization allocation stated and appropriate?	Yes	No	Can't tell	NA	X	
f) Was there an account for the comparability of intervention and control groups with regard to relevant/possible factors that might affect outcome?	Yes	No	Can't tell	NA	X	
g) Compliance reported in an acceptable way, and compliance acceptable?	Yes	No	Can't tell	NA	X	
h) Drop-out rate within an acceptable range? 6mo<30%, 12mo<40%, 24mo<50%	Yes	No	Can't tell	NA	X	
i) The drop-outs did not differ between the groups?	Yes	No	Can't tell	NA	X	

3. Dietary interventions and assessment		
a) Intervention diets clearly defined and characterised (fish intake)?	Yes No Can't tell NA	X X
b) Method used for dietary assessment valid/adequately validated?	Yes No Can't tell NA	X
c) Intervention diets consist of normal foods/relevance to research question?	Yes No Can't tell NA	X
d) Measurement errors in dietary reporting considered?	Yes No Can't tell NA	X
e) Energy adjustment adequately done?	Yes No Can't tell NA	X

4. Anthropometry						
a) Assessment details clearly reported and assessment adequately performed?	Yes	No	Can't tell	NA	X	
5. Outcome, results, and analyses						
a) Acceptable and clear definition of the outcome/endpoint?	Yes	No	Can't tell	NA	X	X
b) Results analysed blind?	Yes	No	Can't tell	NA	X	
c) Attempts in the analysis phase made to adjust for imbalances between treatment arms with regard to important determinants for the outcome (e.g. through multivariate modelling)?	Yes	No	Can't tell	NA	X	
d) Possible use of medication/supplements taken into account?	Yes	No	Can't tell	NA	X	

6. Statistical power		
a) Sample size and power calculation reported/considered (relevant for main outcome variable)?	Yes No Can't tell NA	X
7. Summary of the study quality	A B C	
8. Upgrading elements		
a) Energy intake at a credible level?	Yes No Can't tell NA	

b) No possible conflicts of interest affecting the study quality?	Yes	No	Can't tell	NA	
c) Was the study registered at ClinicalTrials.gov before the start of the study?	Yes	No	Can't tell	NA	
Did other seafood exceeded 10% of the fish intake variable?	Yes	No	Can't tell	NA	
Reasons for grade C, and other comments:					

16.1.1.1 Modifications from the NNR5 questionnaire

The following questions were removed from the questionnaire used in NNR5

- Sample size and power calculation reported/considered (relevant for the main outcome variable)?
- Food composition database reported?
- Biological mechanism for endpoint plausible?
- Valid biomarkers used to study compliance with the dietary exposure?
- Between measurement variation minimised/standardised?
- Smallest effect clinically relevant/reasonable?

The following questions were included in the original questionnaire, but defined as “upgrading elements” in VKM’s version. NNR5 did not have upgrading elements.

- Energy intake at a credible level?
- No possible conflicts of interests affecting the study quality?

The following questions were added to VKM’s version

- Assessment details clearly reported and assessment adequately performed? (Anthropometry)
- Sample size and power calculation reported/considered (relevant for main outcome variable)?
- Was the study registered at ClinicalTrials.gov before the start of the study? (Upgrading element)

16.1.2 Quality assessment tool for nested case-control studies

Authors: _____

Year: _____

Main outcome: _____

1. General questions and study design					Requires yes for level		
					A	B	C
a) Research question clearly formulated?	Yes	No	Can't tell	NA	X	X	
b) Endpoint/outcome clearly formulated?	Yes	No	Can't tell	NA	X	X	
c) Was the study design suited to test the research hypothesis?	Yes	No	Can't tell	NA	X	X	
2. Sampling (Ascertainment of cases and controls)							
a) Source population well defined and recruitment done in an acceptable way?	Yes	No	Can't tell	NA	X	X	

b) Fish intake according to inclusion criteria (individual intake and at least frequency)?	Yes	No	Can't tell	NA	X	X
c) Time period for baseline examinations reported and adequate?	Yes	No	Can't tell	NA	X	X
d) Criteria for inclusion/exclusion clearly formulated and acceptable?	Yes	No	Can't tell	NA	X	X
e) Repeat exposure assessment during follow up adequately done?	Yes	No	Can't tell	NA	X	
f) Endpoint clearly ascertained and validly assessed?	Yes	No	Can't tell	NA	X	X
g) Follow-up period clearly identified?	Yes	No	Can't tell	NA	X	
h) Matching criteria clearly formulated?	Yes	No	Can't tell	NA	X	
i) Criteria for inclusion/exclusion of controls clearly formulated and acceptable?	Yes	No	Can't tell	NA	X	
j) Characteristics of cases versus controls examined and reported?	Yes	No	Can't tell	NA	X	

3. Dietary exposure		
a) Type of exposure (fish intake) reported in sufficient detail?	Yes No Can't tell NA	X X
b) Was the dietary assessment method validated?	Yes No Can't tell NA	X
c) Measurement errors in dietary reporting considered?	Yes No Can't tell NA	X
d) Energy adjustment adequately done?	Yes No Can't tell NA	X
e) Repeat assessment of diet during follow up?	Yes No Can't tell NA	X
4. Anthropometry		

a) Assessment details clearly reported and assessment adequately performed?	Yes	No	Can't tell	NA	X	
5. Confounding						
a) Were important confounders identified/ascertained and considered by authors?	Yes	No	Can't tell	NA	X	X
6. Statistical power						
a) Was the study power considered and power calculations and sample size reported?	Yes	No	Can't tell	NA	X	

b) In view of multiple tests, were by chance findings considered?	Yes	No	Can't tell	NA	X	
7. Statistical analysis						
a) Appropriately handled?	Yes	No	Can't tell	NA	X	
b) Relevant confounders adequately handled; e.g, Restriction, Stratified analyses, Multivariate modelling, Interaction tested?	Yes	No	Can't tell	NA	X	X
c) Ascertainment/detection bias considered (eg. cases detected due to screening)?	Yes	No	Can't tell	NA	X	
d) Cases/corresponding controls detected early during the follow-up period removed?	Yes	No	Can't tell	NA	X	

8. Summary of the study quality	A	B	C	
9. Upgrading elements				
a) Particulars of dietary assessment tool reported in sufficient detail?	Yes	No	Can't tell	NA
b) Energy intake at a credible level?	Yes	No	Can't tell	NA
c) No possible conflicts of interest affecting the study quality?	Yes	No	Can't tell	NA
Did other seafood exceeded 10% of the fish intake variable?	Yes	No	Can't tell	NA
Reasons for grade C, and other comments:				

16.1.2.1 Modifications from the NNR5 questionnaire

The following questions were removed from the questionnaire used in NNR5

- Concurrent validity (validation coeff.) of specific exposures reported?
- Associations/correlations between dietary variables reported?
- Use of dietary biomarkers adequate? Details of assessment and handling reported?
- Coefficient of variation of assay?
- Time period between biomarker assessment and diagnosis acceptable?
- Assessment details clearly reported, and assessment adequately performed? (Physical activity)
- Assessment details clearly reported, and assessment adequately performed?
- The distribution of confounders similar in cases and controls?

The following questions were included in the original questionnaire but defined as “upgrading elements” in VKM’s version. NNR5 did not have upgrading elements.

- Particulars of dietary assessment reported in sufficient detail?
- Energy intake at a credible level?

The following questions were added to VKM's version

- Fish intake according to inclusion criteria (individual intake and at least frequency)?
- Was the dietary assessment method validated?

16.1.3 Quality assessment tool for retrospective case-control studies

Authors: _____ Year: _____ Main outcome: _____

1. General questions and study design					Requires yes for level		
					A	B	C
a) Research question clearly formulated?	Yes	No	Can't tell	NA	X	X	
b) Endpoint/outcome clearly formulated?	Yes	No	Can't tell	NA	X	X	
c) Was the study design suited to test the research hypothesis?	Yes	No	Can't tell	NA	X	X	

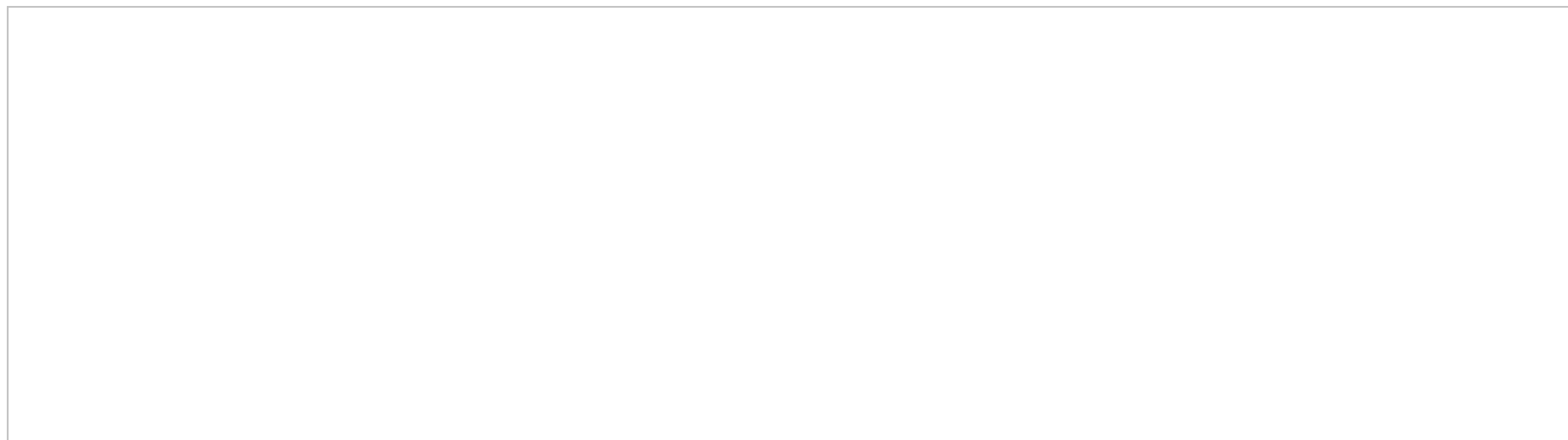
2. Sampling (Ascertainment of cases and controls)						
a) Source population/study-base well defined?	Yes	No	Can't tell	NA	X	X
b) Period of recruitment/ascertainment well defined?	Yes	No	Can't tell	NA	X	X
c) Case status clearly ascertained, and endpoint validly assessed?	Yes	No	Can't tell	NA	X	X
d) Control status clearly defined?	Yes	No	Can't tell	NA	X	
e) Criteria for inclusion/exclusion clearly formulated and acceptable?	Yes	No	Can't tell	NA	X	
f) Matching criteria clearly formulated?	Yes	No	Can't tell	NA	X	
g) Number of non-participating controls and reasons for non-participation reported?	Yes	No	Can't tell	NA	X	

3. Dietary exposure		
a) Fish intake according to inclusion criteria (individual intake and at least frequency)?	Yes No Can't tell NA	X X
b) In retrospective assessment, is the reference (time) period clearly reported?	Yes No Can't tell NA	X
c) Was the dietary assessment method validated?	Yes No Can't tell NA	X
d) Measurement errors in dietary reporting considered?	Yes No Can't tell NA	X
e) Energy adjustment adequately done?	Yes No Can't tell NA	X
4. Anthropometry		

a) Assessment details clearly reported and assessment adequately performed?	Yes	No	Can't tell	NA	X	
5. Confounding						
a) Were important confounders identified/ascertained and considered by authors?	Yes	No	Can't tell	NA	X	X
b) Recall bias considered?	Yes	No	Can't tell	NA	X	X
6. Statistical power						

a) Was the study power considered and sample size and power calculations reported?	Yes	No	Can't tell	NA	X	
b) In view of multiple tests, were by chance findings considered?	Yes	No	Can't tell	NA	X	
7. Statistical analysis						
a) Conditional analysis? Or unconditional with matching variables in the models?	Yes	No	Can't tell	NA	X	X
b) Relevant confounders adequately handled; Restriction, Stratified analyses, Multivariate modelling, Interaction tested?	Yes	No	Can't tell	NA	X	X

8. Summary of the study quality	A	B	C	
9. Upgrading elements				
a) Recruitment done in an acceptable way?	Yes	No	Can't tell	NA
b) Particulars of dietary assessment tool reported in sufficient detail?	Yes	No	Can't tell	NA
c) Energy intake at a credible level?	Yes	No	Can't tell	NA
d) No possible conflicts of interest affecting the study quality?	Yes	No	Can't tell	NA
Did the fish intake variable consist of other seafoods exceeding 10%?	Yes	No	Can't tell	NA
Reasons for grade C, and other comments:				



16.1.3.1 Modifications from the NNR5 questionnaire

The following questions were removed from the questionnaire used in NNR5

- Type of exposure (nutrients, food groups etc) reported in sufficient detail?
- Food composition database reported?
- Concurrent validity (validation coefficients) of specific exposures reported?
- Distribution of confounders similar in cases and controls?

The following questions were included in the original questionnaire, but defined as “upgrading elements” in VKM’s version. NNR5 did not have upgrading elements.

- Particulars of dietary assessment tool reported in sufficient detail?
- Energy intake at a credible level?

The following questions were added to VKM's version

- Fish intake according to inclusion criteria (individual intake and at least frequency)?
- Assessment details clearly reported and assessment adequately performed? (Anthropometry)

16.1.4 Quality assessment tool for prospective cohort studies

Authors: _____ Year: _____ Main outcome: _____

1. General questions and study design					Requires yes for level		
					A	B	C
a) Research question clearly formulated?	Yes	No	Can't tell	NA	X	X	
b) Endpoint/outcome clearly formulated?	Yes	No	Can't tell	NA	X	X	
c) Was the study design suited to test the research hypothesis?	Yes	No	Can't tell	NA	X	X	

2. Sampling (Ascertainment of cases and non-cases)		
a) Source population/study base well defined?	Yes No Can't tell NA	X X
b) Response rate reported and acceptable?	Yes No Can't tell NA	X X
c) Time period of baseline examinations clearly identified?	Yes No Can't tell NA	X
d) Endpoint clearly ascertained and assessed in a valid way?	Yes No Can't tell NA	X X
e) Follow-up period clearly identified?	Yes No Can't tell NA	X

3. Dietary exposure						
a) Fish intake according to inclusion criteria (individual intake and at least frequency)?	Yes	No	Can't tell	NA	X	X
b) Was the dietary assessment method validated?	Yes	No	Can't tell	NA	X	
c) Measurement errors in dietary reporting considered? Mentioned	Yes	No	Can't tell	NA	X	
d) Energy adjustment adequately done?	Yes	No	Can't tell	NA	X	
e) Repeat assessment of diet during follow up, and data considered	Yes	No	Can't tell	NA	X	
4. Anthropometry						
a) Assessment details clearly reported and assessment adequately performed?	Yes	No	Can't tell	NA	X	

5. Confounding						
a) Were important confounders identified/ascertained and considered by authors?	Yes	No	Can't tell	NA	X	X
6. Statistical power						
a) Was the study power considered and power calculations and sample size reported?	Yes	No	Can't tell	NA	X	
b) In view of multiple tests, were by chance findings considered?	Yes	No	Can't tell	NA	X	
c) Sufficient size of study population and no. of outcomes/cases?	Yes	No	Can't tell	NA	X	

7. Statistical analysis		
a) Appropriately handled?	Yes No Can't tell NA	X
b) Relevant confounders adequately handled; e.g, Restriction, Stratified analyses, Multivariate modelling, Interaction tested?	Yes No Can't tell NA	X X
c) Ascertainment/detection bias considered (eg. Cases detected due to screening)?	Yes No Can't tell NA	X
d) Cases detected early during the follow-up period removed?	Yes No Can't tell NA	X
8. Summary of the study quality	A B C	
9. Upgrading elements		

a) Recruitment done in an acceptable way?	Yes	No	Can't tell	NA	
b) Loss to follow up < 20%?	Yes	No	Can't tell	NA	
c) Particulars of dietary assessment tool reported in sufficient detail?	Yes	No	Can't tell	NA	
d) Energy intake at a credible level?	Yes	No	Can't tell	NA	
e) No possible conflicts of interest affecting the study quality?	Yes	No	Can't tell	NA	
Did the fish intake variable consist of other seafoods exceeding 10%?	Yes	No	Can't tell	NA	
Reasons for grade C, and other comments:					

16.1.4.1 *Modifications from the NNR5 questionnaire*

The following questions were removed from the questionnaire used in NNR5

- Criteria for inclusion/exclusion clearly formulated and acceptable?
- Participants and non-participants comparable with Nordic population?
- Time-exposure-variable clearly defined (i.e., period non-cases being exposed)?
- Type of exposure (nutrients, food groups etc) reported in sufficient detail?
- Food composition database reported?
- Concurrent validity (validation coefficients) of specific exposures reported?
- Associations between dietary exposures reported?
- Use of dietary biomarkers adequate? Details of assessment and handling reported?
Valid biomarker assay?
- Time period between biomarker assessment and diagnosis acceptable?
- Assessment details clearly reported, and assessment adequately performed? (Physical activity)

The following questions were included in the original questionnaire but defined as “upgrading elements” in VKM’s version. NNR5 did not have upgrading elements.

- Recruitment done in an acceptable way?
- Loss to follow up < 20%?
- Particulars of dietary assessment tool reported in sufficient detail?
- Energy intake at a credible level?

The following questions were added to VKM’s version

- Fish intake according to inclusion criteria (individual intake and at least frequency)?
- Was the dietary assessment method validated?

16.2 Quality assessment of systematic reviews and meta-analyses. The AMSTAR tool (version 1)

NNR5 modified AMSTAR checklist for quality assessment of systematic reviews

Author: Year: Referencenumber:

1. Was an 'a priori' design provided? The research question and inclusion criteria should be established before the conduct of the review.	☐ Yes ☐ No ☐ Can't answer ☐ Not applicable
2. Was there duplicate study selection and data extraction? There should be at least two independent data extractors and a consensus procedure for disagreements should be in place.	☐ Yes ☐ No ☐ Can't answer ☐ Not applicable
3. Was a comprehensive literature search performed? At least two electronic sources should be searched. The report must include years and databases used (e.g. Central, EMBASE, and MEDLINE). Key words and/or MESH terms must be stated and where feasible the search strategy should be provided. All searches should be supplemented by consulting current contents, reviews, textbooks, specialized registers, or experts in the particular field of study, and by reviewing the references in the studies found.	☐ Yes ☐ No ☐ Can't answer ☐ Not applicable
4. Was the status of publication (i.e. grey literature) used as an inclusion criterion? The authors should state that they searched for reports regardless of their publication type. The authors should state whether or not they excluded any reports (from the systematic review), based on their publication status, language etc.	☐ Yes ☐ No ☐ Can't answer ☐ Not applicable
5. Was a list of studies (included and excluded) provided? A list of included and excluded studies should be provided.	☐ Yes ☐ No ☐ Can't answer ☐ Not applicable
6. Were the characteristics of the included studies provided? In an aggregated form such as a table, data from the original studies should be provided on the participants, interventions and outcomes. The ranges of characteristics in all the studies analyzed e.g. age, race, sex, relevant socioeconomic data, disease status, duration, severity, or other diseases should be reported.	☐ Yes ☐ No ☐ Can't answer ☐ Not applicable

17 Appendix IV Contaminants considered for inclusion

17.1 Description and evaluation of candidate contaminants or contaminant groups proposed for inclusion in the benefit and risk assessment

The process of including or excluding contaminants is described in Chapter 2.3.1, and the flow chart describing the decision process for inclusion or exclusion of candidate contaminants for the benefit and risk assessment of fish in the Norwegian diet I shown in Figure 2.3.1-1.

17.1.1 Arsenic

Arsenic is a metalloid that occurs in many different chemical forms in the environment and in food. Fish and seafood are the main contributors to the dietary exposure to total arsenic, and a high consumption of fish and seafood leads to a high dietary exposure to total arsenic. However, inorganic and organic arsenic have different toxicity, and as organic forms dominate total arsenic in fish, this must be taken into consideration when assessing risk.

Inorganic forms of arsenic are considered more toxic compared to organic arsenic. Inorganic arsenic causes cancer of the lung and urinary bladder in addition to skin. However, in fish (and seafood) the relative proportion of inorganic arsenic is small and tends to decrease as the total arsenic content increases. The total arsenic is reported within a range of 0.2 – 150 µg/g for marine fish and bioaccumulation varies for different tissues. About 90% of arsenic is of organic form, while the content of inorganic arsenic is reported to be only 0.02 – 11% in marine fish (Zhang et al., 2015; Zhang et al., 2016; Pei et al., 2019). Fish is not considered an important source of inorganic arsenic (VKM, 2016).

The dominating species of organic arsenic in fish is arsenobetaine. Arsenobetaine is excreted unchanged and is considered to be of no toxicological concern (EFSA, 2009). However, other forms of organic arsenic are less well characterised. In more recent years, arsenic bound to lipids, i.e. fatty acids, phospholipids etc, have been characterised. Arsenolipids have been found in the lipid phase in several seafoods including cod liver. In a preliminary study by Sechmeisser et al. (2006), the results indicated that ingested arsenolipids are rapidly metabolised to water-soluble compounds and excreted (Sechmeisser et al., 2006).

There is currently no commonly agreed definition of arsenolipids, but among those that contain a long aliphatic chain, five main groups of arsenolipids have been identified: arsenic-containing hydrocarbons (AsHCs), fatty acids (AsFAs), phospholipids, phosphatidylcholines, and fatty alcohols.

In an in vitro study, Schwerdtle, Francesconi, and colleagues investigated the cellular toxicity of AsHCs in cultured human bladder and liver cells (Meyer, et al., 2014). In this study, the cytotoxicity of the three tested AsHCs was comparable to that of inorganic arsenic, in the low μM range. The authors concluded that the study “cannot exclude a risk to human health related to the presence of arsenolipids in seafood”.

Also, arsenosugars have been found in seafood. They are found mainly in algae and shellfish, as organic arsenic in seafood changes in composition throughout the foodweb. Still, regarding organic arsenic compounds, including arsenolipids and arsenosugars, there is still little information on both their occurrence and toxicity.

Evaluation: For inorganic arsenic, fish is not an important source, and the answer to question 1 is ‘no’. For organic arsenic the dominating species, arsenobetaine, is not considered to be a concern, and hence the answer to questions 1 is ‘no’. For arsenolipids and arsenosugars the lack of data makes an evaluation difficult, but a risk assessment would not be possible until more data is available, and therefore the decision for arsenic is to not include it in the benefit and risk assessment, but to highlight the lack of data on some forms of organic arsenic as a data gap.

17.1.2 Methyl mercury

In 2012 EFSA established a TWI for methyl mercury of $1.3 \mu\text{g/kg bw/week}$, expressed as mercury, based on recent findings of neurodevelopmental effects in prenatally exposed children (EFSA, 2012). EFSA calculated that mean exposure in Europe (all population groups) is below the TWI, whereas 95th percentile exposure is in the range of or exceeding the TWI. This was confirmed by reported levels in hair and blood in Europe. Fish is the only substantial source of methyl mercury in Norway. Methyl mercury was part of VKM’s risk-benefit assessment of fish in 2014 (VKM 2014). The assessment concluded that more than 95% of the population of 2-year-olds, adults and pregnant women had methyl mercury intake below the TWI of $1.3 \mu\text{g/kg bw/week}$. The assessment was based on mean levels in fish often consumed and containing background levels of methyl mercury. Some fish species or fish originating from areas contaminated with mercury contain higher levels of methyl mercury. This was further elaborated by VKM in 2019 (VKM 2019). There is also information on association between fish consumption and measured levels in blood from Norwegians (Jenssen et al., 2012, Birgisdottir et al., 2013) and in blood and hair of pregnant women (Brantsæter et al., 2008, Næss et al., 2019). High consumption of certain fish species with relatively high methyl mercury concentration or species from contaminated areas can lead to exposure higher than the current TWI for methyl mercury.

Evaluation: Fish is, together with shellfish, the only relevant sources of methyl mercury, and the answer to question 1 and 2 is ‘yes’. It has been assessed before by EFSA, thus the answer to question 3 is also ‘yes’. However, this risk assessment was published in 2012, and the answer to question 4 is that new information might have become available that need consideration. Exposure to methyl mercury may be exceeding the TWI in some population groups, although it is not exceeded by most part of the population, and the answer is ‘yes’ to

question 5. Based on this, methyl mercury exposure was included in the present benefit and risk assessment.

In order to answer question 4 VKM explored new available information on health effects of methyl mercury, as summarized in Chapter 17.1.2.1 below. It was however considered to be beyond the scope of this benefit and risk assessment to revise the HBGV for methyl mercury.

17.1.2.1 Review of systematic reviews on methyl mercury

Since EFSA's risk assessment of methyl mercury in 2012, many publications have assessed the association between mercury exposure and different endpoints. Their findings may potentially indicate that there is a need to update the risk assessment of methyl mercury. VKM conducted a literature search focussing on systematic reviews published after the EFSA risk assessment in 2012. The search is described in Chapter 3.3, and the search strategy can be found in Appendix II, Chapter 15.5. In addition, a separate search was conducted in order to identify original publications addressing mercury exposure and health outcomes in the Norwegian population. This second search was done in order to capture new information of particular relevance to Norway, in view of a relatively high fish consumption combined with relatively low methyl mercury concentrations in the fish species most often consumed. This search is also briefly described in Chapter 3.3, and the search strategy can be found in Appendix II, Chapter 15.5.

Summary of systematic reviews

In order to get an overview of results from studies published on methyl mercury after the EFSA assessment published in 2012, VKM conducted a literature search for systematic reviews. VKM obtained 106 hits, and a flow chart describing the process can be found in Figure 3.3-1 in Chapter 3.3. The screening of title and abstract was done in accordance with criteria in Table 3.3-1 by two independent reviewers and resulted in 22 papers that were checked in full text. From these 22, 14 reviews fulfilled the inclusion criteria and were summarized in the present narrative review of reviews. The quality of included reviews was assessed by use of AMSTAR, see 3.1.3.2 for more details. In order not to lose information also two reviews graded C were included (AMSTAR grade is indicated in the description of results). The included studies comprised papers covering the topics autism, ADHD, neurodevelopment, neurological disorder, blood pressure/hypertention, foetal growth, birth outcomes, autoimmunity, diabetes and metabolic diseases.

In the following text, the summary and conclusions made by EFSA (2012) for each outcome is described first, then the findings in the included reviews are summarised.

Autism and/or ADHD

EFSA (2012) included studies up to the summer of 2012 on ADHD and autism spectrum disorders (ASD) and concluded that *"studies on autism do not indicate any increased risk from dietary mercury exposure, but for ADHD some studies have found associations with*

mercury. Taken together, however, the results do not provide information to allow conclusions."

Rossignol et al. (2014) summarised association between environmental toxicants (including mercury), and ASD and included studies through November 2013 and covered also genetic mechanisms that might be involved. They pointed out that the biomarker studies contained small sample sizes and the relationships between biomarkers and ASD were inconsistent across studies. The quality of this systematic review is graded 'C'.

Yosibashu et al. (2014) conducted a meta-analysis on prenatal and early infancy exposures to different forms of mercury and childhood autism and ADHD. Only two studies on methyl mercury exposure were included in the meta-analysis, and the summary OR (95% CI) after correcting for publication bias was calculated to be 1.60 (1.10–2.33) with non-significant heterogeneity ($P = 0.26$, $I^2 = 24\%$) for the random effects model. The authors stated that further replicated findings are warranted with an adjustment for fish consumption), since the adverse effects of methyl mercury on childhood neurological development might be diminished by the beneficial effects of seafoods, referring to Sagiv et al. (2012), reporting protective association for fish consumption. The quality of this meta-analysis is graded 'B'.

Perez-Fernandez et al. (2019) published a systematic review on association between xenobiotic (including methyl mercury) exposure and inhibitory control in experimental animals and humans. They reported that the results were inconclusive for methyl mercury in humans. The quality of this systematic review is graded 'B'.

Neurological disorder (adults)

Neurological disorders after high level methyl mercury exposure are well known from previous studies. In the Opinion from 2012, EFSA considered only studies on neurological disorders in adults that had been exposed to methyl mercury in the range of that observed in the Faroe Islands and the Seychelles and lower. EFSA did not consider studies on populations more highly exposed as relevant for their assessment, since there was already a TWI based on neurodevelopmental effects in the Faroe Islands and the Seychelles. EFSA concluded in 2012 that the studies included *"do not show relevant associations between mercury exposure, at low levels, and adverse neurological outcomes in the adult population."*

Puty et al. (2019) conducted a systematic review on associations between methyl mercury environmental exposure and neurological disorders and included populations > 13 years of age. The search covered publications up to December 2017. According to the authors the identified six eligible studies could not form basis for conclusion due to high risk of bias and low evidence level. This systematic review is graded 'B'.

Heart rate variability

Influence of methyl mercury on heart rate variability was reviewed by EFSA and results particularly in adolescents and adults were evaluated in the opinion in 2012. Heart rate

variability can reflect adaptive mechanisms of the autonomic nervous system, influenced by both the sympathetic pathway (cardio-acceleration) and the vagal pathway (cardio-deceleration) and is regulated by feedback from baroreceptors in the arteries. A shift in the sympatho-vagal balance may become a major risk for cardiac events. EFSA concluded that *"Taken together, the studies of cardiac autonomy suggest an influence of mercury on heart rate variability, but the results are not consistent between studies and the implications for health are currently unclear. The well-designed intervention study showed a change in heart rate variability after 14 weeks of a weekly intake of 3.4 µg methyl mercury/kg bw. The variability returned to baseline values after a 15 week washout period."*

Gribble et al. (2015) conducted a systematic review on mercury exposure and heart rate variability. The review did not cover papers on adults that were not already reviewed by EFSA and furthermore concluded that the evidence was too limited. This systematic review is graded 'B'. Karita et al. (2018) conducted a narrative review and identified three more recent papers (Périard 2015, Gump 2010, Miller 2018). None of them found associations between autonomic heart control and methyl mercury exposure. The review is graded 'C'.

Blood pressure, hypertension

In 2012, EFSA concluded the following: *"In all, the observations on blood pressure give a somewhat inconsistent picture, e.g. as regards whether diastolic or systolic blood pressure may be affected. There is no firm basis for assessment of a dose-response relationship"*.

In order to distinguish between effects from inorganic mercury and methyl mercury exposure, EFSA in 2012 considered mainly studies of populations with mercury exposure from seafood. In contrast, the systematic review on mercury exposure, blood pressure and hypertension by Hu et al. (2018) included studies with both predominantly inorganic mercury and predominantly methyl mercury exposure. They included thirty studies, of which one cohort, one case control and the rest with cross sectional design. The quality of the meta-analysis by Hu et al, 2018 was quality assessed by VKM using AMSTAR and is graded B. The authors investigated the observed heterogeneity for mercury species and suggested that mercury exposure level was the main determinant for blood pressure, regardless of mercury species. They concluded that *"The association between mercury exposure and the prevalence of hypertension was nonlinear, with no association in populations exposed to low-to-moderate mercury (hair mercury <2 µg/g) and evident association in populations exposed to high mercury (hair mercury ≥2 µg/g). However, the interpretation of causal association of mercury exposure and hypertension is limited by the cross-sectional design of original studies."*

Gallego Vinas et al. (2019) conducted a systematic review on chronic mercury exposure and blood pressure in children and adolescents. They concluded that few studies assessing chronic mercury exposure and blood pressure in children and adolescents, that the results available are inconsistent and that more research is needed. This systematic review is graded 'B'.

Cardiovascular disease and mortality

After summarizing the evidence regarding blood pressure, heart rate variability and coronary heart disease in 2012, EFSA made the following summarizing comment: *"At the time of the evaluation by the JECFA in 2006, there were only two major epidemiological studies that indicate an association between methyl mercury and increased the risk of cardiovascular disease (Guallar et al., 2002; Virtanen et al., 2005). Both these concern acute coronary events or myocardial infarction. Reported mercury levels ranged from 0.14 to 0.57 mg/kg in toenails (Guallar et al., 2002) and from 0 to 15.7 mg/kg in hair (mean: 1.9 mg/kg) (Virtanen et al., 2005). Results in the same direction were found in a recent study on sudden cardiac death (Virtanen et al., 2012) from a longer follow up of the cohort previously studied by Virtanen et al. (2005). The negative results of Yoshizawa et al. (2002) have been further strengthened by the recent study by Mozaffarian et al. (2011), in which no increased cardiovascular risk was observed even in the group with hair mercury > 2.7 mg/kg. Some other studies have dealt with lower exposure levels and provided negative findings.*

The importance of taking the beneficial effects of fish consumption into account when studying cardiovascular outcomes of methyl mercury has become evident. The studies by Yoshizawa et al. (2002) and Mozaffarian et al. (2011) have based the correction for n-3 LCPUFA confounding on dietary questionnaires, while the studies by Guallar et al. (2002) and Virtanen et al. (2005) have used biochemical measurements, and this may explain part of the discrepancy.

Thus, the observations related to myocardial infarction, HRV and possibly blood pressure are of potential importance, but still not conclusive."

Using the same search string as in Hu et al. (2018) a systematic review and meta-analysis on the relationship between mercury exposure and cardiovascular disease (cardiovascular disease (CVD), ischemic heart disease (IHD), myocardial infarction, stroke) and all-cause mortality was reported by Hu et al., 2021. This systematic review is graded 'B'. 14 studies were included, of which nine were cohort studies, four were case-control and one was cross-sectional. The cohort and case control studies were overall rated "good" by the authors. The included cross-sectional study was however not rated by the authors.

The pooled HR for all-cause mortality was 1.21 (95% CI: 0.90, 1.62), for mortality due to all CVD was 1.68 (95% CI: 1.15, 2.45), for mortality from IHD was 0.92 (95% CI: 0.40, 2.13), mortality from other heart disease was 1.50 (95% CI: 1.07, 2.11), and mortality from stroke was 1.01 (95% CI: 0.51, 2.00). Combining IHD and OHD, the pooled HR for heart disease was 1.22 (95% CI: 0.82, 1.80).

The study authors indicated that there was a high degree of heterogeneity between the included studies due to differences in mercury exposure measurement, exposure levels, study design, analysis methodology, and format of reporting.

The overall conclusion in Hu et al. (2021) was that chronic exposure to mercury was associated with an increased risk of all-cause mortality and fatal/non-fatal IHD, and that the

risk of multiple cardiovascular endpoints starts to increase at hair mercury concentration of 2 µg/g.

VKM noted that three of the cohort studies (Chen et al., 2018, Daneshmand et al., 2016, Larsen et al., 2018) and one of the cross-sectional studies included (Downer et al., 2017) were more recent and not included in the assessment by EFSA (2012). None of these reported significant beneficial or adverse associations between mercury exposure and the investigated endpoints. Given this fact, VKM is of the opinion that the additional information captured by Hu et al. (2021) does not provide evidence that alter the conclusion from EFSA on methyl mercury exposure regarding cardiovascular disease and mortality.

Foetal growth and birth outcomes

EFSA (2012) addressed studies on foetal growth and anthropometric birth outcomes under the heading developmental toxicity other than neurotoxicity and immunotoxicity. Inverse associations and null-associations were reported in some studies, both with and without adjustment for n-3 LCPUFA or maternal fish consumption. EFSA (2012) could not conclude on these endpoints based of the available data.

Dack et al (2021) conducted a systematic search on prenatal mercury exposure and birth weight, birth length, or head circumference. They included 27 studies (16 prospective and 27 cross sectional) from 17 countries, which used 8 types of mercury biomarkers. They summarized the studies narratively due to the heterogeneity in mercury measurements. They concluded that the review did not identify strong evidence that mercury exposure is associated with impaired prenatal growth, although there was some evidence of a negative association of mercury with birth weight. This narrative, systematic review is graded 'B'.

Saavedra et al (2021) conducted a systematic search on prenatal methyl mercury exposure and the health of foetuses, neonates and children up to 8 years of age. The results were summarized in a narrative way. They concluded that exposure was consistently associated with lower birth weight, based on four included studies. They also noted that mercury toxicity may sometimes be mitigated by e.g. polyunsaturated fatty acids in the maternal diet. This narrative, systematic review is graded 'B'.

Diabetes and metabolic disease

A systematic review was performed by Roy et al. (2017) covering literature up to November 2016 on mercury or methyl mercury and diabetes, metabolic syndrome or insulin resistance. The review included 34 studies. Most of the included studies were cross-sectional, three were nested case-control studies and four were prospective cohorts. By a WoE approach, Roy et al. (2017) concluded that the assessment of available data suggests a possible association between mercury exposure and diabetes mellitus or metabolic syndrome, but that the relationship is not consistent. They also considered available support of biological plausibility by *in vitro* and *in vivo* studies but could not conclude that there is a causal

relationship due to the inconsistency of the epidemiological evidence. This systematic review is graded 'B'.

Concluding remarks regarding reviews on methyl mercury

The results from this narrative review of reviews on methyl mercury exposure and different outcomes that have been published after the assessment by EFSA in 2012 does not provide clear indications that the risk assessment of methyl mercury from 2012 needs revision. However, VKM notes that there are probably primary studies and outcomes that have not been captured by the available reviews.

17.1.2.2 Original studies on methyl mercury and health outcomes conducted on the Norwegian population

Summary

The result of this search included one study on association between maternal fish intake and maternal mercury concentration in blood, and metabolic health and inflammatory markers in children at age 6 to 12 years (n=805). This was the Human Early Life Exposome (HELIX) project, which is a collaboration of five European birth cohort studies, including the Norwegian MoBa cohort (Stratakis et al. 2020). The outcomes studied were waist circumference, systolic and diastolic blood pressures, and levels of triglyceride, high-density lipoprotein cholesterol, and insulin. The authors concluded that *"Results of this study suggest that moderate fish intake consistent with current health recommendations during pregnancy was associated with improvements in the metabolic health of children, while high maternal mercury exposure was associated with an unfavourable metabolic profile in children"*.

One paper addressed birth weight in MoBa participants in relation to maternal fish consumption and dietary mercury intake (Vejrup et al 2014). The authors reported that *"Although seafood intake was positively associated with increased birth weight, stratified analyses showed negative associations between mercury exposure and birth weight within strata of seafood intake."*

Three papers addressed neurodevelopmental endpoints (cognitive function, language development) (Kvestad et al 2018, Vejrups 2016 and Vejrups 2020).

Kvestad et al 2018 conducted a randomized controlled trial with an intervention in lunch meals with fatty fish and studied the association between mercury in hair and cognitive function by Wechsler Preschool and Primary Intelligence Scale-III (WPPSI-III). The authors concluded that *"Lunch meals including fatty fish led to a significant increase in THHg, but the values remain below the point of departures used for risk assessment by the EFSA, WHO and US-EPA. We observed no associations between THHg and cognitive function."*

Association between maternal mercury exposure in pregnancy in women participating in MoBa and language development in their children at age three (n=46750) (Vejrup et al., 2016) and five years was reported by (Vejrup et al., 2018). At age 3 years maternal dietary

mercury intake was calculated and the authors concluded that *"significant associations were found between prenatal MeHg exposure above the 90th percentile and delayed language and communication skills in a generally low exposed population"*. The 90-percentile intake was 0.29 µg/kg bw/week. At follow up at 5 years (n=38581) the maternal concentration of mercury in blood was available for a subset of the participants (n=2239). The results showed that blood mercury concentrations were not associated with any measured outcomes. Increased dietary mercury exposure was significantly associated with improved score on the speech and language assessment scale (SLAS) when mothers had a seafood intake below 400 g/week in the adjusted analysis. Sibling matched analysis showed however a small significant adverse association between those above the 90th percentile dietary mercury exposure and the SLAS scores. Maternal seafood intake during pregnancy was positively associated with the language and communication scales. The authors concluded that *"Low levels of prenatal mercury exposure were positively associated with language and communication skills at five years. However, the matched sibling analyses suggested an adverse association between mercury and child language skills in the highest exposure group."*

Concluding remarks regarding studies on methyl mercury in the Norwegian population

VKM is of the view that the studies conducted in the Norwegian population may be of particular relevance since they are conducted in the target population of the present benefit and risk assessment. The few studies identified do not indicate clear concern at the relatively low methyl mercury exposure levels in the population included in the studies. Given uncertainties in dietary intake of mercury, the findings regarding language development would need confirmation in a larger study group with prenatal mercury concentrations measured analytically to be conclusive.

17.1.3 Brominated flame retardants

Brominated flame retardants (BFRs) is a wide group of chemicals that still are, or have previously been, used to prevent fire in different products, including furniture, textiles and electronic equipment. EFSA has previously assessed different classes of BFRs, but health-based guidance values have not been set for any of them because of insufficient information (EFSA 2011a, EFSA 2011b, EFSA 2011c, EFSA 2012a, EFSA 2012b). Instead, EFSA used a margin of exposure (MoE) approach for the flame retardants when feasible. This applied for some of the polybrominated diphenyl ethers (PBDEs, BDE-47, -153, -99 and -209) (EFSA 2011a), hexabromocyclododecanes (HBCDDs) (EFSA 2012b), tetrabromobisphenol A (TBBPA) (EFSA 2011c), and the brominated phenol 2,4,6-TBP (EFSA 2012a). The MoEs were sufficiently large for these BFRs, with the exception of one polybrominated diphenyl ether (BDE-99) for which there was a potential health concern (EFSA 2011a). EFSA is in the progress of updating the assessments of the BFRs, but new information was not available at the time of inclusion or exclusion of substances in 2020. An updated EFSA opinion on HBCDD has later been published (EFSA 2021). The conclusion is still to apply a MoE approach and the MoE is sufficiently large to conclude there is low concern with possible exception of some breastfed infants. The mean concentrations of HBCDDs in fish from Norwegian waters are in similar range as in data submitted to EFSA from different European regions (Nøstbakken et al, 2018, EFSA 2021). The paper by Nøstbakken et al., (2018) also summarizes levels of other BFRs in fish from Norway.

Due to structural and toxicological as well as toxicokinetic similarities it has been suggested that PBDEs should be assessed together with ndl-PCBs (see also sub chapter 4.1.6 ndl-PCBs) since their effects might be additive (Dingemans et al., 2016).

Evaluation: Brominated flame retardants are present in fish as well as in other food, and for some flame retardants fish might be a major source. The answer to question 1 and 2 was therefore 'yes'. They have been assessed by EFSA some years ago, hence the answer to question 3 was 'yes'. The conclusion then was that the MoE was sufficiently large to conclude on low concern for most of the BFRs. However, there is a need for updating the risk assessments of BFRs, leading to a 'yes' in reply to question 4. Updates of the risk assessments of BFRs are under development in EFSA and should not be done by VKM as it would be duplication of work. Due to the lack of an updated risk assessment, brominated flame retardants are not included in the present benefit and risk assessment.

17.1.4 Pesticides

17.1.4.1 DDT/DDE

DDT, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl) ethane, is a synthetic pesticide used for pest control and agriculture since the 1940s. It was strictly regulated in many countries, including EU and USA, in the 1970s due to its toxicity and persistence in the environment. It is however still accepted for use in developing countries to control malaria. Technically, it is composed of *p,p'*-DDT; *o,p'*-DDT and *o,o'*-DDT. The main DDT metabolites, DDE

(dichlorodiphenyl-dichloroethylene) and DDD (dichlorodiphenyl-dichloroethane), are often found in biota together with DDT (EFSA, 2006; ATSDR, 2019). For simplicity, unless otherwise specified, DDT or sumDDT will be used to refer to the combined fraction of DDT and its metabolites in this report, although some studies suggest opposing associations between different DDT metabolites and adverse outcomes (see below).

The main target organs of DDT are the nervous system and the liver, but it also affects hormonal tissues, reproduction, fetal development and the immune system (EFSA, 2006). DDT cause tumors mainly in the liver of experimental animals and is classified by IARC as possibly carcinogenic to humans. The Joint FAO/WHO Meeting on Pesticide Residues derived a provisional tolerable daily intake (PTDI) for DDT of 0.01 mg/kg bw/day (FAO/WHO, 2001). In 2020, ATSDR suggests a minimal risk level (MRL) for chronic exposure of 0.0001 mg/kg bw/day in a document for public consultation. The MRL was increased to 0.0005 mg/kg bw per day in the final version published in 2022 (ATSDR 2022). The MRL was based on liver hypertrophy in experimental animals resulting from chronic oral exposure, based on a BMDL₁₀ of 0.05 mg/kg bw per day and uncertainty factor 100. The epidemiological evidence was deemed insufficient to form basis for the MRL, partly because of inconsistent findings but most importantly lack of control of exposure to other lipophilic persistent compounds as e.g. PCBs, PCDDs and PCDFs.

Several effects related to reproductive toxicity, immunotoxicity, and metabolic disruption have been associated with exposure to DDT and other organochlorine pesticides in epidemiological studies. DDT has been shown to cross the placenta and enter fetal circulation, and epidemiological studies have shown DDT exposure to be associated with maternal hypertensive disorders and birth weight, although the latter being cohort dependent (reviewed in Gingrich et al., 2020). In some cases, opposing associations between different DDT metabolites and adverse pregnancy outcomes such as birth weight and length of gestation have been reported (Kezios et al., 2013).

Data from the Hokkaido study, representing 333 mother and child pairs, indicate that organochlorine pesticides including DDT, even at very low levels, may influence maternal and child thyroid hormone levels, which could modulate child development (Yamazaki et al., 2020).

Immunotoxic effects of DDT have been reviewed by Blakley et al. (1999) and Forawi et al. (2004). The effects are generally seen as a suppression of stimulated immune response in different animal species. DDT can inhibit intercellular communication, suppress lymphocyte proliferation and differentiation, and induce apoptosis in thymocytes, in addition to interference with other molecular and cellular components of the immune system (Forawi et al., 2004; Schjenken et al., 2021). However, specific evidence supporting a clinical impact on immune functions in humans in non-occupational DDT exposure settings seems to be lacking.

A meta-analysis of prospective human studies across the world demonstrated a consistent positive association between maternal exposure to DDT and children with obesity (Cano-

Sancho et al., 2017). Cano-Sancho et al. (2017) concluded that p,p'-DDT and p,p'-DDE can be "presumed" to be obesogenic for humans, based on a moderate level of primary human evidence, a moderate level of primary *in vivo* evidence, and a moderate level of supporting evidence from *in vivo* and *in vitro* studies.

In a follow-up study to Cano-Sancho et al. (2017), the association of maternal exposure to DDT with the risk of obesity in daughters during their mid-life in a prospective birth cohort with up to 53 years follow-up was investigated (La Merrill et al., 2020). Maternal o,p'-DDT was positively associated with a 26% (95% CI: 6–49) to 31% (95% CI: 6–62) higher risk of overweight and the same magnitude of additional risk for obesity, based on waist circumference and BMI definitions respectively, in multivariate models. The data indicate maternal DDT exposure to be significantly associated with an increased risk of obesity in middle-aged daughters independent of the obesity definition, confounding, and obesity risk factors.

Fish is reported to be a major source of DDT in humans. In an estimation of dietary intake of PCB and organochlorine pesticides in a Danish population, Fromberg et al. (2011) reported that fish represent approximately 45% of the mean daily intake of sumDDT. The mean reported intake levels of 3.7 and 6.7 ng/kg body weight per day for adults and children, respectively, are well below the MRL from ATSDR of 0.0005 mg/kg bw per day. Concentrations in Norwegian pregnant women are in similar range as in the rest of Europe (Papadopoulou et al., 2019).

Levels of DDT in farmed salmon in Norway have been decreasing in the period 2000-2015, from 10-15 µg/kg to around 5 µg/kg, mainly due to a change in the feed composition from fish-based oil and meal to plant-based ingredients (sjomatdata.hi.no; Nøstbakken et al., 2015).

Evaluation: As fish has been identified as an important source of DDT the answer to question 1 and 2 was 'yes'. The answer to question 3 was also 'yes' (FAO/WHO, 2001 and ATSDR, 2020). The studies on reproductive, immunologic and metabolic disorders showing associations to DDT exposure also in the lower dose range and through maternal exposure indicate data gaps and a need for more studies on DDT. Thus, the answer to question 4 is also 'yes'. VKM highlights this as a data gap. The answer to question 5 is 'no', and DDT/DDE is not included in the present benefit and risk assessment.

17.1.4.2 Other pesticides

Chlorpyrifos is one of the most widely used agricultural pesticides, used to kill a wide range of insects. It is an organophosphate pesticide that acts on the nervous system of insects by inhibiting the acetylcholinesterase enzyme. As plant ingredients are increasingly used in feedstuff for aquaculture, the presence of pesticides has been documented in such aquafeeds and transfer to fish is of concern. Toxicity of chlorpyrifos is associated with neurological dysfunction, endocrine disruption, and cardiovascular diseases, as well as

developmental and behavioral anomalies, genotoxicity, immunotoxicity and oxidative stress (Ubaid ur Rahman et al., 2021). Dietary exposure is thought to be the most important non-occupational source of chlorpyrifos exposure for humans. However, levels in fish seem to be low and were not detected in fish fillet in the national monitoring program in Norway (Hannisdal et al., 2019), nor in feeding trials with salmon and gilthead seabream fed plant-based diets (Nácher-Mestre et al., 2018).

Evaluation: Fish is not a significant source of CPF, the answer to question 1 is 'no', and chlorpyrifos is not included in the current benefit and risk assessment.

Hexachlorobenzene

Hexachlorobenzene is a legacy organochlorine pesticide, introduced for agriculture in 1945, and banned for agricultural use in EU in 1981. It is regulated in the Stockholm Convention, but still used to some extent and also released to the environment through industrial processes (EFSA, 2006). As a persistent, volatile, and lipophilic POP it is widely spread and accumulated in fatty tissues and can biomagnify in the food chain and is readily absorbed in humans and animals. Hexachlorobenzene has low acute toxicity but is classified by the IARC (2001) as a possible human carcinogen based on tumor development in experimental animals. Hexachlorobenzene primarily targets the liver although immunotoxic, reprotoxic, and genotoxic effects are observed. Long-term exposure can lead to porphyria cutanea tarda, a condition involving changes in skin color, skin sores, arthritis, and neurologic problems.

Hexachlorobenzene levels have been stable around 1-1.5 µg/kg in Norwegian farmed salmon fillet since 2008 (<http://sjomatdata.hi.no>; Hannisdal et al., 2017). The suggested health-based guidance value for hexachlorobenzene is 170 ng/kg bw/day (EFSA, 2006). Human dietary hexachlorobenzene exposure, with salmon as a major contributing factor, ranges up to a few ng/kg bw/day (EFSA, 2006), which is far below the guidance value.

Evaluation: Fish is not a significant source of hexachlorobenzene, the answer to question 1 and 2 is 'no', and hexachlorobenzene is not included in the current benefit and risk assessment.

Hexachlorocyclohexane

Hexachlorocyclohexane is a persistent organochlorine pesticide, extensively used in the past. Although technical HCH consists of at least five stable isomers, it is the isomer known as lindane, that possesses the insecticidal activities. Its production and use have created serious environmental contamination with HCH, and its persistence and lipophilicity make it into a worldwide problem, ending up in water, soil, air, and biota. The toxicity and persistence of the HCH-isomers varies. All isomers cause liver hyperplasia and/or liver tumors in animal studies, and they are classified as possibly carcinogenic to humans by IARC (Berntssen et al., 2017; EFSA, 2005). Several isomers are reported to have endocrine disrupting properties including estrogenicity (Pathak et al., 2009).

Food is the main source of HCH exposure for humans but decreasing concentrations of HCH in breast milk indicate current exposure to be low, in the lower range of 1-10 ng/kg bw/day (EFSA, 2005). Levels in farmed salmon from Norway are in the range from below LOQ to 0.3 µg/kg for the different isomers in salmon fillet over the past 10 years (<http://sjomatdata.hi.no>; Hannisdal et al., 2017), indicating a minor contribution to the total exposure. EFSA (2016) has set an ADI of 0.001 mg/kg bw/day based on NOAEL in a two-year rat study.

Evaluation: Fish is not a major source of HCH, the answer to question 1 and 2 is 'no', and HCH is not included in the current benefit and risk assessment.

Chlordane

Chlordane is an organochlorine pesticide with widespread use until the late 1980s. Chlordane was used extensively to control termites and as a broad-spectrum insecticide on a range of agricultural crops. It is highly persistent and has a reported half-life in the environment of 10-20 years. Chlordane may affect the human nervous system, liver and immune system and is classified as a possible human carcinogen. Chlordane has been banned in the EU since 1981 and is listed under the 12 initial POPs under the Stockholm Convention. Technical chlordane is a mixture of several constituents, with cis-nonachlor and trans-nonachlor (t-NC) being among the most abundant bioaccumulating components, biomagnifying in the food chain. Toxicity data for individual chlordane constituents are generally rare. In a 28-day feeding study comparing trans- and cis-nonachlor with technical chlordane toxicity in rats, t-NC produced the most overt toxicological responses, with liver as the main target organ (Bondy et al., 2000).

A provisional tolerable daily intake of chlordane of 500 ng/kg bw was established by WHO in 1995 (EFSA, 2007). EFSA calculated human dietary exposure to chlordane in the low ng/kg bw range, which is well below the WHO TDI. Norwegian data for these compounds in fish range from 0.7-3 µg/kg in Atlantic salmon and Atlantic halibut for chlordane, with decreasing concentrations in salmon over the past decade (<http://sjomatdata.hi.no>), whereas t-NC was reported at a median of 0.52-0.67 µg/kg in Atlantic salmon and rainbow trout in 2016 (Hannisdal et al., 2017).

Evaluation: Fish can potentially be a source for chlordane and its constituents, including t-NC. Hence the answer to question 1 is 'yes'. The answer to question 2.0 is however 'no', and chlordane is not included in the current benefit and risk assessment.

17.1.5 Dioxins and dl-PCBs

This is a large group of compounds, but here we refer to 29 individual substances belonging to the polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (DL-PCBs). Out of the 29 compounds (congeners), seven belonging to PCDDs and ten belonging to PCDFs are as a group commonly called

“dioxins”. However, in the present assessment we refer to this group of 17 substances as PCDD/Fs.

Evaluation: PCDD/Fs and DL-PCBs are present in fish, and fish is a major source. The answer to question 1 and 2 is ‘yes’. In 2018 EFSA set a new TWI for dioxins and dl-PCBs at 2 pg TEQ_{WHO2005}/kg bw/week. The exposure calculations indicate that a large proportion of the EU population has exposure exceeding the TWI, and similar has been calculated for pregnant women participating in MoBa (Caspersen et al). No new risk assessment is needed (question 4) but a re-evaluation of the TEF-factors which is undertaken by WHO might change the exposure estimate. With the present TEF-factors, exposure from fish can exceed the TWI, the answer to question 5 is ‘yes’, and the substances are included in the benefit and risk assessment, in accordance with the mandate.

17.1.6 Non-dioxin like PCBs

Polychlorinated biphenyls (PCBs) are a group of 209 distinct chemical compounds, called congeners, where 1 to 10 chlorine atoms are attached to a biphenyl molecule. PCBs were commercially produced as complex mixtures containing different compositions of different congeners. PCBs are lipophilic and characterized by chemical stability. These properties are also responsible for their continuing persistence in the environment. Almost fifty years after they were subject to international regulation and almost forty years after global banning, they are still present in all environmental media. In a toxicological context, PCBs are generally divided into dioxin-like PCBs (dl-PCBs, 12 congeners) and non-dioxin-like PCBs (ndl-PCBs, all other congeners) (EFSA, 2005).

In several epidemiological studies associations between exposure to PCBs and effects on various outcomes such as reproduction and (neuro)development, thyroid system, nervous system, immune system, cardiovascular system, growth, lipid metabolism, diabetes, and obesity have been found (e.g., reviews by ATSDR, 2000; EFSA, 2005; Hatch et al., 2010; Korrick and Sagiv, 2009; Meeker and Hauser, 2010; Wang et al., 2010a; Pessah et al., 2019). Many of these associations are still under investigation and debate as they are not expected at the measured exposure levels or could not be confirmed in other studies (Hamers et al., 2011).

Despite the abundance of ndl-PCBs their toxicity is poorly characterized in terms of the spectrum of effects and potency. Due to lack of relevant data for individual congeners the Scientific Panel on Contaminants in the Food Chain of the EFSA was not able to establish health-based guidance values for ndl-PCBs (EFSA, 2005). One important limitation in the database was the low-level contamination of ndl-PCB congeners with dioxins, resulting in non-representative toxicity profiles. The toxicity of ndl-PCBs has been investigated intensely over the following years, e.g. in the EU funded ATHON project (<https://cordis.europa.eu/project/id/22923>), where ultrapure ndl-PCBs were produced and tested.

Based on *in vitro* screening of 24 ultrapure compounds, ndl-PCBs could be separated into two groups (Hamers et al., 2011; Stenberg et al., 2011). The first group, comprising PCB 28, 47, 51, 52, 53, 95, 100, 101, 104 and 136, included mainly smaller, ortho-substituted congeners with higher biological activity in most of the assays, with PCB 95, 101 and 136 distinguished as the most active. The second group included the most abundant congeners of the congeners tested, and was comprised of PCB 19, 74, 118, 122, 128, 138, 153, 170, 180 and 190. The second group had lower activity in many of the assays, except for three assays related to endocrine function and disruption (Stenberg et al., 2011).

Ultrapure PCB 52 and 180 underwent extensive toxicity profiling in 28-day oral toxicity study in rodents, following the OECD 407 Guideline enhanced for detection of endocrine, neurotoxicity, retinoid, bone and DNA damage endpoints. In the PCB-180 study, Viluksela et al. (2014) reported a distinct toxicological profile with altered open field behavior in female rats being the most sensitive endpoint, and induction of certain xenobiotic metabolizing enzymes in liver taking place at the same exposure levels. An MoE approach, when using the WHO default uncertainty factor (UF) of 25 and altered locomotor activity as the critical endpoint, indicated that critical PCB 180 tissue concentration is exceeded in some human cohorts.

More recently, a study by Zhao et al. (2020) using PCB-52 exposure of rats during gestation and lactation found decreased body lengths and weights at birth and abnormal expression of neurotransmission ligand-receptors in male offspring. These findings could provide an insight into the possible mechanisms of ndl-PCB induced neurodevelopmental toxicity.

Generally, a strong correlation between dioxins, dl-PCBs and ndl-PCBs can be found, and it can be argued that the low TWI for dioxins would protect against potential effects of ndl-PCBs.

Although the data on ndl-PCB toxicity is gradually increasing, the complexity of congeners, their physico-chemical properties, behavior, and Mode of Action, as well as the complexity of potential targets and adverse outcomes, make the development of simple risk descriptors challenging, as the debate around neurotoxic equivalent factors values demonstrate (Pradeep et al., 2019). These issues point to the need for more systematic testing and analysis across a range of endpoints to be able to perform in-depth risk assessment of PCB mixtures and to close data gaps.

Evaluation: As fish is an important contributor to PCB exposure, the answer to question 1 and 2 was 'yes'. Although previous risk assessments have been performed, the issue of purity of congeners used in the earlier studies raises questions to their validity. Thus, the answer to question 3 is 'yes', but the answer to question 4 is also 'yes'. A new risk assessment of non-dl PCB was not possible within the scope of the current benefit and risk assessment of VKM, hence this is highlighted as a data gap. Due to the lack of an updated risk assessment, the non-dioxin like PCBs are not included.

17.1.7 Perfluorinated alkyl substances (PFAS)

Perfluorinated alkyl substances (PFASs) is a class of compounds that include perfluoroalkyl carboxylates (PFCAs) and perfluoroalkyl sulfonates (PFASs). Such compounds are water, oil and stain repellent and have been used extensively the last 50 years in a vast variety of applications, including non-stick coating on materials in contact with food, casseroles and pans, impregnation of textiles, firefighting foam, cosmetics and paint.

EFSA assessed the risk of exposure for PFOS and PFOA and set provisional HBGVs for these two substances separately in 2018 (EFSA 2018). In 2020 EFSA assessed 27 PFASs in food and also reviewed the assessment of PFOS and PFOA in order to consider potential mixture effects among the PFASs (EFSA 2020). EFSA set a TWI of 4.4 ng/kg bw/week for the sum of four PFASs (PFOS, PFHxS, PFOA, PFNA). These four PFASs contribute approximately 46% of the exposure to the sum of all PFASs in adults for which exposure from food could be calculated. Fish is an important source for exposure, but also other foods contribute substantially. The calculated dietary exposure of most part of the European population exceeds the TWI for the sum of four PFASs.

Evaluation: The answers to questions 1, 2 3 and 5 are 'yes' and PFASs are included in the present risk-benefit assessment, as is also in line with the terms of reference.

17.1.8 Mycotoxins

Mycotoxins comprise a structurally very diverse group of fungal secondary metabolites which enter human and animal food chains through infected cereal grains used for food or feed (Streit et al., 2013, Rocha et al., 2014). Mycotoxins found to regularly occur in fungal infected goods at toxicologically relevant concentrations are subject to legal regulations or guidance in the EU and Norway (Streit et al., 2014). With plant-based ingredients replacing fishmeal in finished fish feeds, the presence of mycotoxins also was reported in feeds for farmed fish (Nácher-Mestre et al., 2015).

A risk assessment of mycotoxins in cereal grain in Norway highlighted data and knowledge gaps concerning the effects of mycotoxins on fish health and the transfer of mycotoxins from feed to fillet in farmed fish (VKM, 2013). Studies published since, showed that in fish feed levels of 18 different mycotoxins surveyed according to EU regulations, were below maximum residue limits established by the Commission Recommendation 2006/576/EC, and no mycotoxin carry-over was found from feeds to edible fillets of farmed fish (Nácher-Mestre et al., 2015). EFSA concluded that available data on acute exposure to beauvericin and enniatins did not indicate concern for human health. With respect to chronic exposure, EFSA stated that there might be concern, but no firm conclusion could be drawn (EFSA, 2014). Available data on the non-regulated mycotoxins, beauvericin and enniatins showed that, while present in fish feed, all the mycotoxins analyzed were below quantification limits (< 0.1 µg/kg) in fish samples (n = 82) implying no risk for consumers of the fish (Nácher-Mestre et al., 2020).

Evaluation: Fish was not identified an important source of mycotoxins; the answer to question 2 was 'no'. Based on this, mycotoxins is not separately included in the present benefit and risk assessment.

17.1.9 Antioxidants

Synthetic antioxidants are commonly used as preservatives in fish feed and comprise substances including ethoxyquin (EQ), butylhydroxytoluen (BHT), butylhydroxyanisol (BHA) and propylgallate (PG) (VKM, 2014). In contrast to Japan, which set MRLs of 1 mg EQ per kg, 10 mg BHT per kg, and 0.5 mg BHA per kg for fish, in the European Union no MRLs for synthetic antioxidants in products from farmed animals have been established (VKM, 2014). In 2010, Lundebye et al. calculated the intake of EQ, BHT and BHA from fillets of different farmed fish species (cod, salmon, halibut and trout). It was found that EQ, BHT and BHA constitute between 4 - 15%, 34 - 74%, and less than 1%, respectively of their respective ADIs based on daily consumption of a 300 g portion of fish (Lundebye et al., 2010; VKM, 2014).

Ethoxyquin (EQ; 6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline; EQ) is a quinoline-based synthetic antioxidant used in feed components for pets, livestock and aquaculture (Bernhard et al., 2019). EQ or butylated hydroxytoluene (BHT; see below) have commonly been used for the stabilization of fish meal to prevent spontaneous combustion during transport and storage (IMO, 2014). In the European Union (EU), directive 70/524/EEC and regulation EC 1831/2003 authorized the inclusion of EQ as a feed additive for all farmed species with a maximum content of 150 mg/kg; however, concerns regarding the safety of EQ and its transformation products led to a suspension of the previously granted authorization (OJ L 276, 20.10.2010, p. 33–79. (2010)). A transition period granted until March 2020 allowed feed produced from certain materials containing EQ to be placed on the market (VKM, 2019a). Use of EQ in food is not permitted but due to feed to food carry-over, EQ and many of its transformation products were detected in commercially produced Atlantic salmon feeds, in edible parts of commercial Atlantic salmon fillets, and in Atlantic salmon fed graded levels of EQ enriched feed (Regueiro et al., 2017, Merel et al., 2019). EQ itself was not found to be genotoxic, carcinogenic or to elicit developmental toxicity. However, EQ transformation products, such as ethoxyquin quinone imine (EQI), showed structural alerts for mutagenicity, carcinogenicity and DNA binding (EFSA, 2014), and exposure to EQ dimer, 1,8'-di(6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline (EQDM), lead to the development of microvesicular steatosis in a rodent model of toxicity (Bernhard et al., 2018). EQDM is the main and most abundant EQ transformation products identified in farmed fish muscle (Lundebye et al., 2010; Bohne et al., 2008). Potentially mutagenic and carcinogenic substances such as EQI, are in general not considered to have specific threshold limits. The detection and identification of novel EQ transformation products in salmon muscle, even at relatively low levels, as reported in (Merel et al., 2019) therefore, calls for an assessment if these are mutagenic. With the current lack of toxicity and concentration data in most food items except fish (VKM, 2019a) reauthorization or prohibition of use of EQ as feed additive in the EU, will depend on the outcome of risk assessments in which concerns regarding the toxicity of individual EQ transformation products are being addressed (EFSA, 2021).

Butylated hydroxytoluene (BHT) is a synthetic antioxidant authorized as food and feed additive in the EU (VKM, 2019a). Based on a no observed adverse effect level (NOAEL) of 25 mg/kg bw/day derived from the effects of BHT on litter size and pup body weight in two-generation rat studies, EFSA established an acceptable daily intake (ADI) of 0.25 mg/kg bw/day (EFSA, 2012). According to EFSA, exposure of adults to BHT is unlikely to exceed the ADI at the mean and at the 95th percentile (EFSA, 2012). This in accordance with a recent risk assessment of BHT performed in Norway, which found in scenario calculations that only the 95 percentile of the highest exposure scenario (i.e. the worst-case estimation) was above the ADI, and concluded that BHT exposure was unlikely to cause adverse health effects in adults (VKM, 2019b).

Butylated hydroxyanisole (BHA) is a synthetic antioxidant, which in the EU is authorized as both feed and food additive, and is used in fats and oils and in many processed foods such as soups, sauces, breakfast cereals and fine bakery products (VKM, 2019). BHA was evaluated by EFSA in 2011 and, based on a NOAEL of 100 mg/kg, an ADI was set at 1.0 mg/kg bw (VKM, 2019). A reevaluation of BHA by EFSA in 2018 concluded that the previously set ADI can be retained (EFSA, 2018). In the same report it was stated that for consumer safety no concern would arise from the use of BHA as a feed additive at the maximum concentration of 150 mg/kg feed as this, in a highly conservative estimate of consumer exposure, results in an exposure of 5 mg BHA/person/day, corresponding to about 8% of the ADI.

Evaluation: According to literature and previous risk assessments, farmed fish can be a source of synthetic antioxidants due to feed to fillet carry-over (EQ, BHA and BHT); for BHA and BHT the answer to question 1 is 'yes'. For EQ, the answer to question 1 and 2 is a tentative 'no' since at the time of writing, the use of EQ in fish feed is not permitted. EQ is therefore not included in the present assessment.

All three compounds have been risk assessed previously; the answer to question 3 is 'yes' for BHA and BHT. Exposure to BHA and BHT through farmed fish is reported to be below their respective ADIs for adults. For children under 3 years and pregnant women/unborn children the situation is less clear. In addition, to date, no assessments of the presence and risk of transformation products of BHA or BHT have been performed. Risk assessments of BHA and BHT transformation products are highlighted as data gaps. The risk from synthetic antioxidants in fish was not separately included in the present benefit and risk assessment.

17.1.10 Erucic acid

Erucic acid is a long-chained fatty acid (22:1 n-9) occurring in high levels in some cultivars of rape seeds and mustard seeds. The cultivars used for rape seed oil production for human consumption (Canola oil) are low in erucic acid, and exposure assessments performed by EFSA showed that the exposure in the European population was below the TDI for erucic acid of 7 mg/kg bw/day set by EFSA (EFSA 2016). The TDI was based on a no observed adverse effect level of 0.7 g/kg bw/day for myocardial lipidosis (accumulation of triacylglycerols in myocardium) in young rats and newborn piglets and UFs of uncertainty

factor of 100 to account for intra- and interspecies differences. The lipidosis observed was transient and reversible, and EFSA considered that the approach followed for the establishment of the TDI is conservative and more likely to overestimate than to underestimate the risk.

Mean and high (95-percentile) chronic exposure of the different age groups across different European populations did not exceed the TDI. In adults the mean and high (95-percentile) exposure ranged from 0.3 to 2.2 and 0.9 to 4.3 mg/kg bw/ay (minimum LB to maximum UB). The maximum 95-percentile intake was seen in infants with 5.8/7.4 mg/kg bw/day (LB/UB) and other children with 5.3/9.5 mg/kg bw/day (LB/UB). Fine bakery wares was the main food group contributing to exposure, in particular in younger age groups. EFSA had few samples on erucic acid concentration in fish, but still fish was an important contributor in some adult populations with contribution up to 41% in adult populations. Sissner et al. in 2018 reported more information on concentration in fish in Norway. They reported that consumption of 200g of e.g. mackerel at mean erucic acid concentration would lead to exposure equal to the TDI for a person weighing 60 kg, confirming that fish can be an important source to erucic acid in adults.

Evaluation: The answer to question 1 is 'yes'. As fish was identified by EFSA as an important source to erucic acid the answer to question 2 is also 'yes'. The answer to question 3 is 'yes' and since EFSA's assessment is relatively new, no updated risk assessment is needed and the answer to question 4 is 'no'. The data from Sissner (2018) indicate that concentrations in fish from Norway is in similar range as those used for exposure assessment from EFSA, and the fish consumption in Norway is in a similar range as in countries with high fish consumption that are included in the consumption surveys used by EFSA. Therefore, VKM deduce that the exposure to erucic acid from fish and other food in Norway is expected to be in similar range as estimated by EFSA in 2016. Thus, the exposure is expected to be below the TDI, and the answer to question 5 is 'no'. Based on this, the risk from erucic acid in fish was not separately included in the present benefit and risk assessment.

17.1.11 3-MCPD esters, glycidyl esters and glycidol

3-MCPD, 3-MCPD esters, glycidyl esters and glycidol are contaminants that can be formed during food processing, e.g. fermentation and/or oil refining, and are therefore not relevant for the present assessment (EFSA 2016, EFSA 2018). 3-MCPD esters, glycidyl esters and glycidol can be present in refined fish oil, but this is not part of the risk-benefit assessment.

Evaluation: These compounds are not of concern in relation to fish intake and the answer to question 1 is 'no', and these substances were not considered further in the present assessment.

17.1.12 Siloxanes

Siloxanes is a group of chemicals with widespread use in the pharmaceutical, medical, cosmetic and food industries (Mojsiewicz-Pienkowska and Krenczkowska, 2018). Siloxanes

are considered to be non-toxic (ECHA). However, for certain siloxane sub-groups, several publications challenge this view (Mojsiewicz-Pienkowska and Krenczkowska, 2018). For example, due to their predicted persistence and/or bioaccumulation, cyclic volatile methylsiloxanes (cVMS) were identified as potential emerging contaminants of concern (Howard et al, 2010). Recently, based on persistent, bioaccumulative and toxic properties, octamethylcyclotetrasiloxane (D4), decamethylcyclopentasiloxane (D5) and dodecamethylcyclohexatetra-siloxane (D6) were classified as REACH substances of very high concern (https://chemycal.com/news/a52776ad-59a6-490c-9f2e-a9d49557e2e3/ECHA_Siloxanes_D4_D5_and_D6_Classified_as_SVHC). Both human toxicity data and data on concentration of these substances in fish and seafood is limited (VKM, 2019; Lille-Langøy et al., 2015). In Norway, levels D4, D5 and D6 were analyzed in the food web of Lake Mjøsa (Borgå et al., 2013) in Atlantic cod (Warner et al., 2014) and the Arctic environment (Magali et al., 2016). Large variations in concentrations were observed between the different studies performed, albeit higher concentrations usually were correlated with proximity of anthropogenic point sources (Magali et al., 2016). In 2019, VKM evaluated the cVMS for priority setting of monitoring efforts in foods, drinks and dietary supplements; for D4, D5 and D6, fish and seafood were identified as possible sources of exposure (VKM, 2019). Below, a brief summary of main findings concerning toxicity and occurrence of D4, D5, and D6 in seafood and fish is provided; a more comprehensive account is provided in the report (VKM, 2019).

Data on human toxicity of D4, D5, and D6 is limited. Margin of safety (MOS) values derived from Monte Carlo (MC) simulations for most groups exposed to D4 were higher than 60,000; when modeling exposure through ingestion of food containing D4, MOS were expected to be higher than 1 000 000 (Gentry et al, 2017). Only limited information of D4 concentrations in food is available. Based on a MC simulation, ingestion of fish, root crops or ingestion of food containing residual antifoam, and indoor air exposure resulted in the greatest intake; in subsistence fishermen the 90th percentile of oral D4 intake was approximately 0.009 mg/kg bw/day (Gentry et al., 2017). For D5, MOS values derived from MC simulation studies were above 15 000 000 for the general public and fishermen (Franzen et al., 2016). Information on concentrations of D5 in fish is limited. MC analysis indicated that consumer product use resulted in much greater exposure than that occurring through exposure through ingestion. According to MC simulations, the 90th percentile of oral intake to D5 is 0.011 mg/kg/day for males in the general public or subsistence fisherman 20 to 59 years of age (Franzen et al., 2016). For the general Population in Canada for D6, a MOE of 40 000 was reported (EHC, 2008). Data on levels of D6 in the environment are scarce and little information on exposure in humans is available; the most significant contribution to daily intake is inhalation of indoor air (Danish EPA, 2014). The persistence and lack of human toxicity data for the cVMS D4, D5, and D6 is a cause for concern and warrants further research (Mojsiewicz-Pienkowska and Krenczkowska, 2018).

Evaluation: The general population is predominantly exposed to siloxanes from the use of consumer products (EHC, 2008) and levels of the occurrence data of cVMS in seafood and fish seem low by comparison; the answer to question 1 therefore is 'no'. However, lack of occurrence data in fish and seafood are data gaps which need to be addressed to allow for

more comprehensive future risk assessments of these substances. Based on this, siloxanes are not separately included in the present benefit and risk assessment.

17.1.13 Phthalates

Phthalates are added to plastics during manufacture, and may during use, disposal or recycling leach into the environment and biota where they can persist degradation and bioaccumulate (Salvaggio et al., 2019). In commercial fish species (*Lepidopus caudatus*) in the Mediterranean, relatively high concentrations of di-isononyl phthalate (DIDP), bis(2-ethylhexyl)phthalate (DEHP), butyl-benzyl-phthalate (BBP), di-butylphthalate (DBP), and monobutyl phthalate (MBP) were detected in liver and intestine of fish and trace amounts were measured in muscle tissue (i.e. fillet) (Salvaggio et al., 2019). In 2005, EFSA issued five separate opinions (EFSA, 2005a; b; c; d; e) on a group of phthalates listed and authorised in the positive list in Annex 614 I (Table 1) of Regulation (EC) No 10/20117 on plastic materials and articles intended to come into contact with food namely, di-butylphthalate (DBP), butyl-benzyl-phthalate (BBP), bis(2-ethylhexyl)phthalate (DEHP), di-isodecyl phthalate (DINP) and di-isononyl phthalate (DIDP). After having reviewed these phthalates individually, EFSA published an additional statement regarding the possibility of allocating a group TDI for those five phthalates (EFSA, 2005f). An evaluation of phthalates performed by VKM in Norway highlighted data gaps for all five phthalates (i.e. for DBP, BBP, DEHP, DINP, and DIDP) (VKM, 2019). Based on a study performed in Norwegian foods (Sakhi, 2014), VKM summarized dietary phthalate exposure (ng/kg bw/day) for the Norwegian adult population as follows: DBP (30), BBP (18), DEHP (384), DINP (402), and DIDP (33). In the Norwegian adult population, grain and meat products were found to be the major contributors of exposure; estimated dietary exposures to these chemicals were considerably lower than their respective TDI values established by EFSA in 2005 (Sakhi, 2014). At the end of 2019, EFSA published an updated opinion on phthalates in which the re-evaluation of DBP, BBP, DEHP, DINP and DIDP was presented (EFSA, 2019). In the updated opinion, the Panel on Food Contact Materials, Enzymes and Processing Aids (CEP Panel) derived the same critical effects and individual tolerable daily intakes (TDIs) (mg/kg bw/day) as in 2005 (EFSA, 2005f), i.e. reproductive effects for DBP (0.01), BBP (0.5), DEHP (0.05), and liver effects for DINP and DIDP (0.15 each). Based on aggregated dietary exposure estimates (mean and high (P95)), which were obtained by combining consumption data from the EFSA Comprehensive Database with occurrence data in literature, exposure for DBP, BBP, DEHP and DINP was estimated to be 0.9–7.2 and 1.6–11.7 µg/kg bw/day for mean and high consumers, respectively. In other words, in the worst-case exposure scenario calculation, a contribution of up to 23% of the group-TDI is achieved. For DIDP, which was not included in the group-TDI, estimated dietary exposure was always determined to be below 0.1 lg/kg bw/day; i.e. far below the TDI of 150 lg/kg. The CEP Panel acknowledged in its report that due to the large amount of new emerging evidence for DBP, BBP, and DEHP more sensitive endpoints compared to their effects on reproductive toxicity (and liver toxicity for DINP and DIDP) may exist; the panel therefore considered "*that the current assessment of the five phthalates, individually and collectively, should be on a temporary basis*" (EFSA, 2019).

Evaluation: Fish may be a source of phthalates, but concentration in fish fillets seems to be low. The answer to question 1 and 2 is therefore a tentative 'no'. As only limited data on occurrence in fish is available a data gap is highlighted. Phthalates are not separately included in the present benefit and risk assessment.

17.1.14 Bisphenols

Bisphenols such as bisphenol A (BPA), bisphenol B (BPB), bisphenol F (BPF), and other related bisphenols are chemicals mainly used in the manufacturing of plastics, resins, dental sealants, adhesives, thermal paper, etc. For example, BPA is used in polycarbonate plastics used to make food and drink containers. BPA is also used to make protective epoxy resin coatings for food and beverage cans and vats. BPA is ubiquitous within the environment (Repossi et al., 2016; Wells, 2019). BPA is an endocrine disrupting compound acting among other mechanisms through several nuclear receptors (Gore et al., 2015; Lille-Langøy et al., 2015). Due to its observed migration into foodstuffs, and increased concern over its toxicity and endocrine disrupting properties, other bisphenols have started to replace BPA in the manufacturing of plastics, resins, and other products. These replacement compounds have shown to varying extents to have similar toxicities to BPA (Wells, 2019).

According to EFSA (2013) diet is the main source of BPA exposure, with canned food and non-canned meat and meat products as major contributors. While canned food can be contaminated through contact migration. Limited data exist on the occurrence of bisphenols in fish. In a literature review by Repossi et al. (2016), canned seafood was found to be generally more contaminated with BPA than non-canned seafood (46.2 µg/kg vs. 14.9 µg/kg). However, higher BPA concentrations were reported in some fish species from Europe, such as flounder, cod and herring (100-430 µg/kg), and canned tuna was reported with higher levels of BPA than other canned fish. However, no data were presented on non-canned tuna. In its 2015 report, EFSA (2015) concluded that BPA does not pose any risk for consumer health at the current exposure levels, but nevertheless reduced the TDI levels from 50 to 4 µg/kg bw/day. This TDI was made temporary, and EFSA committed to reevaluate BPA toxicity again, with an updated assessment scheduled for autumn 2022. Akhbarizadeh et al. (2020) analyzed several bisphenols in fish from the Persian Gulf, finding highest levels of BPA and BPB in several fish species, especially at the higher trophic levels. Based on a calculation deriving average daily intake of BPA, and also average daily intake of estrogenic equivalents from four bisphenols (BPA, BPB, BPF, BPAF), they concluded that the hazard ratio of bisphenols intake via seafood consumption was low compared to the temporary TDI of 4 µg/kg bw/day.

Evaluation: Fish is a source of bisphenols, but concentration in fish fillets is low in relation to the existing temporary TDI for BPA. The answer to question 1 and 2 is therefore a tentative 'no'. However, a new assessment of bisphenol A toxicity is underway from EFSA. Pending the new risk assessment of BPA from EFSA, the risk of bisphenol exposure from fish was not separately included in the present benefit and risk assessment.

17.2 Original list of suggested compounds

Group	Compound name
Brominated flame retardants	ΣPBDEs, including DecaBDE
Brominated flame retardants	1,2-Bis(2,4,6-tribromophenoxy)ethane
Brominated flame retardants	DBDPE
Brominated flame retardants	HBB
Brominated flame retardants	HBCDD
Brominated flame retardants	2,4,6-Tribromophenol (TBP)
Pesticides	Chlorpyrifos
Pesticides	Phosphoric acid-phosphates
Pesticides	HCB
Pesticides	HCH
Pesticides	<i>trans</i> -Nonachlor
Pesticides	DDT

Pesticides	DDE
Mycotoxins	Aflatoxin
Mycotoxins	Beauvericin
Mycotoxins	Enniatin B
Perfluorinated compounds	PFOS
Perfluorinated compounds	PFOA
Perfluorinated compounds	PFHxS
Perfluorinated compounds	PFNA
Perfluorinated compounds	PFDA
Perfluorinated compounds	PFUnDA
Perfluorinated compounds	PFHpS
Dioxins and dl-PCBs; group of compounds	
non-dioxin-like PCBs; group of compounds	Σ PCB6
non-dioxin-like PCBs; group of compounds	PCB153

non-dioxin-like PCBs; group of compounds	PCB138
Phthalates; group of compounds	
Glycidyl fatty acid esters (GEs)	Glycidyl fatty acid esters (GEs)
MeHg	MeHg
Antioxidants	BHT (butylhydroxytoluene)
Antioxidants	Ethoxyquin (EQ)
Erucic acid	Erucic acid

Total arsenic

Total arsenic

Inorganic arsenic	Inorganic arsenic
Organic arsenic	Organic arsenic
Siloxanes	Siloxan D4
Siloxanes	Siloxan D5
Siloxanes	Siloxan D6
Bisphenols	Bisphenol A
Bisphenols	Bisphenol G

Bisphenols	Bisphenol TMC
Bisphenols	Bisphenol F

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18 Appendix V Deviations from protocol

18.1 Literature searches

Initially a search for systematic reviews was performed, as described in the protocol, to check if any systematic reviews were thorough enough to replace our own search. Based on this, none of these publications met the criteria, and a new search for primary studies was performed. However, the systematic reviews and meta-analyses from this search were later screened and quality assessed as described in Chapter 3 and included in the Weight of Evidence process.

18.2 Inclusion and exclusion of contaminants

The flow chart presented in Figure 3.2-1 in the protocol, describing the process for inclusion or exclusion of candidate contaminants, is slightly modified, and the process was modified accordingly. A new version of the flow chart is presented in Figure 2.3.1-1 in the assessment. Additional risk assessments or scoping reviews was not feasible within this project.

18.3 Quality assessment tool for systematic reviews and meta-analyses

For quality assessment of systematic reviews and meta-analyses the Amstar 1 tool is used instead of Amstar 2 as described in the protocol. Amstar 2 was used for reference to aid interpretation of questions in Amstar 1.

19 Appendix VI: Technical details for the intake estimates

19.1 Data imputation in dietary surveys

19.1.1 Adults

The following background variables had missing values and were imputed: Sex, Age, Weight, Height, Education, Region, household size and the number of children in the household. While the two household variables and height are not directly used in the present assessment, their inclusion may improve imputation accuracy for the variables of interest. There were 1, 1, 32, 2, 6, 1, 11, and 4 missing observations, respectively.

Imputations were implemented in R using `mice` package v.3.13.0, setting the seed for imputation to 123 (reported for complete replicability). The number of iterations was set to 150.

In imputation, the variables were used as predictors for each other (without intercepts). The models used for imputation were *logreg*, *pmm*, *pmm*, *pmm*, *polr*, *polyreg*, *polr*, and *polr*, respectively. “logreg” stands for Logistic regression, “pmm”—for Predictive mean matching, “polr”—for Proportional odds model, and “polyreg”—for Polytomous logistic regression.

19.1.2 1-, 2-, 4-, 9-, and 13-year-olds

The following variables had missing values and were imputed: Weight, education, and region. There were 607, 25, and 2 missing observations, respectively. Of missing weight observations, 1-, 2-, 4-, 9-, and 13-year-olds contribute 155, 380, 17, 29, and 26, respectively. The same parameters and approach were used as for adults. The models used for imputation were *pmm*, *polr*, and *polyreg*, respectively.

19.2 Nutritional databases

Intake of nutrients and exposure to contaminants at the individual level is based on two categories of information: survey responses and information on concentration of nutrients/contaminants in the coded foods and drinks that have been consumed. In some cases, a code refers to an elementary ingredient, e.g., a tomato. In other cases, a code refers to processed food with multiple ingredients, e.g., ketchup or a ready-to-eat tomato soup.

There are three available food-composition databases, from newest to oldest: AE-18, AE-14, and N3. Each subsequent database includes updates to product formulations. Using the most

recent database is thus desirable. However, more recent databases do not cover all foods of earlier surveys.

A hybrid food-composition database is adopted. It is a superset of all three food-composition databases, where the most recent available data is selected for each food code. This ensures the broadest coverage of reported food intake along with the most recent food-composition data available.

Note, that the N3 food-composition database covers a shorter list of nutrients. Notably, it contains no information on LC n-3 FA and iodine. For individuals with intake of foods not covered in AE-14/18, the corresponding values are underestimated. The N3 food-composition database covers vitamin D, vitamin B12, and selenium. Spedkost 3 and Småkost 3 are structured to follow AE-14, while Ungkost 3 is structured to follow AE-18, so there are no problems of missing LC n-3 FA or iodine for 1-, 2-, 4-, 9-, and 13-year-olds.

For the composite products that had a recipe in the food composition database, ingredient amounts were calculated based on the list of ingredients in the recipe and the nutrient content of the product and its ingredients. If the recipe ingredient list did not allow to find product weights that would give a good correspondence between the composite product and the ingredients, as measured by the nutritional content, the recipe was rejected, and the product remained as a composite product. Otherwise, the optimal ingredient weights, the weights with the best correspondence between the composite product and the ingredients, were used to translate the composite product into its ingredients. The exception was made for fish composite products. Even when the recipe was otherwise deemed to be of insufficient quality, the fish fillet was extracted from the dish using the best fitting value match between the nutrient values of the product and the nutrient values of the fish. Splitting composite dishes into ingredients, whenever possible, allows a tighter match between dietary intake and occurrence data, as well as better attribution of sources of nutrients and contaminants grouped by food categories.

19.3 Technical details for mixed models

There are some further technical ingredients in mixed models beyond those that are introduced in Chapter 7. In implementing the MM approach for the current assessment, the MCMCglmm v.2.32 library in R v.4.1.0 was used, a package for fitting generalised linear mixed models using Markov chain Monte Carlo techniques. While not strictly necessary, it is recommended to specify priors. Priors set original values used in the variance-covariance matrices of the random effects and residuals.

Four alternative priors were considered. The code used to specify the priors is as follows:

```
priors<-list(
  list(R = list(R1 = list(V = diag(2)/10, nu=3)),
    G = list(G1 = list(V = diag(2)/10, nu=3))),
  list(R = list(R1 = list(V = diag(2)/1, nu=1)),
    G = list(G1 = list(V = diag(2)/1, nu=1))),
```

```
list(R = list(R1 = list(V = diag(2), nu=1.002)),
      G = list(G1 = list(V = diag(2), nu=2,alpha.mu=c(0,0),
                        alpha.V=diag(2)*a))),
list(R = list(R1 = list(V = diag(2)*1e-6, nu=3)),
      G = list(G1 = list(V = diag(2), nu=2,alpha.mu=c(0,0),
                        alpha.V=diag(2)*a))))
```

Model estimates showed little dependence on the priors. The first prior gave marginally better fit (as measured by the DIC measure), so it is the model results under the first prior that are reported in the MM tables, in all cases.

The model was estimated after setting the seed to 1234 (set and reported for reproducibility). For adults the model was run as follows:

```
MCMCglmm(
  as.formula(paste0(transformed.nutr.cols.MM[i],
    "~Sex + Age + Education.High+Landsdel+Weekday+Month")),
  prior = p,
  random = ~idh(Sex):BB.ID, rcov = ~idh(Sex):units,
  data = df[grepl("^N",BB.ID) & Month!=0,],
  nitt=35000, thin=10, burnin=5000)
```

where *BB.ID* is the respondent identifier, *random* specifies the random component, and *rcov* is the residual component introduced in Chapter 7.5.4.1.

For minors (4-, 9-, and 13-year-olds), the model was run as follows:

```
MCMCglmm(
  as.formula(paste0(transformed.nutr.cols.MM[i],
    "~Sex + Education.High+Landsdel+Weekday")),
  prior = p,
  random = ~idh(Sex):BB.ID, rcov = ~idh(Sex):units,
  data = df[Age==a,], nitt=35000, thin=10, burnin=5000)
```

For nutrients affected by supplementation the corresponding codes were

```
MCMCglmm(as.formula(
  paste0(transformed.nutr.cols.MM.S[i],
    "~Sex + Age + Education.High+Landsdel+Weekday+Month+",
    gsub(".Scenario.[12]", "", nutr.cols.MM.S[i]), ".Supplement.Dummy")),
  prior = p,
  random = ~idh(Sex):BB.ID, rcov = ~idh(Sex):units,
  data = df[grepl("^N",BB.ID),], nitt=35000, thin=10, burnin=5000))
```

and

```
MCMCglmm(as.formula(
  paste0(transformed.nutr.cols.MM.S[i],
    "~Sex + Education.High+Landsdel+Weekday+", gsub(".Scenario.[12]", "",
    nutr.cols.MM.S[i]), ".Supplement.Dummy")),
  prior = p,
```

```
random = ~idh(Sex):BB.ID, rcov = ~idh(Sex):units,
data = df[Age==a,], nitt=35000, thin=10, burnin=5000))
```

for adults and minors, respectively.

The next step is to generate a table comprising fixed-effect structures for each survey participant for one year:

```
simulation.data<-copy(df)[,.(BB.ID,Sex,Age,Weight,Education.High,Landsdel)]
simulation.data[,Sex:=as.factor(Sex)][,Landsdel:=as.factor(Landsdel)][,Education.High:=as.factor(Education.High)]
simulation.data<-simulation.data[rep(1:N,each=365),][,Date:=seq(as.Date("2021-01-01"),by=1,length.out=365),by="BB.ID"]
simulation.data[,Weekday:=relevel(as.factor(format(Date,"%u")),ref = 1)][,Month:=as.factor(as.integer(format(Date,"%m")))]
simulation.data[,Date:=NULL]
```

Note, in the simulations, the days are set from Monday to Sunday, from January 1 through December 31.

This fixed-effect table along with the previously estimated model results (*y* below) are combined to generate simulated results:

```
simulate(object=y, nsim = 100, seed = 1234L,
newdata=(simulation.data[grep("^N",BB.ID)][,
c("BB.ID",strsplit(as.character(y$Fixed$formula)[3]," + ",fixed = TRUE)
[[1]])],with=FALSE)[,
(gsub("log\\(([^\\)]*)\\).*$", "\\1",y$Fixed$formula[2])):=0]),
type = "response", it=NULL, posterior = "all", verbose=TRUE)
```

Note, 100 vectors of the left-hand-side variable (LHS) are simulated, 100 years' worth of data for each survey participant.

To get back to the original scale of the LHS, the transformation is reversed. The result is merged with the respondent IDs, for example, for the log transformation:

```
data.table(data.frame(
BB.ID=simulation.data[grep(paste0("^N"),BB.ID)][,BB.ID],
Prior=p, V1=exp(sim.try.A[[c]][[p]]))
```

Each year of data is then converted to daily averages for that year:

```
sim.A.yr<-lapply(1:length(sim.try.A), function(c) lapply(1:length(priors),function(p) unique(copy(sim.try.A[[c]][[p]])),
(setdiff(names(sim.try.A[[c]][[p]]),c("BB.ID","Prior")):=lapply(.SD,function(x) sum(x)/365),
by=c("BB.ID","Prior"),.SDcols=setdiff(names(sim.try.A[[c]][[p]]),c("BB.ID","Prior")))))
```

Thus, there are 100 annual averages for each respondent.

As an indication of estimate reliability at the level of each participant, the mean and the standard deviation of the annual averages were computed. It was done for each person, each compound, and each prior. The average mean and average standard deviation across all participants, for the chosen prior, is then considered. For compounds where this average standard deviation is high (relative to the average mean), it can be concluded that the probabilistic nature of the model structure adds more uncertainty to the estimate, by forcing an ill-suited distributional assumption, than it takes away, by modelling the within-person day-to-day variation (something that reduces long-term estimate variability). In the present assessment, after adopting the Box-Cox transformation, all estimated distributions exhibited improved statistical properties compared to the OIM-based distributions: in particular, the MM-based distributions had thinner tails.

Having 100 observations per participant is equivalent to having 100 cross-sections. The starting point of the analysis is one observed cross-section, the survey. The result is 100 simulated cross-sections based on the original data and the model structure. From each cross-section arises one distribution and corresponding percentiles of interest. The distribution is weighted using demographic weights. Calendar weights are unnecessary, as the simulated data are annual averages. All percentiles of interest are averaged and reported in the tables. Furthermore, for each percentile, the 5th lowest and the 5th highest values are taken. As there are 100 values altogether, the two values correspond to the 5th and 95th percentile, or 90% confidence interval, for the considered percentile.

19.3.1 Variable transformations and their reversals

The dependent variable (the modelled compound) was transformed to have a model specification where the fitted residuals are closer to normality. In addition, a desired property of a transformation function (in the relevant case of modelling a non-negative exposure) is to have a model where the simulated values are also non-negative. There are two commonly used transformation functions, the log transformation and the Box-Cox transformation. The former is easier to work with, but inflexible. The latter has a λ parameter that allows to adapt the transformation to the data. Note, that the Box-Cox transformation with $\lambda = 0$ is equivalent to the log transformation. Due to its flexibility, the Box-Cox transformation can produce significantly better results for some compounds. The Box-Cox transformation has been used in other studies of exposure. For example, SPADE: Statistical Program to Assess habitual Dietary Exposure developed by the RIVM, while adopting a different MM approach to their estimations, incorporates the Box-Cox transformation as the first step of the modelling procedure (Dekkers et al., 2014).

Some distributions cannot be transformed in such a way that residuals become approximately normal. For example, distributions with a sizeable probability mass on zero will have a strong deviation from normality even after transformation. For them, the chosen model approach cannot be used. This is the reason why MMs were not used to assess mean contribution from food groups or food items. In contrast, all considered measures for total intakes/exposures except for methyl mercury were well-suited for Box-Cox transformation. The effect of transforming the modelled exposure is illustrated using the example of Q-Q

plots for residuals of PCDD/Fs and DL-PCBs exposure (without fruits, vegetables, and potatoes), under LB for the VKM dataset for adults. Q-Q plots present quantiles of residuals versus quantiles of a normal distribution. If residuals are normal, all residuals will be on the diagonal line, where quantiles for residuals are equal to quantiles of the normal distribution. In Figure 19.3.1-1 below, the dashed lines represent the best fitting lines to the observed quantile combinations. The line for untransformed exposure significantly diverges from the diagonal, while the residual values themselves exhibit significant deviation from the straight line. The residuals for the transformed exposure clearly have much better alignment with the diagonal, and, thus, greatly diminished divergence from normality.

Q-Q Plots: Residual Normality for Dioxin Exposure of Adults

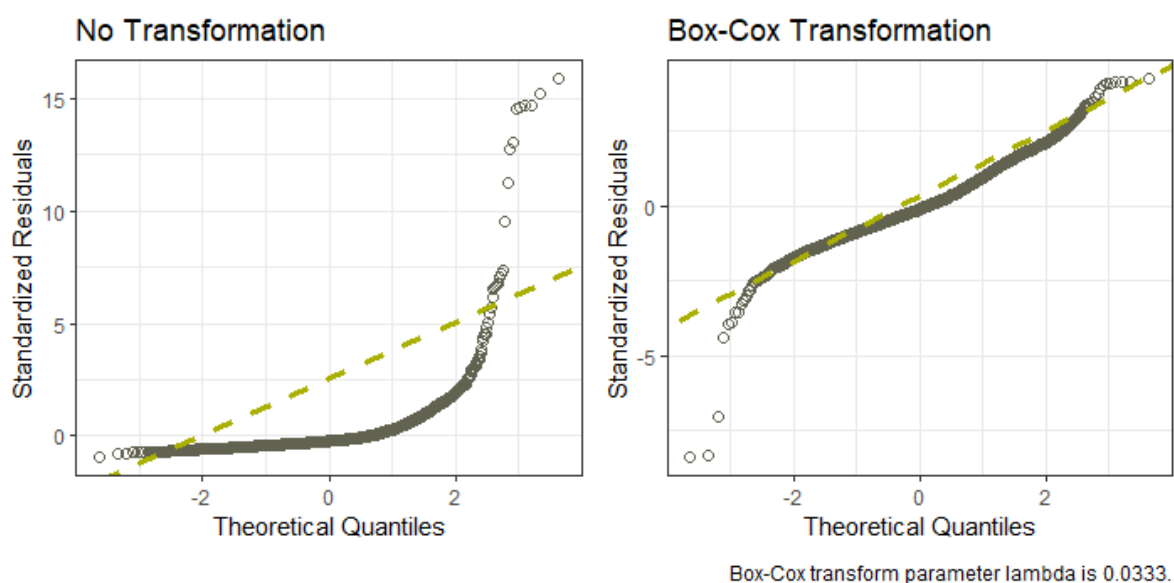


Figure 19.3.1-1. Q-Q plots for total PCDD/Fs and DL-PCBs exposure residuals (excluding fruits, vegetables, and potatoes), LB VKM dataset, for Norkost 3. The left subplot is for raw, untransformed exposure. The right subplot is for Box-Cox-transformed exposure using the optimal lambda found as per procedure in Chapter 19.3.2. In both cases, the dashed lines represent the best fitting lines to the residuals.

The Box-Cox transformation, $z = (y^\lambda - 1)/\lambda$, can be performed for all non-negative values. All exposure values are non-negative, and, thus, the transform is defined for all values in the database. After the transformed values are modelled and simulated, the transformation has to be reversed before the estimated distribution can be reported. The reversal is performed as follows: $y = (z\lambda + 1)^{1/\lambda}$. The reverse transformation is only defined for such z that $z\lambda + 1 \geq 0$ (assuming $1/\lambda$ is non-integer). For $\lambda > 0$, when simulated z s are lower, the function is undefined. Such values of the simulated transformed daily exposure are mapped back to zero. For $\lambda < 0$, when simulated z s are higher, the function is undefined, and the resulting daily exposures in that range are set to two times the maximum observed daily value. Additionally, the resulting values above double the maximum observed daily value are also set to two times the maximum observed daily value. This is a quite conservative approach

that affects only a very small share of simulations. The choice of the upper bound has no effect on any percentiles of interest, but it does preserve interpretability of the mean values.

The log transformation, $z = \log(y)$ is defined for all positive values. Zero values of the reported exposure are shifted up by a small amount away from zero prior to transformation. The corresponding reverse transformation, $y = \exp(z)$, is defined for all real values.

19.3.2 Finding the optimal λ

The standard Box-Cox transformation is of the form $(y^\lambda - 1)/\lambda$. The regression, for example, of the form

$$\begin{aligned} & (Compound_i^\lambda - 1)/\lambda \\ &= \alpha + \beta_1 Sex + \beta_2 Age + \beta_3 Education.High + \beta_4 Landsdel + \beta_5 Weekday \\ &+ \beta_6 Month + \beta_7 Compound.S.Dummy + \epsilon_i \end{aligned}$$

is repeatedly run using the maximum-likelihood estimation, for different values of λ . The process is implemented using the `boxcox` function of MASS package v.7.3.54 in R. λ associated with the highest log-likelihood is selected and utilized in the corresponding mixed model.

20 Appendix VII WHO TEF-values

Table 20-1 Toxic equivalency factors established by WHO in 2005 (van den Berg et al., 2006; see reference list in Chapter 6).

Congener	WHO2005-TEFs
PCDDs	
2,3,7,8-TCDD	1
1,2,3,7,8-PeCDD	1
1,2,3,4,7,8-HxCDD	0.1
1,2,3,6,7,8-HxCDD	0.1
1,2,3,7,8,9-HxCDD	0.1
1,2,3,4,6,7,8-HpCDD	0.01
1,2,3,4,6,7,8,9-OCDD	0.0003
PCDFs	
2,3,7,8-TCDF	0.1
1,2,3,7,8-PeCDF	0.03
2,3,4,7,8-PeCDF	0.3
1,2,3,4,7,8-HxCDF	0.1
1,2,3,6,7,8-HxCDF	0.1
2,3,4,6,7,8-HxCDF	0.1
1,2,3,7,8,9-HxCDF	0.1
1,2,3,4,6,7,8-HpCDF	0.01
1,2,3,4,7,8,9-HpCDF	0.01
1,2,3,4,6,7,8,9-OCDF	0.0003
Non- <i>ortho</i> PCBs	
PCB-77	0.0001
PCB-81	0.0003
PCB-126	0.1
PCB-169	0.03
Mono- <i>ortho</i> PCBs	
PCB-105	0.00003
PCB-114	0.00003
PCB-118	0.00003
PCB-123	0.00003
PCB-156	0.00003
PCB-157	0.00003
PCB-167	0.00003
PCB-189	0.00003

21 Appendix VIII Previous assessments

21.1 VKM 2006

In 2006, the Scientific Steering Committee of the VKM published "A comprehensive assessment of fish and other seafood in the Norwegian diet" (VKM, 2006). In this assessment, Norwegian dietary data was used to estimate the intake of nutrients and contaminants by children and adults when they consumed fish and other seafood. Relevant Norwegian data on nutritional significance, toxicology and hygienic factors was used, as was international assessments and scientific literature.

In the assessment from 2006, VKM found that the consumption of fish, lean or fatty, has a beneficial effect on health. Even though there was no widely accepted method of conducting a quantitative risk-benefit comparison at the time, VKM stated that an integration of the nutritional and toxicological assessments would clearly show that Norwegians in general should eat more fish and that fish consumption should include both lean and fatty fish. It was evident that the adult population, especially the group with the highest risk of developing cardio-vascular disease, will gain the greatest health-related benefits from increasing their consumption of fatty fish. VKM also stated that marine n-3 fatty acids are important for pregnancy and foetal development. Consumption of fish and other seafood was not shown to increase or reduce the risk of any common form of cancer.

The content of dioxins and dioxin-like PCBs in fatty fish was the only potential limiting factor for fish consumption. This is because eating fatty fish in an amount equivalent to more than two meals per week, with the levels of dioxins and dioxin-like PCBs present at that time, over time, would lead to the tolerable intake level being exceeded. Fertile women are particularly vulnerable but based on knowledge about young women's consumption of fatty fish, there was little reason to believe that a general recommendation to increase fish consumption would result in fertile women consuming so much fatty fish that the intake of dioxins and dioxin-like PCBs over a long period would exceed the tolerable intake (TWI) and consequently constitute a health risk for the foetus. Children may exceed the TWI, but for most children (2-13 years), foods other than fish was the predominant source of these contaminants.

Hence, in 2006 VKM supported the general Norwegian recommendation to eat more fish both for dinner and on sandwiches.

21.2 VKM 2014

In 2014, the Scientific Steering Committee of the VKM published an update of the benefit-risk assessment published by VKM in 2006 (VKM, 2014). The assessment consisted of three parts; a benefit assessment, a risk assessment, and a semi-quantitative benefit-risk assessment.

In line with the request from the NFSA, VKM did a reassessment of fish consumption in Norway with focus on some specific nutrients and contaminants. These nutrients were n-3 fatty acids, vitamin D and minerals iodine and selenium, and the contaminants were mercury, dioxins and dl-PCBs. VKM was asked to address changes in the use of raw materials in farmed fish feed, and how these changes affect levels of nutrients and contaminants in fish. VKM was also requested to consider benefits and risks of eating fish with regard to intake of nutrients and contaminants, and comment on whether this changed the conclusions from 2006.

Fish consumption was based on Småbarnskost 2007 and Norkost 3, as well as information for pregnant women who had answered the Norwegian Mother and Child Cohort study food frequency questionnaire. The fish consumption appeared to be unchanged from the 2006 assessment for all age groups. In 2014, lean and fatty fish contributed with about 60 and 40 percent, respectively, of the total fish consumption. This was similar to 2006.

The benefit characterisation of nutrients in the fish was based on recommended daily intakes (national or European). VKM concluded that the current (2014) intake of fish would give a contribution of EPA and DHA sufficient to reach recommended levels for adults and 2-year-olds. For pregnant women the European recommendations would be met, but the national recommendations only for DHA. For vitamin D, fish consumption contributed to approximately 20% of the national recommended intakes for adults, and less for pregnant women and 2-year-olds. For selenium and iodine, fish contributed with only low intakes.

In the risk characterisation, a rough comparison of contaminant concentrations between 2006 and 2014 indicated minor or no changes in concentrations of mercury, dioxins and dl-PCBs in wild fish species. However, in farmed Atlantic salmon concentrations had changed. Concentrations of dioxins and dl-PCBs and mercury had been reduced to about 30 and 50%, respectively, of the corresponding levels in 2006 (due to changes in the feed).

Briefly, after VKM compared the benefits and the risks of eating fish and fish products they concluded that “the benefits clearly outweigh the negligible risk presented by current levels of contaminants and other undesirable substances in fish”. They stated that adults, including pregnant women, may miss the beneficial effects if they consume less than one serving of fish per week. The beneficial effects were on cardiovascular diseases, cardiac mortality, and optimal neurodevelopment (VKM, 2014).

21.3 EFSA Risk benefit 2014

In 2014, the EFSA Panel on Dietetic Products, Nutrition, and Allergies (the NDA Panel) addressed the risks and benefits of seafood (fish and shellfish) consumption with regards to the intake of nutrients and exposure to methylmercury (EFSA, 2014). The NDA Panel evaluated the health benefits of seafood consumption and concluded that the consumption of one to two servings of seafood per week, and up three to four servings per week during pregnancy, was associated with better neurodevelopmental outcomes in children compared to no consumption of seafood. In adults, similar weekly serving sizes were associated with a

lower risk of coronary heart disease mortality. The NDA Panel noted that these associations refer to the consumption of seafood as such, i.e. they include beneficial effects of nutrients and adverse effects of contaminants (e.g. methylmercury).

21.4 EFSA Risk benefit 2015

Following the EFSA Scientific Opinions of 2012 and 2014, the EFSA Scientific Committee (SC) published, in 2015, a Scientific Opinion on the benefits of fish/seafood consumption compared to the risks of methylmercury in fish/seafood (EFSA, 2015). Considering the TWI for methylmercury (EFSA, 2012) and the dietary reference value (DRV) for n-3 long chained polyunsaturated fatty acids (LCPUFA) (EFSA, 2010) the SC used scenarios to calculate the number of servings per week needed to reach the TWI and DRV, respectively. If fish species with high concentrations of methylmercury was consumed the TWI for mercury was reached before the DRV for n-3 LCPUFA. The TWI was reached when consuming one to two servings of fish species with a high mercury content per week. The SC concluded that the consumption of fish species with a high concentration of methylmercury should be limited. They stated that due to regional variation in the type of fish consumed, it is not possible to give a general recommendation for the European population on the consumption of fish. Each country needs to assess benefits and risks associated with consumption of fish considering national consumption patterns.

21.5 VKM 2019

In 2019, VKM published an opinion on "Scenario calculations of mercury exposure from fish and overview of species with high mercury concentrations" (VKM, 2019). VKM was requested by the NFSA to evaluate human exposure to mercury from fish, with focus on fish with elevated mercury concentrations and vulnerable groups of the population.

For the assessment, VKM received from NFSA 26 361 measurements of total mercury in 36 different species from 305 locations (in Norway). VKM chose a six-by-four set of scenarios for the estimation of exposure to methylmercury from fish consumption. The six-by-four matrices were based on six levels of consumption (ranging from 150 to 1000 g fish per week) and four different compositions of the diet (varying from a diet consisting of only fish with low mercury concentrations to a diet of only fish with high concentrations). The scenario exposures to mercury were compared with the TWI. VKM used inverse modelling to estimate the concentration of mercury in fish leading to an exposure reaching the TWI given different compositions of fish in the diet and number of portions of fish consumed. The estimated concentrations were compared to the estimated mean or 95-percentile concentrations of mercury in fish. When eating three weekly portions of fish consisting of only fish with an assumed high concentration of mercury, the fish can contain up to 0.28 mg/kg ww before the TWI is reached. Three portions of fish per week is in line with the current upper recommendations of fish consumption from the Norwegian Directorate of Health.

Further, VKM identified species with estimated mean and 95-percentile concentrations of mercury above 0.28 mg/kg ww. Species with an estimated mercury concentration above 0.28 mg/kg at the 95-percentile were the marine species Atlantic cod, tusk, blue ling, common ling, rosefish, European hake, and Atlantic halibut, and the freshwater species burbot, brown trout, Northern pike, European perch, and Arctic charr. Atlantic cod is a species commonly caught by recreational fishing. The estimated mercury concentration in Atlantic cod rarely exceeds 0.28 mg/kg ww, i.e. the estimated mean concentration and the estimated 95-percentile concentration are 0.12 and 0.33 mg/kg ww, respectively. The concentration of mercury increased with fish length in several species. This was in particular evident for Atlantic cod, tusk, haddock, saithe, Atlantic halibut and brown trout.

21.6 References

EFSA (2012) Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food. EFSA Journal 10:2985. DOI: 10.2903/j.efsa.2012.2985.

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VKM (2006) Alexander J., Frøyland L., Hemre G-O., Jacobsen B.K., Lund E., Meltzer H.M and Skåre J.U. A comprehensive assessment of fish and other seafood in the Norwegian Diet. Norwegian Scientific Committee for Food Safety (VKM), Oslo, Norway.

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VKM (2019) Amlund H., Rakkestad K.E., Ruus A., Starrfelt J., Beyer J., Brantsæter A.L., Bremer S., Eriksen G.S., Mariussen E., Samdal I.A., Thomsen C. and Knutsen H.K. (2019) Scenario calculations of mercury exposure from fish and overview of species with high mercury concentrations. Opinion of the Panel on Contaminants of the Norwegian Scientific Committee for Food and Environment. VKM report 2019:3, ISBN: 978-82-8259-319-9, ISSN: 2535-4019. Norwegian Scientific Committee for Food and Environment (VKM), Oslo, Norway.

22 Appendix IX PFAS exposure

22.1 Lower bound contribution from food groups to single PFASs based on the EFSA dataset

Table 22.1-1 Dietary exposure from different food groups (ng/kg bw per week) in adults (18-70 years. n=1787). to PFOS, PFOA, PFNA and PFHxS and the sum of these four PFASs (lower bound values, unweighted OIM, based on EFSA dataset).

	PFHxS	PFNA	PFOA	PFOS	Sum of PFASs
Fish	0.01	0.05	0.37	2.37	2.79
Shellfish	0.01	0.02	0.02	0.26	0.32
Meat	0.01	0.01	0.31	0.81	1.14
Dairy	0.00	0.00	0.03	0.03	0.07
Eggs	0.00	0.00	0.28	0.71	0.99
Grain	0.00	0.00	0.00	0.00	0.00
Fruit Veg Potato	0.36	0.19	0.26	0.50	1.31
Drinking water	0.32	0.01	0.23	0.11	0.67
Other*	0.07	0.00	0.14	0.01	0.23

*Other = other food with detected levels (e.g. fat, sugar, condiments)

Table 22.1-2 Dietary exposure from different food groups (ng/kg bw per week) in women (18-45 years. n=466) to PFOS, PFOA, PFNA and PFHxS and the sum of these four PFASs (lower bound values, unweighted OIM, based on EFSA dataset).

	PFHxS	PFNA	PFOA	PFOS	Sum of PFASs
Fish	0.00	0.03	0.34	1.56	1.93
Shellfish	0.00	0.01	0.01	0.20	0.22
Meat	0.00	0.01	0.24	0.61	0.86
Dairy	0.00	0.00	0.03	0.03	0.07
Eggs	0.00	0.00	0.25	0.63	0.89
Grain	0.00	0.00	0.00	0.00	0.00
Fruit Veg Potato	0.37	0.19	0.27	0.51	1.35
Drinking water	0.36	0.02	0.26	0.12	0.77
Other*	0.05	0.00	0.10	0.01	0.17

*Other = other food with detected levels (e.g. fat, sugar, condiments)

Table 22.1-3 Dietary exposure from different food groups (ng/kg bw per week) in 13-year-olds (n=687) to PFOS, PFOA, PFNA and PFHxS and the sum of these four PFASs (lower bound values, unweighted OIM, based on EFSA dataset).

	PFHxS	PFNA	PFOA	PFOS	Sum of PFASs
Fish	0.01	0.02	0.24	1.18	1.45
Shellfish	0.00	0.01	0.01	0.06	0.08
Meat	0.00	0.01	0.31	0.78	1.11
Dairy	0.00	0.00	0.04	0.05	0.09
Eggs	0.00	0.00	0.23	0.59	0.82
Grain	0.00	0.00	0.00	0.00	0.00
Fruit Veg Potato	0.21	0.11	0.18	0.30	0.79
Drinking water	0.13	0.01	0.09	0.04	0.26
Other*	0.00	0.00	0.03	0.03	0.06

*Other = other food with detected levels (e.g. fat, sugar, condiments)

Table 22.1-4 Dietary exposure from different food groups (ng/kg bw per week) in 9-year-olds (n=636) to PFOS, PFOA, PFNA and PFHxS and the sum of these four PFASs (lower bound values, unweighted OIM, based on EFSA dataset).

	PFHxS	PFNA	PFOA	PFOS	Sum of PFASs
Fish	0.00	0.04	0.34	1.79	2.18
Shellfish	0.00	0.00	0.00	0.06	0.07
Meat	0.00	0.02	0.36	0.90	1.29
Dairy	0.00	0.00	0.05	0.07	0.13
Eggs	0.00	0.00	0.36	0.91	1.27
Grain	0.00	0.00	0.00	0.00	0.00
Fruit Veg Potato	0.40	0.21	0.29	0.56	1.46
Drinking water	0.17	0.01	0.12	0.06	0.36
Other*	0.00	0.00	0.03	0.04	0.07

*Other = other food with detected levels (e.g. fat, sugar, condiments)

Table 22.1-5 Dietary exposure from different food groups (ng/kg bw per week) in 4-year-olds (n=399) to PFOS, PFOA, PFNA and PFHxS and the sum of these four PFASs (lower bound values, unweighted OIM, based on EFSA dataset).

	PFHxS	PFNA	PFOA	PFOS	Sum of PFASs
Fish	0.01	0.09	0.76	5.24	6.11
Shellfish	0.01	0.01	0.01	0.08	0.11
Meat	0.01	0.02	0.38	1.01	1.42
Dairy	0.00	0.03	0.09	0.12	0.25
Eggs	0.00	0.00	0.61	1.54	2.15
Grain	0.00	0.00	0.00	0.00	0.00

	PFHxS	PFNA	PFOA	PFOS	Sum of PFASs
Fruit Veg Potato	1.15	0.58	0.63	1.48	3.85
Drinking water	0.29	0.01	0.21	0.10	0.62
Other*	0.00	0.00	0.04	0.04	0.08

*Other = other food with detected levels (e.g. fat, sugar, condiments)

Table 22.1-6 Dietary exposure from different food groups (ng/kg bw per week) in 2-year-olds (n=1413) to PFOS, PFOA, PFNA and PFHxS and the sum of these four PFASs (lower bound values, unweighted OIM, based on EFSA dataset).

	PFHxS	PFNA	PFOA	PFOS	Sum of PFASs
Fish	0.02	0.11	0.95	6.14	7.22
Shellfish	0.00	0.00	0.00	0.00	0.00
Meat	0.00	0.02	0.24	0.59	0.84
Dairy	0.00	0.01	0.09	0.22	0.31
Eggs	0.00	0.00	0.77	1.93	2.69
Grain	0.00	0.00	0.00	0.00	0.00
Fruit Veg Potato	1.72	0.87	0.98	2.21	5.79
Drinking water	0.56	0.03	0.41	0.19	1.19
Other*	0.00	0.00	0.03	0.03	0.06

*Other = other food with detected levels (e.g. fat, sugar, condiments)

Table 22.1-7 Dietary exposure from different food groups (ng/kg bw per week) in 1-year-olds (n=1957) to PFOS, PFOA, PFNA and PFHxS and the sum of these four PFASs (lower bound values, unweighted OIM, based on EFSA dataset).

	PFHxS	PFNA	PFOA	PFOS	Sum of PFASs
Fish	0.01	0.11	1.03	5.46	6.60
Shellfish	0.00	0.00	0.00	0.00	0.00
Meat	0.00	0.02	0.22	0.49	0.74
Dairy	0.00	0.00	0.06	0.07	0.13
Eggs	0.00	0.00	0.73	1.82	2.55
Grain	0.00	0.00	0.00	0.00	0.00
Fruit Veg Potato	1.58	0.81	1.05	2.12	5.56
Drinking water	0.61	0.03	0.44	0.21	1.28
Other*	0.00	0.00	0.02	0.02	0.04

*Other = other food with detected levels (e.g. fat, sugar, condiments)

22.2 Lower bound exposure based in concentrations in the VKM dataset

Exposure to the PFASs (PFOS, PFOA, PFNA and PFHxS and the sum of the four PFASs) based on concentration data in the VKM dataset is shown in table 22.2-1.

Table 22.2-1 Mean, median and 95th percentile of PFAS exposure (LB) from total diet (ng/kg bw per week) for adults (Norkost 3), 13- 9-, and 4-year-olds (Ungkost 3), mixed model, and 2- and 1-year-olds (Spedkost 3 and Småbarnskost 3), weighted OIM. Exposures are based on concentrations in the VKM-dataset.

	PFOS			PFOA			PFNA			PFHxS			Sum of PFASs		
	Mean	Med.	P95	Mean	Med.	P95	Mean	Med.	P95	Mean	Med.	P95	Mean	Med.	P95
Adults, 18-70	2.84	2.46	5.89	1.29	1.21	2.21	0.18	0.17	0.31	0.26	0.23	0.55	4.37	4.02	7.86
Women, 18-45	2.17	1.94	4.23	1.30	1.23	2.20	0.20	0.18	0.33	0.23	0.21	0.49	3.73	3.49	6.43
13-year-olds	1.62	1.43	3.23	1.02	0.95	1.88	0.21	0.18	0.42	0.10	0.07	0.25	2.98	2.71	5.61
9-year-olds	2.30	2.12	4.20	1.50	1.38	2.83	0.32	0.30	0.60	0.16	0.13	0.37	4.35	4.04	7.87
4-year-olds	5.48	4.68	11.85	2.39	2.27	4.05	0.68	0.63	1.30	0.40	0.35	0.84	8.87	8.11	16.65
2-year-olds	6.32	5.10	15.05	2.60	2.09	5.86	0.80	0.71	1.63	0.65	0.56	1.37	10.37	9.42	21.37
1-year-olds	5.19	3.89	12.61	2.35	1.92	5.65	0.62	0.51	1.44	0.56	0.47	1.34	8.73	7.34	19.07

The relative contributions of the four PFASs to the sum of PFASs was quite similar in different age groups as illustrated in figure 22.2-1 (VKM dataset)

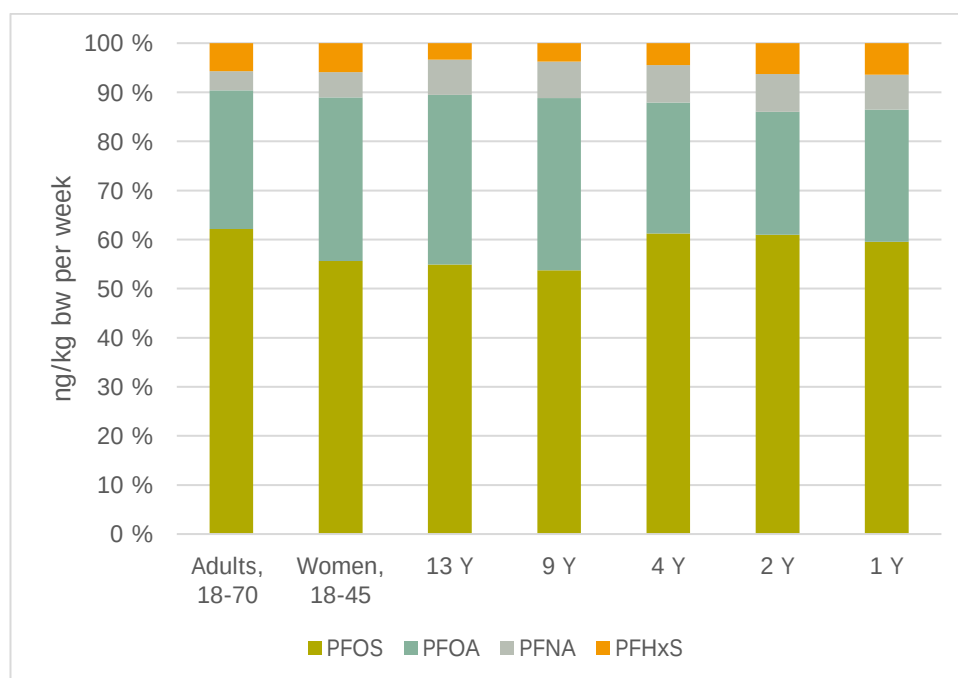


Figure 22.2-1 Mean relative contribution of PFOS, PFOA, PFNA and PFHxS (LB) to the sum of 4 PFASs for adults (Norkost 3), 13- 9-, and 4-year-olds (Ungkost 3), mixed model, and 2- and 1-year-olds (Spedkost 3 and Småbarnskost 3), weighted OIM. Intakes are based on concentrations in the VKM-dataset.

22.2.1 Contribution from food groups at lower bound based on the VKM dataset

All food groups contribute to the exposure to the sum of 4 PFASs, as illustrated in Figure 22.2.1-1 (VKM dataset). Fish contributed most in adults, followed by meat and drinking water. Of note, the concentration in drinking water was also calculated into exposure from tea and coffee. In children, grains and fruit/vegetables/potatoes also contribute considerably in addition to fish.

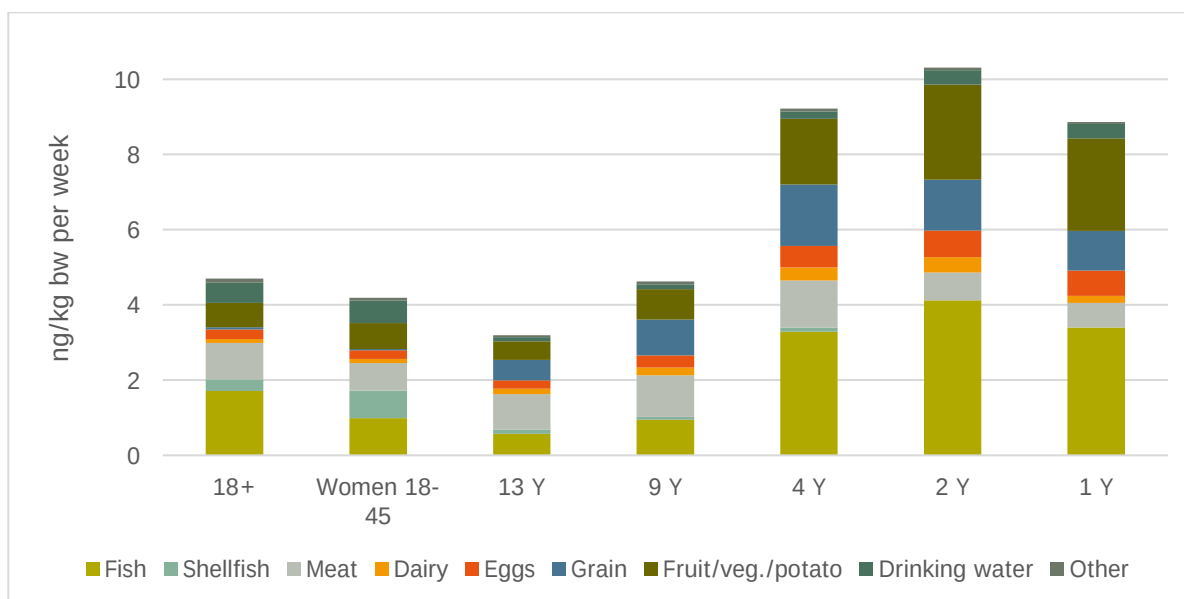


Figure 22.2.1-1 Contribution of food groups to the total dietary exposure of sum of 4 PFASs in adults (Norkost 3), 13- 9-, and 4-year-olds (Ungkost 3), 2- and 1-year-olds (Spedkost 3 and Småbarnskost 3). Intakes are based on mean OIM and concentrations in the VKM dataset.

More information regarding the contribution of different food groups to the intake of each of the 4 PFASs are shown in Tables 22.2.1-1 - 22.2.1-7.

Lean fish contributed most to the exposure from fish when exposure was based on the VKM dataset. Fish liver and roe concentration data were obtained from the EFSA database. Although the mean consumption is low, exposures from such fish liver and roe appear to constitute a substantial share of the PFAS exposure from fish based on the present calculation.

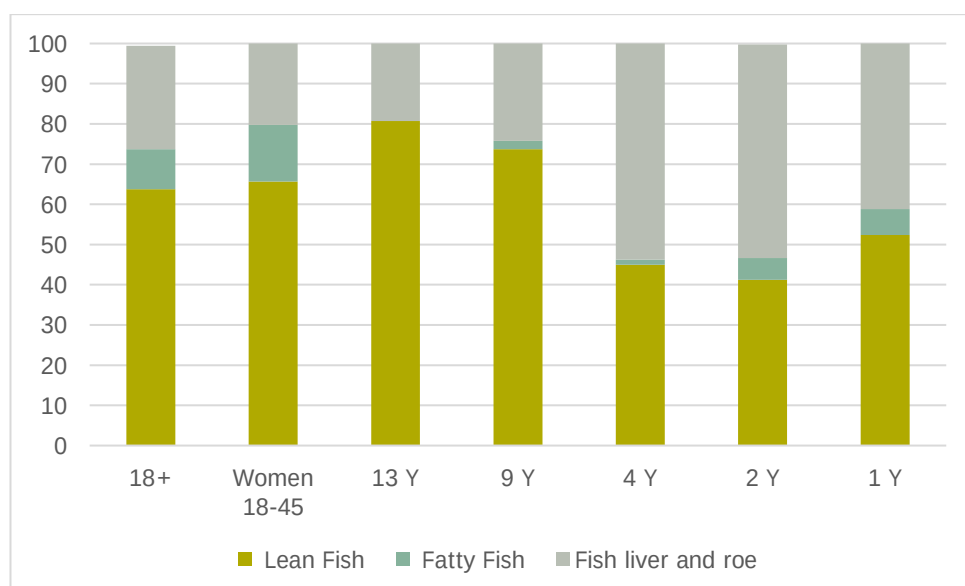


Figure 22.2.1-2 Mean relative contribution of fish categories to the sum 4 PFASs from fish based on the VKM dataset.

Table 22.2.1-1 Dietary exposure from different food groups (ng/kg bw per week) in adults (18-70 years. n=1787) to PFOS, PFOA, PFNA and PFHxS and the sum of these four PFASs (lower bound values, unweighted OIM, based on VKM dataset).

	PFHxS	PFNA	PFOA	PFOS	Sum of PFASs
Fish	0.01	0.03	0.04	1.63	1.71
Shellfish	0.01	0.02	0.02	0.26	0.31
Meat	0.01	0.04	0.29	0.63	0.97
Dairy	0.00	0.03	0.03	0.04	0.10
Eggs	0.01	0.00	0.04	0.21	0.26
Grain	0.00	0.01	0.04	0.01	0.06
Fruit Veg Potato	0.08	0.05	0.32	0.19	0.64
Drinking water	0.10	0.00	0.48	0.04	0.54
Other*	0.03	0.00	0.07	0.01	0.11

*Other = other food with detected levels (e.g. fat, sugar, condiments)

Table 22.2.1-2 Dietary exposure from different food groups (ng/kg bw per week) in women (18-45 years. n=466) to PFOS, PFOA, PFNA and PFHxS and the sum of these four PFASs (lower bound values, unweighted OIM, based on VKM dataset).

	PFHxS	PFNA	PFOA	PFOS	Sum of PFASs
Fish	0.00	0.02	0.03	0.94	0.99
Shellfish	0.00	0.04	0.24	0.45	0.73
Meat	0.00	0.04	0.24	0.45	0.73
Dairy	0.00	0.04	0.03	0.05	0.11
Eggs	0.01	0.00	0.04	0.19	0.23
Grain	0.00	0.00	0.03	0.01	0.04
Fruit Veg Potato	0.10	0.06	0.32	0.22	0.69
Drinking water	0.08	0.00	0.53	0.04	0.59
Other*	0.02	0.00	0.05	0.01	0.08

*Other = other food with detected levels (e.g. fat, sugar, condiments)

Table 22.2.1-3 Dietary exposure from different food groups (ng/kg bw per week) in 13-year-olds (n=687) to PFOS, PFOA, PFNA and PFHxS and the sum of these four PFASs (lower bound values, unweighted OIM, based on VKM dataset).

	PFHxS	PFNA	PFOA	PFOS	Sum of PFASs
Fish	0.01	0.02	0.00	0.55	0.57
Shellfish	0.00	0.00	0.00	0.11	0.11

	PFHxS	PFNA	PFOA	PFOS	Sum of PFASs
Meat	0.00	0.05	0.28	0.62	0.95
Dairy	0.00	0.04	0.03	0.06	0.14
Eggs	0.01	0.00	0.03	0.17	0.22
Grain	0.01	0.06	0.36	0.11	0.55
Fruit Veg Potato	0.07	0.04	0.23	0.16	0.49
Drinking water	0.01	0.00	0.10	0.01	0.11
Other*	0.00	0.00	0.03	0.02	0.05

* Other = other food with detected levels (e.g. fat, sugar, condiments)

Table 22.2.1-4 Dietary exposure from different food groups (ng/kg bw per week) in 9-year-olds (n=636) to PFOS, PFOA, PFNA and PFHxS and the sum of these four PFASs (lower bound values, unweighted OIM, based on VKM dataset).

	PFHxS	PFNA	PFOA	PFOS	Sum of PFASs
Fish	0.00	0.04	0.01	0.91	0.95
Shellfish	0.00	0.00	0.00	0.06	0.07
Meat	0.00	0.06	0.32	0.72	1.11
Dairy	0.00	0.06	0.05	0.09	0.20
Eggs	0.01	0.00	0.05	0.27	0.33
Grain	0.02	0.11	0.62	0.20	0.95
Fruit Veg Potato	0.12	0.07	0.36	0.27	0.81
Drinking water	0.01	0.00	0.11	0.01	0.13
Other*	0.00	0.00	0.04	0.03	0.07

*Other = other food with detected levels (e.g. fat, sugar, condiments)

Table 22.2.1-5 Dietary exposure from different food groups (ng/kg bw per week) in 4-year-olds (n=399) to PFOS, PFOA, PFNA and PFHxS and the sum of these four PFASs (lower bound values, unweighted OIM, based on VKM dataset).

	PFHxS	PFNA	PFOA	PFOS	Sum of PFASs
Fish	0.01	0.13	0.02	3.12	3.29
Shellfish	0.01	0.01	0.01	0.08	0.11
Meat	0.01	0.06	0.35	0.83	1.25
Dairy	0.00	0.13	0.08	0.15	0.35
Eggs	0.02	0.00	0.09	0.46	0.57
Grain	0.03	0.19	1.07	0.34	1.63
Fruit Veg Potato	0.31	0.19	0.62	0.63	1.75
Drinking water	0.01	0.00	0.17	0.02	0.19
Other*	0.00	0.00	0.05	0.03	0.08

*Other = other food with detected levels (e.g. fat, sugar, condiments)

Table 22.2.1-6 Dietary exposure from different food groups (ng/kg bw per week) in 2-year-olds (n=1413) to PFOS, PFOA, PFNA and PFHxS and the sum of these four PFASs (lower bound values, unweighted OIM, based on VKM dataset).

	PFHxS	PFNA	PFOA	PFOS	Sum of PFASs
Fish	0.02	0.18	0.07	3.85	4.12
Shellfish	0.00	0.00	0.00	0.00	0.00
Meat	0.00	0.05	0.23	0.45	0.74
Dairy	0.00	0.10	0.08	0.24	0.41
Eggs	0.03	0.00	0.11	0.57	0.71
Grain	0.03	0.15	0.90	0.27	1.35
Fruit Veg Potato	0.52	0.31	0.83	0.87	2.53
Drinking water	0.04	0.00	0.31	0.04	0.38
Other*	0.00	0.00	0.05	0.01	0.07

*Other = other food with detected levels (e.g. fat, sugar, condiments)

Table 22.2.1-7 Dietary exposure from different food groups (ng/kg bw per week) in 1-year-olds (n=1957) to PFOS, PFOA, PFNA and PFHxS and the sum of these four PFASs (lower bound values, unweighted OIM, based on VKM dataset).

	PFHxS	PFNA	PFOA	PFOS	Sum of PFASs
Fish	0.01	0.14	0.06	3.18	3.40
Shellfish	0.00	0.00	0.00	0.00	0.00
Meat	0.00	0.05	0.23	0.36	0.65
Dairy	0.00	0.06	0.05	0.09	0.19
Eggs	0.03	0.00	0.10	0.54	0.67
Grain	0.02	0.12	0.70	0.21	1.06
Fruit Veg Potato	0.48	0.25	0.98	0.75	2.46
Drinking water	0.02	0.00	0.33	0.03	0.38
Other*	0.00	0.00	0.03	0.01	0.05

*Other = other food with detected levels (e.g. fat, sugar, condiments)

22.3 Comparison of LB exposures performed with the VKM dataset and the EFSA dataset

The mean exposures to each of the PFASs and to the sum of 4 PFASs based on the VKM dataset and the EFSA dataset is illustrated in figure 22.3-1.

The exposures estimated using the EFSA dataset (Table 8.4.2-1) were 1.4 to 1.9 times higher than the exposures based on the VKM dataset (Table 22.2-1). This reflects the higher LB concentrations in the EFSA database than in the VKM database (see Chapter 7). For PFOS, the mean exposures based on the EFSA database were 1.6-2 times higher than the corresponding values based on the VKM database. The difference was less for PFOA (1-1.4 times) and PFNA (0.7-1.6 times), but higher for PFHxS (3.0-3.6 times).

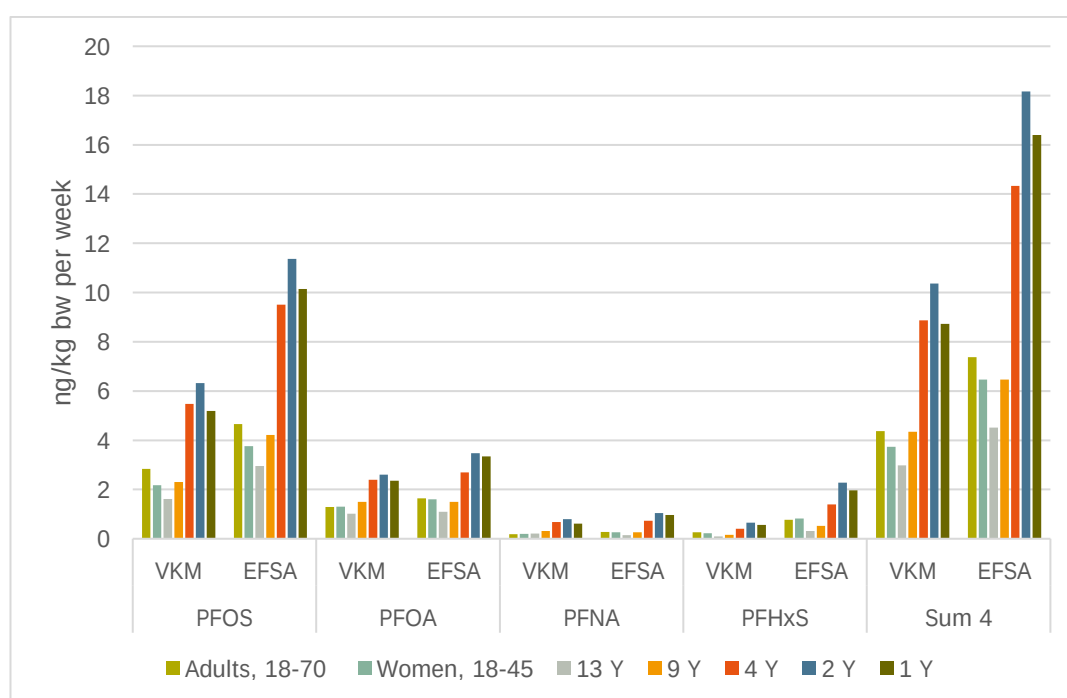


Figure 22.3-1 Mean dietary exposure to PFOS, PFOA, PFNA and PFHxS and the sum of the 4 PFASs (LB) from total diet for adults (Norkost 3), 13- 9-, and 4-year-olds (Ungkost 3), mixed model, and 2- and 1-year-olds (Spedkost 3 and Småbarnskost 3), weighted OIM.

The relative contributions of the four PFASs to the sum of PFASs was quite similar in different age groups and between the datasets (Figures 8.4.2-1 and 22.2-1)

In both datasets several food groups contribute to the exposure to the sum of 4 PFASs, as illustrated in Figure 8.4.2-2 (EFSA dataset) and 22.2.1-1 (VKM dataset). In both datasets fish was important contributor in adults and the 4, 2 and 1-year-old children. In children, fruit/vegetables/potatoes also contribute considerably. The intake from grain, which is particularly evident in children age groups in the VKM dataset, is attributed to detected levels in grains and bread in the VKM dataset. In the EFSA dataset grains is not contributing because of the high percentage of samples with concentrations below the LOQ. The

contribution from eggs is less prominent in the exposures based on the VKM dataset than when based on the EFSA dataset.

The relative contribution from fish to the total exposure to individual PFASs and the sum of four PFASs based on the VKM dataset is shown in Table 22.3-1 and for the EFSA dataset in Table 22.3-2. For the VKM dataset, in all age groups, fish contributed 32-61% of the total PFOS intake. For PFNA, fish contributed up to 18% of the intakes (highest in 4-year-olds) and fish contributed only minor shares of the total dietary intake of PFOA and PFHxS. As PFOS contributes the largest share of intake of the sum of four PFASs, and fish is a major source of PFOS, fish contributes a significant part (up to 36%) of the total intake of the sum of four PFASs. For exposures based on the EFSA dataset (Table 22.3-1), the contribution from fish to PFOS exposure was in similar range as for the VKM dataset. However, the mean contribution from fish to mean PFOA exposure was also substantial (22-29%). Regarding PFOA, the differences in contribution of fish to the total exposure may be due to a lower proportion of samples with detected concentrations of PFOA in fish from Norway (VKM database), in particular for fatty fish. This leads to a lower PFOA concentration in fish in the VKM database than in fish in the EFSA database. The EFSA database includes fish from Norway and the rest of Europe, whereas the VKM database includes also additional Norwegian fish samples that were not present in the EFSA database. Whether there are true concentration differences between fish marketed and commonly consumed in Norway and fish commonly consumed in Europe is not known.

Table 22.3-1 Contribution from fish to total PFAS dietary exposure (%). Calculations are based on concentrations in the VKM dataset.

	PFOS	PFOA	PFNA	PFHxS	Sum 4 PFASs
Adults, 18-75	54	2	17	0	36
Women, 18-45	45	2	12	0	27
13-year-olds	32	0	9	0	18
9-year-olds	36	0	9	0	21
4-year-olds	55	1	18	5	36
2-year-olds	50	3	3	3	26
1-year-olds	61	2	0	2	7

Table 22.3-2 Contribution from fish to total PFAS dietary exposure (%). Calculations are based on concentrations in the EFSA dataset.

	PFOS	PFOA	PFNA	PFHxS	Sum 4 PFASs
Adults 18-75	49	23	17	0	37
Women 18-45	43	22	12	0	31
13-year-olds	39	22	13	0	31

9-year-olds	41	22	14	0	32
4-year-olds	55	28	12	1	42
2-year-olds	54	27	2	1	30
1-year-olds	54	29	0	0	12

Lean fish contributed most to the exposure from fish when exposure was based on the VKM dataset but contributed equally as fatty fish for exposure based on the EFSA dataset (Figures 22.2.1-2 and 8.4.2-3). This can be explained by low detection rate in fatty fish in the VKM database, as mentioned above. Fish offal (category 'other fish') was a major contributor based on the VKM dataset but less so based on the EFSA dataset. In both dataset fish liver and roe concentration data were obtained from the EFSA database.

22.4 Comparison of the lower bound PFAS exposure estimates from Norway to exposure estimates made by EFSA for dietary surveys from European countries

Weekly mean (95th-percentile) LB intake of PFASs for all dietary surveys available to EFSA ranged from 3-22 (9-70) ng/kg bw per week in adult age groups, from 6 to 21 (19-68) ng/kg bw per week for children age groups and 10-46 (23-96) ng/kg bw per week for toddlers (EFSA 2020).

The mean (95th-percentile) intake in adults estimated by VKM is 4.4 (7.9) ng/kg bw per week based on the VKM-dataset and 7.4 (13.1) ng/kg bw per week based on the EFSA dataset. In children age groups the mean (95th-percentile) LB intake ranged from 3.0-10 (5.6-21) ng/kg bw per week based on the VKM dataset and from 4.5-18 (8.8-35) ng/kg bw per week based on the EFS dataset.

Thus, lower bound intake estimates based on both the VKM and EFSA datasets fall within the range of exposures estimated provided by EFSA for other European countries.

22.5 PFAS exposure based on upper bound concentration data

22.5.1 Upper bound exposure based on concentrations in the EFSA dataset

Table 22.5.1-1 Mean, median and 95-percentile PFAS exposure (UB) from total diet (ng/kg bw/week) among adults (Norkost 3), 13- 9-, and 4-year-olds (Ungkost 3) (mixed model) and 2- and 1-year-olds (Spedkost 3 and Småbarnskost 3, weighted OIM). Intakes are based on EFSA-dataset

	PFOS			PFOA			PFNA			PFHxS			Sum of PFASs		
	Mean	Med.	P95	Mean	Med.	P95	Mean	Med.	P95	Mean	Med.	P95	Mean	Med.	P95
Adults, 18-70	36.47	34.53	61.92	34.63	32.69	59.27	30.21	28.26	53.25	28.01	25.99	50.42	129.37	121.52	224.79
Women, 18-45	36.69	34.93	61.97	35.42	33.63	60.30	30.76	28.94	54.01	28.64	26.72	51.38	131.53	124.22	227.58
13-year-olds	37.31	34.75	66.97	35.84	33.40	64.54	32.02	29.41	59.56	29.49	26.72	56.58	134.44	124.50	245.52
9-year-olds	54.03	51.09	92.05	52.19	49.26	89.54	46.01	42.82	81.72	42.09	38.87	76.59	194.28	182.13	339.34
4-year-olds	93.35	89.14	149.00	89.69	85.52	143.98	77.29	72.78	128.96	70.29	65.79	119.74	330.74	313.17	541.78
2-year-olds	117.39	107.37	205.74	114.89	104.83	202.19	93.41	84.76	166.40	83.08	74.28	150.47	408.77	370.68	725.96
1-year-olds	79.79	72.18	153.64	76.73	69.09	148.07	63.15	56.77	122.04	56.41	50.16	110.89	276.09	247.00	535.98

22.5.1.1 Contribution from food groups

Table 22.5.1.1-1 Dietary exposure from different food groups (ng/kg bw per week) in adults (18-70 years. n=1787). to PFOS, PFOA, PFNA and PFHxS and the sum of these four PFASs (upper bound values, unweighted OIM, based on EFSA dataset).

	PFHxS	PFNA	PFOA	PFOS	Sum of PFASs
Fish	2.78	3.59	3.59	5.01	14.98
Shellfish	0.07	0.07	0.06	0.28	0.47
Meat	1.33	1.71	2.21	2.49	7.75
Dairy	3.18	3.85	5.07	4.83	16.94
Eggs	0.15	0.26	0.56	0.92	1.89
Grain	1.39	1.66	1.83	2.25	7.13
Fruit Veg Potato	18.60	18.85	20.92	20.57	78.93
Drinking water	0.66	0.40	0.58	0.47	2.11
Other*	0.81	0.87	0.94	0.80	3.43

*Other = other food with detected levels (e.g. fat, sugar, condiments)

Table 22.5.1.1-2 Dietary exposure from different food groups (ng/kg bw per week) in women (18-45 years. n=466) to PFOS, PFOA, PFNA and PFHxS and the sum of these four PFASs (upper bound values, unweighted OIM, based on EFSA dataset).

	PFHxS	PFNA	PFOA	PFOS	Sum of PFASs
Fish	2.08	2.58	2.56	3.47	10.70
Shellfish	0.03	0.04	0.03	0.21	0.31
Meat	1.22	1.59	1.98	2.16	6.96
Dairy	3.09	3.79	4.95	4.71	16.54
Eggs	0.13	0.23	0.50	0.82	1.69
Grain	1.41	1.68	1.85	2.28	7.23
Fruit Veg Potato	20.67	20.99	23.18	22.82	87.65
Drinking water	0.75	0.45	0.66	0.54	2.41
Other*	0.91	0.99	1.02	0.93	3.85

*Other = other food with detected levels (e.g. fat, sugar, condiments)

Table 22.5.1.1-3 Dietary exposure from different food groups (ng/kg bw per week) in 13-year-olds (n=687) to PFOS, PFOA, PFNA and PFHxS and the sum of these four PFASs (upper bound values, unweighted OIM, based on EFSA dataset).

	PFHxS	PFNA	PFOA	PFOS	Sum of PFASs
Fish	1.68	2.10	2.02	2.74	8.54
Shellfish	0.02	0.02	0.01	0.06	0.11
Meat	1.50	2.02	2.54	2.79	8.84

	PFHxS	PFNA	PFOA	PFOS	Sum of PFASs
Dairy	4.59	5.53	7.29	6.94	24.36
Eggs	0.12	0.22	0.46	0.76	1.56
Grain	2.19	2.62	2.88	3.55	11.24
Fruit Veg Potato	17.64	17.80	19.10	18.86	73.40
Drinking water	0.26	0.16	0.23	0.19	0.83
Other*	1.93	2.15	2.07	2.11	8.26

*Other = other food with detected levels (e.g. fat, sugar, condiments)

Table 22.5.1.1-4 Dietary exposure from different food groups (ng/kg bw per week) in 9-year-olds (n=636) to PFOS, PFOA, PFNA and PFHxS and the sum of these four PFASs (upper bound values, unweighted OIM, based on EFSA dataset).

	PFHxS	PFNA	PFOA	PFOS	Sum of PFASs
Fish	2.46	3.13	3.04	4.10	12.72
Shellfish	0.01	0.01	0.01	0.07	0.11
Meat	1.79	2.45	3.06	3.33	10.63
Dairy	7.50	8.99	11.90	11.32	39.71
Eggs	0.19	0.33	0.71	1.18	2.41
Grain	3.25	3.89	4.28	5.26	16.67
Fruit Veg Potato	25.14	25.42	27.73	27.35	105.64
Drinking water	0.35	0.21	0.31	0.25	1.13
Other*	2.66	2.92	2.80	2.86	11.25

*Other = other food with detected levels (e.g. fat, sugar, condiments)

Table 22.5.1.1-5 Dietary exposure from different food groups (ng/kg bw per week) in 4-year-olds (n=399) to PFOS, PFOA, PFNA and PFHxS and the sum of these four PFASs (upper bound values, unweighted OIM, based on EFSA dataset).

	PFHxS	PFNA	PFOA	PFOS	Sum of PFASs
Fish	5.82	7.54	7.88	10.75	32.00
Shellfish	0.02	0.02	0.02	0.09	0.15
Meat	1.90	2.54	3.20	3.51	11.16
Dairy	12.54	15.37	19.94	18.94	66.78
Eggs	0.33	0.56	1.20	2.00	4.09
Grain	4.66	5.58	6.13	7.55	23.92
Fruit Veg Potato	40.47	41.26	47.10	46.30	175.13
Drinking water	0.61	0.37	0.53	0.44	1.94
Other*	3.07	3.35	3.22	3.29	12.94

*Other = other food with detected levels (e.g. fat, sugar, condiments)

Table 22.5.1.1-6 Dietary exposure from different food groups (ng/kg bw per week) in 2-year-olds (n=1413) to PFOS, PFOA, PFNA and PFHxS and the sum of these four PFASs (upper bound values, unweighted OIM, based on EFSA dataset).

	PFHxS	PFNA	PFOA	PFOS	Sum of PFASs
Fish	6.68	8.74	9.43	12.57	37.42
Shellfish	0.00	0.00	0.00	0.00	0.00
Meat	1.43	1.93	2.33	2.45	8.14
Dairy	24.97	30.10	39.47	37.43	131.97
Eggs	0.41	0.71	1.50	2.50	5.13
Grain	5.46	6.54	7.19	8.86	28.05
Fruit Veg Potato	40.17	41.56	50.62	49.36	181.71
Drinking water	1.17	0.70	1.02	0.84	3.73
Other*	1.34	1.50	1.37	1.45	5.67

*Other = other food with detected levels (e.g. fat, sugar, condiments)

Table 22.5.1.1-7 Dietary exposure from different food groups (ng/kg bw per week) in 1-year-olds (n=1957) to PFOS, PFOA, PFNA and PFHxS and the sum of these four PFASs (upper bound values, unweighted OIM, based on EFSA dataset).

	PFHxS	PFNA	PFOA	PFOS	Sum of PFASs
Fish	6.58	8.46	8.86	11.71	35.62
Shellfish	0.00	0.00	0.00	0.00	0.00
Meat	1.35	1.82	2.15	2.20	7.52
Dairy	7.77	9.87	12.32	11.67	41.63
Eggs	0.39	0.67	1.43	2.37	4.86
Grain	4.05	4.85	5.33	6.56	20.79
Fruit Veg Potato	33.73	35.11	44.11	42.73	155.68
Drinking water	1.26	0.75	1.10	0.90	4.01
Other*	0.95	1.03	0.95	1.05	3.98

*Other = other food with detected levels (e.g. fat, sugar, condiments)

An overview of the UB mean contribution from food groups are shown in figure 22.5.1.1-1.

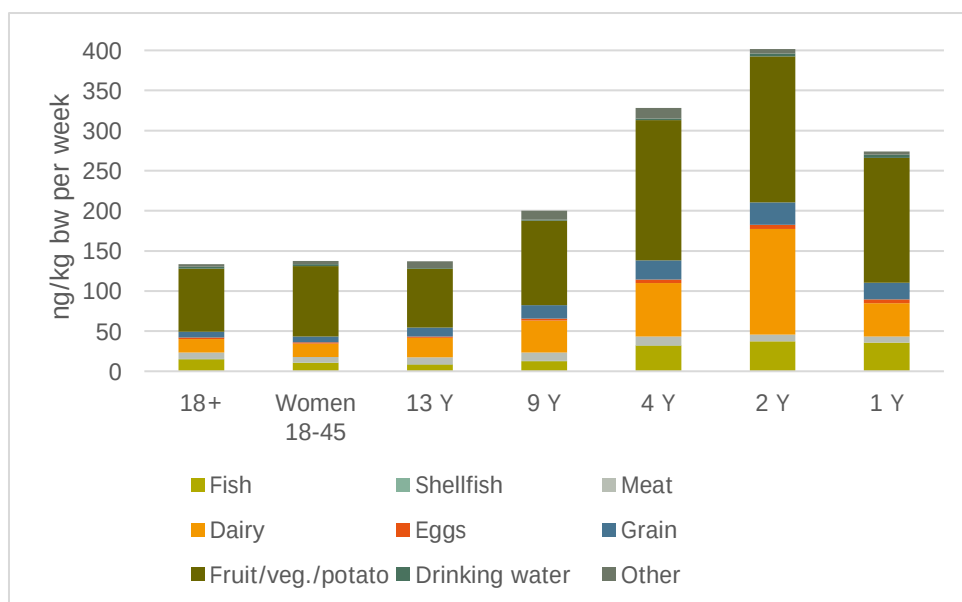


Figure 22.5.1.1-1 Contribution of food groups to the upper bound total dietary exposure of sum of 4 PFASs in adults (Norkost 3), 13- 9-, and 4-year-olds (Ungkost 3), 2- and 1-year-olds (Spedkost 3 and Småbarnskost 3). Intakes are based on mean OIM and concentrations in the EFSA dataset.

22.5.2 Upper bound exposure based on concentrations in the VKM dataset

Table 22.5.2-1 Mean, median and 95-percentile PFAS exposure (UB) from total diet (ng/kg bw/week) among adults (Norkost 3), 13- 9-, and 4-year-olds (Ungkost 3) (mixed model) and 2- and 1-year-olds (Spedkost 3 and Småbarnskost 3, weighted OIM). Intakes are based on VKM-dataset

	PFOS			PFOA			PFNA			PFHxS			Sum of PFASs		
	Mean	Med.	P95	Mean	Med.	P95	Mean	Med.	P95	Mean	Med.	P95	Mean	Med.	P95
Adults, 18-70	20.43	18.38	40.09	20.41	18.43	39.61	17.12	15.04	35.32	17.25	15.15	35.61	74.76	66.88	148.67
Women, 18-45	21.12	19.14	41.16	21.18	19.26	40.93	18.25	16.16	37.40	17.87	15.79	36.87	78.03	70.31	154.22
13-year-olds	21.02	18.39	44.31	21.33	18.54	45.30	18.49	15.89	40.21	17.72	14.99	39.68	78.51	67.71	169.48
9-year-olds	29.19	26.95	53.44	29.81	27.52	54.43	25.27	22.98	47.83	23.96	21.57	46.56	107.95	99.03	200.71
4-year-olds	48.29	45.77	80.77	48.57	45.94	81.39	40.44	38.01	68.78	38.54	36.17	65.84	175.65	165.89	295.44
2-year olds	67.41	61.97	128.72	68.72	62.99	130.65	54.66	49.42	104.23	50.06	45.05	94.73	240.85	218.89	452.06
1-year old	35.41	30.52	74.92	35.71	30.81	76.11	28.83	24.86	60.96	27.66	23.93	58.60	127.60	110.10	268.95

22.5.2.1 Contribution from food groups

Table 22.5.2.1-1 Dietary exposure from different food groups (ng/kg bw per week) in adults (18-70 years. n=1787) to PFOS, PFOA, PFNA and PFHxS and the sum of these four PFASs (upper bound values, unweighted OIM, based on VKM dataset).

	PFHxS	PFNA	PFOA	PFOS	Sum of PFASs
Fish	3.92	2.88	3.78	4.01	14.59
Shellfish	0.07	0.08	0.06	0.28	0.47
Meat	0.51	0.65	1.01	1.22	3.39
Dairy	2.69	3.27	4.28	4.05	14.29
Eggs	0.01	0.02	0.05	0.21	0.29
Grain	0.74	0.95	1.06	1.11	3.85
Fruit Veg Potato	8.85	8.97	9.56	9.41	36.80
Drinking water	0.20	0.05	0.50	0.07	0.64
Other*	0.67	0.74	0.75	0.62	2.79

*Other = other food with detected levels (e.g. fat, sugar, condiments)

Table 22.5.2.1-2 Dietary exposure from different food groups (ng/kg bw per week) in women (18-45 years. n=466) to PFOS, PFOA, PFNA and PFHxS and the sum of these four PFASs (upper bound values, unweighted OIM, based on VKM dataset).

	PFHxS	PFNA	PFOA	PFOS	Sum of PFASs
Fish	2.78	2.05	2.51	2.66	10.01
Shellfish	0.03	0.04	0.03	0.21	0.31
Meat	0.39	0.52	0.79	0.91	2.61
Dairy	2.63	3.23	4.20	3.96	14.03
Eggs	0.01	0.01	0.04	0.19	0.25
Grain	0.79	1.00	1.11	1.19	4.08
Fruit Veg Potato	10.65	10.77	11.43	11.28	44.13
Drinking water	0.16	0.05	0.54	0.07	0.69
Other*	0.82	0.89	0.87	0.77	3.36

*Other = other food with detected levels (e.g. fat, sugar, condiments)

Table 22.5.2.1-3 Dietary exposure from different food groups (ng/kg bw per week) in 13-year-olds (n=687) to PFOS, PFOA, PFNA and PFHxS and the sum of these four PFASs (upper bound values, unweighted OIM, based on VKM dataset).

	PFHxS	PFNA	PFOA	PFOS	Sum of PFASs
Fish	2.45	1.67	2.03	1.97	8.12

	PFHxS	PFNA	PFOA	PFOS	Sum of PFASs
Shellfish	0.02	0.02	0.01	0.06	0.11
Meat	0.77	1.07	1.44	1.65	4.93
Dairy	3.87	4.68	6.13	5.79	20.48
Eggs	0.01	0.01	0.04	0.17	0.24
Grain	0.90	1.20	1.58	1.42	5.10
Fruit Veg Potato	8.21	8.31	8.75	8.66	33.93
Drinking water	0.01	0.02	0.10	0.02	0.15
Other*	1.99	2.16	2.01	1.96	8.11

*Other = other food with detected levels (e.g. fat, sugar, condiments)

Table 22.5.2.1-4 Dietary exposure from different food groups (ng/kg bw per week) in 9-year-olds (n=636) to PFOS, PFOA, PFNA and PFHxS and the sum of these four PFASs (upper bound values, unweighted OIM, based on VKM dataset).

	PFHxS	PFNA	PFOA	PFOS	Sum of PFASs
Fish	3.60	2.48	3.04	2.99	12.10
Shellfish	0.01	0.02	0.01	0.07	0.11
Meat	0.93	1.32	1.74	1.98	5.97
Dairy	6.59	7.91	10.43	9.86	34.79
Eggs	0.01	0.02	0.06	0.27	0.36
Grain	1.26	1.69	2.33	2.03	7.31
Fruit Veg Potato	10.08	10.19	10.88	10.77	41.92
Drinking water	0.01	0.02	0.12	0.02	0.17
Other*	2.60	2.80	2.62	2.59	10.61

*Other = other food with detected levels (e.g. fat, sugar, condiments)

Table 22.5.2.1-5 Dietary exposure from different food groups (ng/kg bw per week) in 4-year-olds (n=399) to PFOS, PFOA, PFNA and PFHxS and the sum of these four PFASs (upper bound values, unweighted OIM, based on VKM dataset).

	PFHxS	PFNA	PFOA	PFOS	Sum of PFASs
Fish	7.93	5.96	7.41	8.03	29.33
Shellfish	0.02	0.02	0.02	0.09	0.15
Meat	1.07	1.48	1.96	2.24	6.75
Dairy	10.76	13.30	17.13	16.14	57.33
Eggs	0.02	0.04	0.10	0.46	0.62
Grain	1.75	2.36	3.42	2.91	10.44
Fruit Veg Potato	14.30	14.42	15.69	15.58	59.98
Drinking water	0.01	0.04	0.17	0.04	0.26

	PFHxS	PFNA	PFOA	PFOS	Sum of PFASs
Other*	2.89	3.11	2.88	2.89	11.77

*Other = other food with detected levels (e.g. fat, sugar, condiments)

Table 22.5.2.1-6 Dietary exposure from different food groups (ng/kg bw per week) in 2-year-olds (n=1413) to PFOS, PFOA, PFNA and PFHxS and the sum of these four PFASs (upper bound values, unweighted OIM, based on VKM dataset).

	PFHxS	PFNA	PFOA	PFOS	Sum of PFASs
Fish	8.68	6.81	8.35	9.47	33.32
Shellfish	0.00	0.00	0.00	0.00	0.00
Meat	0.65	0.94	1.20	1.28	4.07
Dairy	23.73	28.64	37.59	35.51	125.47
Eggs	0.03	0.04	0.13	0.57	0.77
Grain	2.16	2.90	3.86	3.40	12.32
Fruit Veg Potato	13.28	13.50	15.44	15.27	57.49
Drinking water	0.07	0.10	0.33	0.11	0.61
Other*	0.72	0.85	0.70	0.71	2.99

*Other = other food with detected levels (e.g. fat, sugar, condiments)

Table 22.5.2.1-7 Dietary exposure from different food groups (ng/kg bw per week) in 1-year-olds (n=1957) to PFOS, PFOA, PFNA and PFHxS and the sum of these four PFASs (upper bound values, unweighted OIM, based on VKM dataset).

	PFHxS	PFNA	PFOA	PFOS	Sum of PFASs
Fish	0.61	0.89	1.12	1.12	3.75
Shellfish	0.00	0.00	0.00	0.00	0.00
Meat	6.97	8.95	11.14	10.46	37.52
Dairy	0.03	0.04	0.12	0.54	0.73
Eggs	1.59	2.13	2.88	2.52	9.13
Grain	8.76	9.13	11.28	10.89	40.06
Fruit Veg Potato	0.02	0.08	0.33	0.08	0.51
Drinking water	0.48	0.55	0.49	0.55	2.07
Other*	0.00	0.00	0.00	0.00	0.00

*Other = other food with detected levels (e.g. fat, sugar, condiments)

An overview of the UB mean contribution from food groups are shown in figure 22.5.2.1-1.

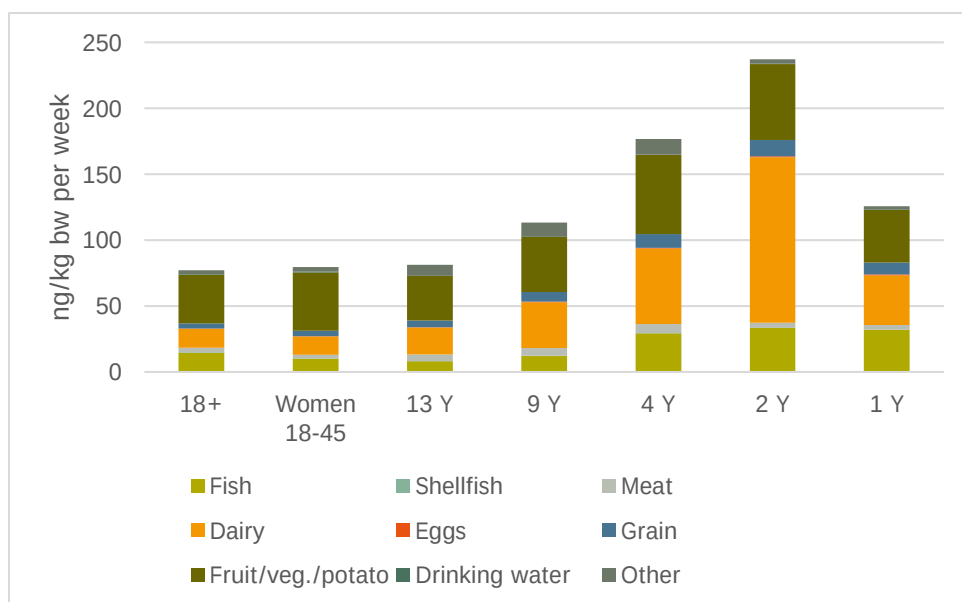


Figure 22.5.2.1-1 Contribution of food groups to the upper bound total dietary exposure of sum of 4 PFASs in adults (Norkost 3), 13- 9-, and 4-year-olds (Ungkost 3), 2- and 1-year-olds (Spedkost 3 and Småbarnskost 3). Intakes are based on mean OIM and concentrations in the VKM dataset.

22.6 Basis for focusing on the four PFASs PFOA, PFNA, PFHxS, and PFOS

A TWI for the sum of the four PFASs PFOA, PFNA, PFHxS, and PFOS has been set by EFSA (2020). Fish is a major contributor to intake of three out of four PFASs included in the TWI (PFOA, PFNA, PFOS). According to EFSA (2020, Table 10), the mean intake in adults was highest for PFOS, followed by PFBA, PFHxA, PFOA and PFHpA. The intakes of PFNA and PFHxS were considerably lower. Other food groups than fish had a more prominent contributing role for PFBA (starchy roots and tubers, although highly uncertain due to a very low number of samples), PFHxA (fruit and fruit products, drinking water, starchy roots and tubers), and PFHpA (drinking water, starchy roots and tubers). For PFHxS, which is part of sum 4 PFASs covered by the EFSA TWI, fruit and fruit products and drinking water were major contributors.

According to EFSA (2020) fish is also a major contributor to FOSA, PFPeA, PFDA, PFUnDA, PFDoDA, PFTrDA and PFTeDA intake. The concentration of these substances in fish from Norway was checked against a database that has previously been used as basis for dietary intake assessment (Papadopoulou et al., 2017). FOSA was detected above the LOQ in several samples of muscle of Greenland halibut, mackerel, salmon, as well as liver of cod. PFPeA was detected in 8 samples, of which 5 samples were from herring and 3 samples

were of cod liver. PFDA was detected in one muscle sample of halibut and 14 samples of cod liver. PFUnDA was detected in several muscle samples of halibut and Greenland halibut and in many cod liver samples. PFTrDA was not in the fish database. PFTeDa was detected in only one sample of Greenland halibut and one cod liver. Based on the above information, FOSA was the only PFAS with fish as a major source (in addition to PFOS, PFOA and PFNA) with quantified results in species that are more commonly consumed. The estimated intake of FOSA in adults is similar as PFNA according to EFSA's assessment, but the contribution of FOSA to concentration in human blood is much lower than for PFNA (EFSA 2020, Table 10 and Figure 10). This may reflect that FOSA is transformed to PFOS in the body.

Based on the above information indicating that the PFASs other than PFOA, PFNA, PFHxS, and PFOS are either not major contributors to total PFAS intake or not having fish as a major source, and also taking time and resources available into consideration, it was decided that intake of PFOA, PFNA, PFHxS, and PFOS (individually and as sum) is sufficient for the present benefit and risk assessment.

22.7 References

EFSA (2020), Risk to human health related to the presence of perfluoroalkyl substances in food. EFSA Journal 18:e06223. DOI: <https://doi.org/10.2903/j.efsa.2020.6223>.

Papadopoulou E., Poothong S., Koekkoek J., Lucattini L., Padilla-Sánchez J.A., Haugen M., Herzke D., Valdersnes S., Maage A., Cousins I.T., Leonards P.E.G., Småstuen Haug L. (2017) Estimating human exposure to perfluoroalkyl acids via solid food and drinks: Implementation and comparison of different dietary assessment methods. Environ Res 158:269-276. DOI: 10.1016/j.envres.2017.06.011.