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## Risk assessment of "other substances" – Collagen from fish skin

**Opinion of the Panel Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics of the Norwegian Scientific Committee for Food Safety**

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Opinion of the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact  
with Food and Cosmetics of the Norwegian Scientific Committee for Food Safety  
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# **Risk assessment of "other substances" – Collagen from fish skin**

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## **Assessed and approved**

The opinion has been assessed and approved by Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics. Members of the panel are: Inger-Lise Steffensen (Chair), Ellen Bruzell, Berit Granum, Ragna Bogen Hetland, Trine Husøy, Jens Rohloff, Trude Wicklund.

(Panel members in alphabetical order after chair of the panel)

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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 Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM) has, at the request of the Norwegian Food Safety Authority (Mattilsynet; NFSA), assessed the risk of "other substances" in food supplements and energy drinks sold in Norway. VKM has assessed the risk of doses in food supplements and concentrations in energy drinks given by NFSA. These risk assessments will provide NFSA with the scientific basis while regulating the addition of "other substances" to food supplements and other foods.

"Other substances" are described in the food supplement directive 2002/46/EC *as substances other than vitamins or minerals that have a nutritional and/or physiological effect*. It is added mainly to food supplements, but also to energy drinks and other foods. VKM has not in this series of risk assessments of "other substances" evaluated any claimed beneficial effects from these substances, only possible adverse effects.

The present risk assessment is based on a previous risk assessment of collagen from fish skin and articles retrieved from literature searches.

According to information from NFSA, collagen from fish skin is an ingredient in food supplements sold in Norway. The food supplements on the Norwegian market may contain collagen hydrolysate. NFSA has requested a risk assessment of 750 mg/day of collagen from fish skin in food supplements. The intake of collagen from fish skin was estimated for the age groups children (10 to <14 years), adolescents (14 to <18 years) and adults ( $\geq 18$  years).

Other sources of collagen from fish skin, such as foods and cosmetics, have not been included in the present risk assessment.

Collagen is the major insoluble fibrous protein in the extracellular matrix and in connective tissue in vertebrates. The various collagens and the structures they form all serve the same purpose, to help tissues withstand stretching. All collagens contain an abundance of the amino acids glycine, proline and hydroxyproline. Fish gelatins are produced by extraction and hydrolysis of fibrous, insoluble collagen from skin or bones. Collagen and gelatin hydrolysate are processed forms, which are more water-soluble.

No studies on metabolism of fish collagen, gelatin or collagen/gelatin hydrolysates in animals or humans have been found in the literature. However, as collagens or gelatins are proteins of variable solubility that will be partly absorbed from the gastrointestinal tract after digestion, it is anticipated that the absorbed parts will become building blocks of new proteins in the body. Hydroxyproline, which is a non-proteinogenic amino acid, will be metabolized to glycine and pyruvate and eventually oxidized.

There were no toxicity studies found on fish collagen or gelatin or collagen/gelatin hydrolysates in the general human population. A 2-year oral toxicity study in rats on effects

of marine collagen peptides prepared from chum salmon (*Oncorhynchus keta*) skin showed that there were no adverse effects of collagen up to 8.6 g/kg bw per day, which was the highest dose tested. One study on chromosomal aberrations and another study on allergic sensitization in Guinea pigs reported no effects of fish collagen.

The value used for comparison with the estimated exposure in the risk characterisation is the NOAEL of 8.6 g/kg bw per day taken from the chronic oral toxicity study in rats.

From a daily dose of 750 mg collagen from fish skin, the exposure is 17.3 mg/kg bw per day for children (10 to <14 years), 12.2 mg/kg bw per day for adolescents (14 to <18 years) and 10.7 mg/kg bw per day for adults (≥18 years).

The margin of exposure (MOE), the ratio of the NOAEL value to the exposure, was calculated. An acceptable MOE value based on an animal study is ≥100. For a daily intake of 750 mg/day of collagen from fish skin, the MOE values were above 100 for all age groups.

VKM concludes that it is unlikely that 750 mg/day of collagen from fish skin in food supplements causes adverse health effects in children (10 to <14 years), adolescents (14 to <18 years) or adults (≥18 years).

Collagen from fish has been identified as a fish allergen. Persons allergic to fish are therefore vulnerable and might experience adverse effects from fish collagen. Two studies in humans indicate that individuals allergic to fish may also have allergic reactions to fish gelatin, which is processed fish collagen.

### **Short summary**

The Norwegian Scientific Committee for Food Safety (VKM) has, at the request of the Norwegian Food Safety Authority, assessed the risk of intake of 750 mg/day of collagen from fish skin in food supplements.

The value used for comparison with the estimated exposure in the risk characterisation is the NOAEL of 8.6 g/kg bw per day taken from a chronic oral toxicity study in rats. The margin of exposure (MOE), the ratio of the NOAEL value to the exposure, was calculated. An acceptable MOE value based on an animal study is ≥100. For a daily intake of 750 mg/day of collagen from fish skin, the MOE values were above 100 for all age groups.

VKM concludes that it is unlikely that 750 mg/day of collagen from fish skin in food supplements causes adverse health effects in children (10 to <14 years), adolescents (14 to <18 years) or adults (≥18 years).

Collagen from fish has been identified as a fish allergen. Persons allergic to fish are therefore vulnerable and might experience adverse effects from fish collagen. Two studies in humans indicate that individuals allergic to fish may also have allergic reactions to fish gelatin, which is processed fish collagen.

**Key words:** Adverse health effect, collagen from fish skin, gelatin from fish skin, hydrolysed collagen/-gelatin, negative health effect, Norwegian Food Safety Authority, Norwegian Scientific Committee for Food Safety, other substances, risk assessment, VKM



# Sammendrag på norsk

På oppdrag for Mattilsynet har Vitenskapskomiteen for mattrygghet (VKM) vurdert risiko ved tilsetning av «andre stoffer» i kosttilskudd og energidrikk som selges i Norge. VKM har risikovurdert ulike bruksdoser oppgitt fra Mattilsynet. Disse risikovurderingene vil gi Mattilsynet vitenskapelig grunnlag for å regulere andre stoffer.

«Andre stoffer» er beskrevet i kosttilskuddsdirektivet 2002/46/EC som *stoffer som har en ernæringsmessig og/eller fysiologisk effekt, og som ikke er vitaminer og mineraler*. De tilsettes i hovedsak til kosttilskudd, men også til energidrikker og andre næringsmidler. I disse risikovurderingene har VKM ikke sett på påståtte gunstige helseeffekter, men kun vurdert mulige negative helseeffekter.

Denne rapporten er en risikovurdering av kollagen fra fiskeskinn, og den er basert på en tidligere risikovurdering og artikler hentet fra litteratursøk.

I følge informasjon fra Mattilsynet er kollagen fra fiskeskinn en ingrediens i kosttilskudd som selges i Norge. Kosttilskudd på det norske markedet kan inneholde hydrolysert kollagen. Oppdraget fra Mattilsynet var å risikovurdere inntak av 750 mg/dag av kollagen fra fiskeskinn i kosttilskudd for den generelle befolkningen fra 10 år og eldre.

Andre kilder til kollagen fra fiskeskinn, som mat og kosmetikk, er ikke inkludert i denne risikovurderingen.

Kollagen er det viktigste uløselige fibrøse proteinet i ekstracellulær matriks og bindevev i virveldyr. De ulike typene av kollagen hjelper kroppens vev til å tåle strekk. Alle typer kollagen har et høyt innhold av aminosyrene glycin, prolin og hydroksyprolin. Fiskegelatin produseres ved ekstraksjon og hydrolyse av kollagen fra skinn og bein. Kollagen- og gelatinhydrolysater er prosesserte former som er mer løselige i vann.

Ingen humane studier eller dyrestudier av metabolismen til fiske-kollagen, gelatin eller kollagen-/gelatin-hydrolysater ble funnet i litteraturen. Siden dette er proteiner med varierende løselighet som vil bli delvis absorbert fra mage-/tarm-kanalen etter fordøyelsen, er det forventet at de absorberte aminosyrene inngår som byggesteiner i nye proteiner i kroppen. Hydroksyprolin, som ikke er en naturlig aminosyre i protein, blir metabolisert til glycin og pyruvat og eventuelt oksidert.

Studier av metabolismen av fiske-kollagen, gelatin eller kollagen-/gelatin-hydrolysater ble ikke funnet, hverken på den generelle befolkningen eller i dyr.

Det ble ikke funnet studier av eventuelle toksiske effekter på den generelle befolkningen av fiske-kollagen, gelatin eller kollagen-/gelatin-hydrolysater. I en 2-års oral toksisitetstudie på rotter av marine kollagen-peptider, fremstilt fra hud fra 'chum salmon' (*Oncorhynchus keta*), ble det ikke funnet skadelige effekter av kollagen opp til 8,6 g/kg kroppsvekt per dag (høyeste testede dose). I en studie av kromosomavvik og en annen studie av allergisk sensitivisering i marsvin ble det ikke funnet effekter av fiske-kollagen.

I risikokarakteriseringen ble den estimerte eksponeringen sammenlignet med NOAEL-verdien (null-effektsnivå) på 8,6 g/kg kroppsvekt per dag fra den kroniske orale toksisitetsstudien i rotter.

Det estimerte inntaket fra en dose på 750 mg/dag av kollagen fra fiskeskinn var 17.3 mg/kg kroppsvekt for barn (10 til <14 år), 12.2 mg/kg kroppsvekt for ungdom (14 til <18 år) og 10.7 mg/kg kroppsvekt for voksne.

VKM har beregnet eksponeringsmargin ('margin of exposure' (MOE)), som er ratio mellom NOAEL-verdien og eksponeringen. En akseptabel MOE-verdi for en risikovurdering som er basert på NOAEL fra en dyrestudie er  $\geq 100$ . De beregnede MOE-verdiene for de ulike aldersgruppene var over 100.

VKM konkluderer med at det er usannsynlig at et daglig inntak av 750 mg kollagen fra fiskeskinn fører til skadelige helseeffekter hos barn (10 til <14 år), ungdom (14 til <18 år) eller voksne ( $\geq 18$  år).

Kollagen fra fisk har blitt identifisert som et fiske-allergen. Personer med fiskeallergi kan derfor være sårbare for negative effekter av fiske-kollagen. I to studier av fiskeallergikere indikerte resultatene at personer som var allergiske mot fisk også kunne få allergiske reaksjoner mot fiske-gelatin, som er prosessert fiske-kollagen.

### **Kort sammendrag**

På oppdrag fra Mattilsynet har Vitenskapskomiteen for mattrygghet (VKM) vurdert risiko ved daglig inntak av 750 mg kollagen fra fiskeskinn som kosttilskudd.

VKM har beregnet eksponeringsmargin ('margin of exposure' (MOE)), som er ratio mellom NOAEL-verdien og eksponeringen. En akseptabel MOE-verdi for en risikovurdering som er basert på NOAEL fra en dyrestudie er  $\geq 100$ . De beregnede MOE-verdiene for de ulike aldersgruppene var over 100.

VKM konkluderer med at det er usannsynlig at et daglig inntak av 750 mg kollagen fra fiskeskinn fører til skadelige helseeffekter hos barn (10 til <14 år), ungdom (14 til <18 år) eller voksne ( $\geq 18$  år).

Personer med fiskeallergi kan være sårbare for negative effekter av fiske-kollagen. I to studier av fiskeallergikere indikerte resultatene at fiske-gelatin kan være et allergen i denne befolkningsgruppen.

# Abbreviations and glossary

## Abbreviations

bw	- body weight
EFSA	- European Food Safety Authority
MCP	- marine collagen peptides
MOE	- margin of exposure
NFSA	- Norwegian Food Safety Authority [ <i>Norw.</i> : Mattilsynet]
NOAEL	- no observed adverse effect level
VKM	- Norwegian Scientific Committee for Food Safety [ <i>Norw.</i> : Vitenskapskomiteen for Mattrygghet]
WHO	- World Health Organization

## Glossary

"Other substances": a substance other than a vitamin or mineral that has a nutritional or physiological effect (The European Parliament and the Council of the European Union, 2006).

"Negative health effect" and "adverse health effect" are broad terms. VKM uses the definition endorsed by EFSA for "adverse effect": a change in morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other influences (EFSA, 2006; WHO, 1994).

# Background as provided by the Norwegian Food Safety Authority

«Other substances» are substances other than vitamins and minerals, with a nutritional and/or physiological effect on the body. "Other substances" are mainly added to food supplements, but these may also be added to other foods and beverages, such as sports products and energy drinks. Ingestion of these substances in high amounts presents a potential risk for consumers.

In Norway, a former practice of classification of medicines had constituted an effective barrier against the sale of potentially harmful "other substances". Ever since this practice was changed in 2009, it has become challenging to regulate and supervise foods with added "other substances". Meanwhile, in the recent years, the Norwegian market has witnessed a marked growth in the sales of products containing "other substances". In 2011, food supplements containing "other substances" constituted more than 50% of the market share.

While within the European Economic Area, these substances fall under the scope of the European Regulation (EC) No. 1925/2006 on the addition of vitamins, minerals and certain other substances to foods and the European Regulation (EC) No 258/97 concerning novel foods and novel food ingredients, "other substances" remain largely unregulated. In order to ensure safe use of "other substances" many countries have regulated their use at a national level. For example, Denmark regulates these substances in a positive list, i.e. a list of substances with maximal daily doses, permitted for use in food supplements and other foods (FVM, 2014).

The Norwegian Food Safety Authority (NFSA) is working on the establishment of a regulation on the addition of "other substances" to foods at a national level. The regulation will include a list of substances with permitted maximal doses, based on the substances and doses found in products on the Norwegian market. In preparation for a regulation, NFSA has therefore requested the Norwegian Scientific Committee for Food Safety (VKM) to assess the safety of "other substances" found on the Norwegian market. NFSA, in consultation with the industry, has compiled a list of "other substances" found in products marketed in Norway. Only substances with a purity of minimum 50% or concentrated 40 times or more have been included in the list. Substances regulated by other legislations like those for novel foods, food additives, flavourings, foods for special medical purposes etc., have been excluded from the list.

# Terms of reference as provided by the Norwegian Food Safety Authority

The Norwegian Food Safety Authority (NFSA) has requested the Norwegian Scientific Committee for Food Safety (VKM) to assess the safety of collagen from fish skin in food supplements at the following dose: 750 mg per day.

NFSA requested VKM to assess the safety of "other substances" (in accordance to the guidance document developed in Phase 2) at the doses specified (Phase 3). The safety assessments of "other substances" present in food supplements shall be carried out for the general population, ages 10 years and above.

# Assessment

## 1 Introduction

"Other substances" are described in the food supplement directive 2002/46/EC *as substances other than vitamins or minerals that have a nutritional or physiological effect*, and may be added to food supplements or e.g. energy drinks (The European Parliament and the Council of the European Union, 2006).

This risk assessment regards the substance collagen from fish *per se*, and no specific products.

VKM has in this series of risk assessments of "other substances" not evaluated documentation of any claimed beneficial effects from these substances, but merely possible adverse effects at specified doses used in Norway. Thus, potential high intake consumer groups of the substance may not be identified and included in this assessment.

According to information from the Norwegian Food Safety Authority (NFSA), collagen from fish skin is an ingredient in food supplements purchased in Norway. NFSA has requested a risk assessment of the intake of 750 mg collagen from fish skin per day from food supplements. The total exposure to collagen from fish skin from other sources than food supplements, such as foods and cosmetic products, is not included in the risk assessment.

Collagen is the major insoluble fibrous protein in the extracellular matrix and in connective tissue (Lodish et al., 2000). There are at least 16 types of collagen, 80-90% of the collagen in the body consists of type I, II and III, and type I and III are the most common types in the skin. The various collagens and the structures they form all serve the same purpose, to help tissues withstand stretching. All collagens contain three-stranded helical segments of similar structure. The triple-helical structure of collagen arises from an abundance of three amino acids: glycine, proline and hydroxyproline. The collagen molecules are packed together to form long, thin fibrils of similar structure.

Fish collagen can be isolated from fish skin. Typical fishes used are tilapia, tuna and perch (EFSA, 2004). Type I collagen was the main collagen retrieved by extraction of collagen from brown backed toadfish (*Lagocephalus gloven*) and leather jacket (*Odonus niger*) fish skin (Muralidharan et al., 2013; Senaratne et al., 2006). Collagen hydrolysate was prepared from cod (*Gadus microcephalus*) skin by enzymatic hydrolysis (Shigemura et al., 2014).

Collagen from fish has been identified as a fish allergen (Hamada et al., 2001). Persons allergic to fish are therefore vulnerable and might experience adverse effects from fish collagen. Two studies in humans indicate that individuals allergic to fish may also have

allergic reactions to fish gelatin, which is processed fish collagen (Hansen et al., 2004; Sakaguchi et al., 2000).

Fish gelatins are produced by extraction and hydrolysis of fibrous, insoluble collagen from skin or bones (EFSA, 2004). Fish gelatin is commonly used in food and pharmaceutical products replacing mammalian gelatins. Based on the assumed concentrations of fish gelatin in different foods given by industry, the exposure levels are expected to be up to 1 g per day (EFSA, 2004). When collagen was denatured to gelatin by heating in boiling water for 120 minutes, the collagen retained 90% of its original binding ability to the IgE in three human sera (Hamada et al., 2001). Gelatin allergenicity must, therefore, be considered in the discussion of collagen allergenicity.

By further processing of gelatin, involving breakdown (hydrolysis) of the protein structure in smaller parts, collagen/gelatin hydrolysate is produced. Collagen/gelatin hydrolysates are more water-soluble than collagen and gelatin.

In this risk assessment, the intake of 750 mg collagen from fish skin per day from food supplements is assessed.

# 2 Hazard identification and characterisation

## 2.1 Literature

The present risk assessment is based on a previous risk assessment of collagen from fish and articles retrieved from literature searches.

### 2.1.1 Previous risk assessments

#### **Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to a notification from Givaudan Schweiz AG on fish gelatine used as carrier for flavour pursuant to Article 6 paragraph 11 of Directive 2000/13/EC (EFSA, 2004)**

EFSA was requested to consider the likelihood of adverse reactions triggered in susceptible individuals by the consumption of fish gelatin used as carrier for flavour.

Based on the assumed concentrations of fish gelatin in different foods given by industry, the exposure levels were expected to be up to 1 g per day.

Experimental studies found that the IgE reactivity of collagen from fish was very thermostable and was preserved also in peptide fragments (Hamada et al., 2001; Hamada et al., 2003). When collagen was denatured to gelatin by heating in boiling water for 120 minutes, the collagen retained 90% of its original binding ability to the IgE in three human sera (Hamada et al., 2001). Therefore, gelatin allergenicity must be considered in the discussion of collagen allergenicity. Fish collagens from different species appear to be broadly cross-reactive, and it seems reasonable to treat fish collagens from different species as similar hazard.

Sakaguchi et al. (2000) investigated the presence of specific IgE to fish gelatin in children with fish allergy. Specific IgE to fish gelatin was found in

- 3 out of 10 patients with fish allergy and specific IgE to fish
- 2 out of 2 patients with fish and bovine gelatin allergy and specific IgE to fish and bovine gelatin
- 5 out of 15 patients with atopic dermatitis and specific IgE to fish

All patients with specific IgE to gelatin also had specific IgE to fish. It was unclear how the gelatin used was prepared, and therefore, whether it may have been contaminated by fish flesh. A strong cross-reactivity was observed between gelatins from different fish, but no cross-reactivity was observed between fish gelatin and bovine gelatin.



In a double-blind placebo-controlled food challenge study by Hansen et al. (2004), thirty fish-allergic patients aged 9 to 50 years were included. Skin prick test and histamine release tests were performed with fish gelatin made from codfish skin and with fresh raw codfish. Codfish specific IgE was measured. None of the patients reacted to the cumulative dose of 3.61 g of fish gelatin. The authors concluded that the NOAEL for fish gelatin in this study was 3.61 g (see 2.4.6 for full description of the study).

EFSA concluded that the scientific data provided by the applicant were insufficient to predict the likelihood of adverse reactions in fish-allergic individuals. Nevertheless, taking all the information into account, EFSA considered that it was not very likely that fish gelatin, under the conditions of use specified by the applicant, would cause a severe allergic reaction in the majority of fish-allergic individuals. The reasoning behind this conclusion by EFSA is not clear to VKM.

## **2.1.2 Literature search**

### ***2.1.2.1 Search strategy***

To retrieve publications on adverse effects caused by collagen from fish skin, three literature searches were performed in Embase and Medline. The search strategies are included in Appendix 1.

### ***2.1.2.2 Publication selection and data extraction***

In the primary screening, titles and abstracts of all publications retrieved were independently screened by two persons against the inclusion criteria checklist.

#### **Inclusion criteria checklist:**

- Adverse effects in relation to the substance alone are addressed
- Route of exposure for humans is oral
- Route of exposure for animals is oral, in addition, subcutaneous exposure is included if the toxicokinetics is equal to oral exposure
- Human studies are performed in apparently healthy individuals or patient groups assumed to have normal absorption and metabolism of the assessed substance
- Animal model studies address adverse effects relevant to human health

The inclusion criteria checklist was developed by members of the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics and the Panel on Nutrition, Dietetic Products, Novel Food and Allergy. Articles that did not appear to meet the inclusion criteria were excluded from further analysis. In situations where it was unclear whether the publication was of relevance to the study, it was retained for further screening. The primary screening was performed independently by two persons.

The full text of articles that passed the primary screening was retrieved for secondary screening. In this screening, the full text articles were reviewed and compared against the inclusion criteria checklist. The secondary screening was performed by one person.

The secondary screening resulted in 4 publications. Additionally, 2 publications from a manual search were identified and included. A final total of 6 publications were identified and included in the results in this report (see Figure 2.1.2.1-1).

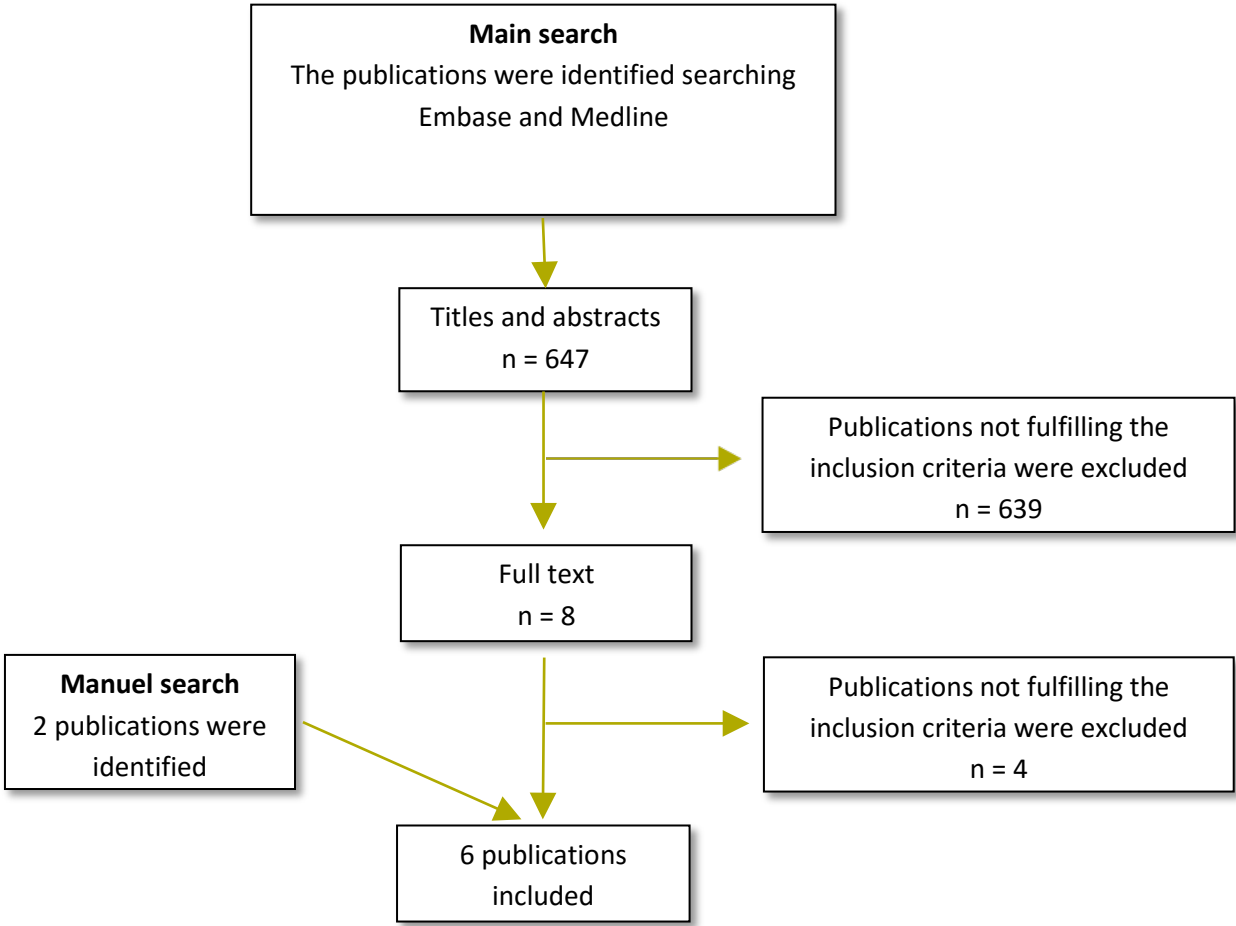


Figure 2.1.2.1-1: Flowchart for literature search for collagen from fish skin and the subsequent publication selection.

## **2.2 General information**

### **2.2.1 Chemistry**

Collagen is the major insoluble fibrous protein in the extracellular matrix and in connective tissue (Lodish et al., 2000). The amino acid composition of collagen deviates substantially from that of other structural proteins in an animal organism. Various fish collagens have shown about equal content of proline and hydroxyproline each of about 11%. Alanine content is about twice as high, about 25% (Piez et al., 1969). The various collagens and the structures they form all serve the same purpose, to help tissues withstand stretching. There are at least 16 types of collagen, but 80-90% of the collagen in the body consists of type I, II and III, where type I and III are the most common types in the skin. All collagens contain three-stranded helical segments of similar structure. The triple-helical structure of collagen arises from an unusual abundance of the amino acids glycine, proline and hydroxyproline. The characteristic repeating motif can be glycine-proline-X and glycine-X-hydroxyproline, where X is any amino acid other than glycine, proline or hydroxyproline. Hydrogen bonds linking the peptide amino group of a glycin residue with a peptide carbonyl group in an adjacent peptide help hold the three chains together. These collagen molecules are packed together to form long, thin fibrils of similar structure.

Fish gelatins are produced by extraction and hydrolysis of fibrous, insoluble collagen from skin or bones. Collagen/gelatin hydrolysates are more processed forms of collagen or gelatin.

### **2.2.2 Occurrence**

Collagen is the most abundant protein in vertebrates, it constitutes about 25% of the total protein (Ogawa et al., 2004), and is therefore a normal constitute of foods.

## **2.3 Absorption, distribution, metabolism and excretion (ADME)**

Collagen and gelatin are proteins of variable solubility that will be partly absorbed from the gastrointestinal tract after digestion. Collagen and gelatin hydrolysate are processed forms, which are more water-soluble.

Used as a nutritional supplement, the role of the gelatin will mainly be as a supply of amino acids. Most amino acids in collagen may be used in protein synthesis. This is not the case for hydroxyproline which is a non-proteinogenic amino acid produced from proline after incorporation into a peptide chain by post-translational hydroxylation. Most dietary hydroxyproline appears to be absorbed in small peptides by the so-called IMINO system transporters (Broer et al., 2009). Absorbed hydroxyproline will be oxidized in the body after conversion to glycine and pyruvate (Wu et al., 2011).

No human or animal studies on metabolism and excretion of collagen, gelatin or collagen/gelatin hydrolysates from fish were found. However, as collagen and gelatin are proteins of variable solubility that will be partly absorbed from the gastrointestinal tract, it is anticipated that the absorbed parts will become building blocks of new proteins in the body.

### **2.3.1 In humans**

#### Collagen/gelatin hydrolysate

To measure the absorption of the collagen hydrolysate from cod skin, the concentration of hydroxyproline-containing peptides in human blood was determined after ingestion of the collagen hydrolysate (Shigemura et al., 2014). Healthy volunteers (two females and two males, average age 27 years) fasted for 12 hours before ingesting 30.8, 153.8 and 384.6 mg/kg bw of collagen hydrolysate dissolved in 100 ml of water (that is 2, 10 and 25 g for a 65 kg person). All four volunteers ingested the three different doses of collagen hydrolysate with a week-long washout between the ingestions. Approximately 10 ml blood was collected from each participant before and 15, 30, 60, 120, 240 and 360 min after ingestion. The hydroxyproline-containing peptide levels in human plasma were measured. A dose-dependent increase of free hydroxyproline in plasma was found after ingestion of collagen hydrolysate.

The quantity and structures of food-derived gelatin hydrolysates in human blood from three sources of type I collagen were compared by Ohara et al. (2007) in a single-blind crossover study. Five healthy male volunteers ingested type I gelatin hydrolysates from fish scale, fish skin or porcine skin after 12 hours of fasting. Amounts of free form hydroxyproline and hydroxyproline-containing peptide were measured over a period of 24 hours. Hydroxyproline-containing peptides comprised approximately 30% of all detected hydroxyproline. For free form hydroxyproline and for hydroxyproline-containing peptide, the AUC<sub>0-24 h</sub> varied in order of fish scale gelatin hydrolysate ≥ porcine skin gelatin hydrolysate ≥ fish skin gelatin hydrolysate. Proline-hydroxyproline was a major constituent of hydroxyproline-containing peptides. The quantity and structure of hydroxyproline-containing peptides in human blood after oral administration of gelatin hydrolysate depended on the gelatin source.

### **2.3.2 In animals**

#### Collagen

The digestion and absorption of collagen, native and artificially cross-linked, were examined in rats by measuring the hydroxyproline content of the faeces and the gut (Harkness et al., 1978). The results showed that native collagen was digested and absorbed in the small intestine.

#### Gelatin

Healthy female Sprague-Dawley rats (500-600 g, n = 6/dose) were given an oral dose of gelatin (4000 mg/kg bw) or hydroxyproline (400 mg/kg bw) either orally or intravenously (Wang et al., 2015). The control groups were given oral or intravenous dose of isometric normal saline. Blood samples were collected at 0.5, 1, 2, 4, 8 and 24 hours after treatment. The plasma concentration of hydroxyproline was determined at each time point. A negligible amount of hydroxyproline in plasma was observed before ingestion of gelatin. After ingestion of gelatin, the hydroxyproline concentration in plasma increased significantly and reached a maximum after 4 hours, which indicated that gelatin was digestible and absorbed from the gastrointestinal tract. The relative and the absolute bioavailability were measured. The relative bioavailability is the hydroxyproline in blood from ingested gelatin relative to hydroxyproline in blood from ingested hydroxyproline. The absolute bioavailability is the hydroxyproline in blood from ingested gelatin relative to hydroxyproline in blood from hydroxyproline given intravenously. The relative and the absolute bioavailability of gelatin was 74% and 86%, respectively.

## **2.4 Toxicological data/Adverse effects**

### **2.4.1 Genotoxicity**

The induction of chromosomal aberrations was studied in CHL/IU cells (a cell line consisting of fibroblasts derived from the lungs of newborn female Chinese hamsters) exposed to fish collagen (produced by solubilized tilapia skin) at concentrations of 1.3, 2.5 and 5 µl/ml for a short treatment (time not indicated) with and without metabolic activation, and for 24 hours without metabolic activation (chromosomal aberration test ISO 10993-3:2003) (Yamamoto et al., 2014). There were no significant differences in structural or numerical chromosomal aberrations between treatment groups and control.

### **2.4.2 Human studies**

No toxicity studies of collagen from fish skin on the general human population were found.

#### ***2.4.2.1 Interactions***

There was no information concerning interactions in the literature reviewed in the present risk assessment. The absence of information in the selected literature does not document an absence of interactions.

#### ***2.4.2.2 Allergic sensitisation (including adjuvant effects)***

Parts of the population that can be especially affected by fish collagen are persons that are allergic to fish. However, no studies were found that investigated the sensitisation in the general population. The only publications found were studying allergic elicitation, and these are described in the section "Vulnerable groups" (2.4.6).

### 2.4.3 Animal studies

An acute systemic toxicity test (ISO 10993-11:2006) was performed by Yamamoto et al. (2014). Fish collagen gel extracted in physiological saline or sesame oil for 72 hours at 37°C was used. The extract was injected once into the caudal vein (physiological saline) or abdominal cavity (sesame oil) in five Crlj:CD1 male mice (50 mL/kg). In the control group (five mice), extract solvents (physiological saline and sesame oil) were injected using similar methods to those applied in the experimental group. The animals were observed at 4, 24, 48 and 72 hours after injection. No deaths were observed. Normal general condition, normal changes in weight and normal autopsy findings were observed in both experimental and control animals.

A two year chronic oral toxicity study of marine collagen peptides (MCP) prepared from Chum Salmon (*Oncorhynchus keta*) skin was performed by Liang et al. (2012). Two hundred four weeks old Sprague Dawley rats were randomly assigned to five groups (20 animals/sex/group). Control rats were fed a basal rodent diet containing about 25% protein, 4% fat, 5% fiber and 60% carbohydrate. Rats in the experimental groups were fed 2.25%, 4.5%, 9% and 18% (wt/wt) MCP added to the control diet, respectively. The food intake and body weight of the individual animals were recorded periodically until sacrifice. The MCP intake of 2.25%, 4.5%, 9% and 18% MCP-treated groups were estimated to be 1.063, 2.216, 4.609 and 8.586 g/kg bw per day for females, and 0.907, 1.798, 3.418, 6.658 g/kg bw per day for males, respectively. Blood and urine samples were collected for serum chemistry evaluations and urinalysis. There was no toxicologically significant difference between the vehicle and MCP-treated animals with respect to the survival rate, body weight, food consumption, urinalysis, clinical biochemistry parameter, including serum levels of alanine aminotransferase, aspartate aminotransferase, albumin, total protein, blood urea nitrogen and creatinine, and relative organ weight in either sex.

Regarding non-neoplastic lesions, most lesions related to inflammatory changes and age-related degenerations (fibrosis, emphysema, atrophy and hyperplasia) were found in all groups. Compared with the control group, the incidence of hepatocyte fatty vacuolation decreased in MCP-treated groups of both sexes, but without statistical significance ( $P > 0.05$ ). No significantly increased incidence of pathologic change or lesion was observed in MCP-treated groups compared with the control group ( $P > 0.05$ ).

No significant MCP-related difference was indicated from the relative organ weights of brain, heart, lungs, liver, spleen, kidneys, adrenal glands, testis or ovaries.

Overall, the effects seen in the study were incidental and not treatment-related. VKM derived a NOAEL from this study of 8.586 g/kg bw per day, which was the highest dose tested in the study.

#### **2.4.3.1 Allergic sensitisation (including adjuvant effects)**

Sensitisation of fish collagen (produced by solubilized tilapia skin) was investigated using a Guinea pig maximisation test (sensitization test ISO 10993-10:2010). Ten Scl:Hartley male guinea pigs (5 weeks old) were injected intracutaneously with 0.1 ml/site of 0.1% fish collagen with Freund's complete adjuvant. Seven days after intracutaneous sensitisation, 10 w/w% sodium lauryl sulphate ointment as an inflammatory chemical was openly applied to the same sensitised site and removed after 24 hours. Subsequently, 0.2 ml/site of 0.1% fish collagen was applied for 48 hours to induce sensitisation. Fourteen days after sensitisation, 0.1 ml/site of either 0.1% fish collagen or sterile water was applied for 24 or 48 hours. As a positive control, 0.1 w/w% 2,4-dinitrochlorobenzene was used, and skin reactions such as swelling and/or erythema was observed. With the negative control, no skin reactions were observed. No skin reactions were observed at the sites treated with 0.1% fish collagen or control water (Yamamoto et al., 2014).

#### **2.4.4 *In vitro* studies**

In a study by Kuzan et al. (2015), the cytotoxicity of fish collagen was measured in human fibroblast from gingiva. Fish collagen was extracted from the skin of silver carp at 16 °C. The fibroblasts were treated for 96 hours with 0.1%, 0.5% and 1.0% experimental collagen formulation to induce endogenous collagen production. Cell viability was assessed by measuring mitochondrial activity in MTT assay (a colorimetric assay for assessing cell metabolic activity) after 24 hours followed by 24 hours of incubation with experimental collagen formulation. Qualitative analysis was performed by immunocytochemical staining of collagen type I and III. Preparations of fish collagen were not cytotoxic at concentrations below 1%.

The effect of various kinds of gelatins, from porcine skin, cold water fish skin and bovine bone, on murine cell proliferation *in vitro* was studied by Kojima et al. (2001). The magnitude of suppression of the proliferation by cold water fish skin or bovine bone gelatin was lower than that by porcine skin gelatin. Bovine bone gelatin stimulated proliferation of murine spleen cells. The magnitude of stimulation of the proliferation by cold water fish skin gelatin was lower than that by bovine bone gelatin. Porcine skin gelatin slightly suppressed proliferation of murine spleen cells. Porcine skin gelatin induced apoptosis but not necrosis of MH134 tumor cells. Cold water fish skin gelatin induced weaker apoptosis of the cells than porcine skin gelatin. DNA histogram indicated that porcine skin and cold water fish skin gelatins acted on MH134 tumor cells to increase ratios of cells in G2 + M-phase.

#### **2.4.5 Vulnerable groups**

A part of the population that can be especially affected by collagen from fish skin are the persons allergic to fish. Although 90% of the allergenicity seems to remain when collagen is extracted and hydrolysed to gelatin, it is not known how hydrolysis of collagen or gelatin will affect the allergenic properties of the substances.

A randomized, double-blinded, placebo-controlled oral challenge study was performed to evaluate the allergenicity of commercial, food-grade fish gelatin in clinically fish-allergic individuals (Hansen et al., 2004). Thirty clinically fish-allergic patients (15 females and 15 males, age 9-50 years) were included in this study. All kept a strict diet avoiding fish and hidden fish according to the dietician's advice. The fish gelatin used was specifically purified gelatin with an average MW of 60000 Da and was prepared from skins of codfish. A titrated double-blind placebo-controlled food challenge was performed with fish gelatin and placebo on separate days (>48 h) and only when patients were free from symptoms. Active challenge preparations contained doses from 0.01 g up to 7 g of fish gelatin (cumulated 0.01, 0.11, 0.61, 1.61, 3.61, 7.61, 14.61 g). Prior to challenge, an intravenous line was inserted and relevant baseline parameters recorded. Every dose was given with an interval of 30 min. Skin prick tests and histamine release tests were performed with fish gelatin and codfish, and codfish-specific IgE was measured in all patients. In all 30 patients, skin prick test, histamine release tests and specific IgE to codfish were positive. Skin prick test and histamine release tests with fish gelatin were positive in 3 out of 30 and 7 out of 30, respectively. One patient showed mild reaction to placebo and no reaction to the active challenge. Two patients reported mild subjective reactions to active challenge. A confirmed, mild subjective reaction was elicited in 1 of 30 clinically codfish-allergic subjects by the ingestion of a cumulative dose of 7.61 g of fish gelatin derived from codfish skins. According to the author the no-observed-adverse-effect level (NOAEL) in this study was a cumulative dose of 3.61 g of fish gelatin, which amounts to 52 mg/kg bw per day for an adult of 70 kg.

In a study by Sakaguchi et al. (2000), the presence of IgE antibody to fish gelatin was investigated in children with fish allergy. Serum samples were taken from patients stratified into three different groups: (1) 10 patients with fish allergy and specific IgE to fish meat; (2) two patients with allergies to both fish meat and bovine gelatin and specific IgE to fish meat and bovine gelatin; (3) 15 patients with atopic dermatitis and specific IgE to fish meat. Various fish gelatins (denaturated type I collagen) were prepared from skin of the fish species tuna, salmon, saurel and mackerel. Of 10 patients with fish allergy, 3 had specific IgE to fish gelatin. Of 2 patients with fish allergy and bovine gelatin allergy, all had specific IgE to fish gelatin. Of 15 patients with atopic dermatitis and specific IgE to fish meat, 5 had specific IgE to fish gelatin. The results indicate that fish gelatin (denaturated type I collagen) might be an allergen in subjects with fish allergy.

## **2.5 Summary of hazard identification and characterisation**

Collagen is the major insoluble fibrous protein in the extracellular matrix and in connective tissue in vertebrates. The various collagens and the structures they form all serve the same purpose, to help tissues withstand stretching. All collagens contain an abundance of the amino acids glycine, proline and hydroxyproline. Fish gelatins are produced by extraction and hydrolysis of fibrous, insoluble collagen from skin or bones. Collagen and gelatin hydrolysates are processed forms, which are more water-soluble.



No studies on metabolism of fish collagen, gelatin or collagen/gelatin hydrolysates in animals or humans have been found in the literature. However, as collagen or gelatins are proteins of variable solubility that will be partly absorbed from the gastrointestinal tract after digestion, it is anticipated that the absorbed parts will become building blocks of new proteins in the body. Hydroxyproline, which is a non-proteinogenic amino acid, will be metabolized to glycine and pyruvate and eventually oxidized.

There were no toxicity studies found on fish collagen or gelatin or collagen/gelatin hydrolysates in the general human population. A 2-year oral toxicity study in rats on effects of marine collagen peptides prepared from chum salmon (*Oncorhynchus keta*) skin showed that there were no adverse effects of collagen up to 8.6 g/kg bw per day, which was the highest dose tested. One study on chromosomal aberrations and another study on allergic sensitization in Guinea pigs reported no effects of fish collagen.

Collagen from fish has been identified as a fish allergen (Hamada et al., 2001). Persons allergic to fish are therefore vulnerable and might experience adverse effects from fish collagen. Two studies in humans indicate that individuals allergic to fish may also have allergic reactions to fish gelatin, which is processed fish collagen.

The value used for comparison with the estimated exposure in the risk characterization is 8.6 mg/kg bw per day based on the 2-year oral toxicity study in rats (Liang et al., 2012).

# 3 Exposure / Intake

NFSA requested VKM to perform a risk assessment of 750 mg/day of collagen from fish skin as food supplement for children (10 years and above), adolescents and adults. The default body weights (bw) for these age groups determined by EFSA were used to calculate the intake for these age groups (EFSA, 2012): 10 to <14 years; 43.4 kg, 14 to <18 years; 61.3 kg and adults ( $\geq 18$  years); 70.0 kg.

From a daily dose of 750 mg collagen from fish skin, the daily exposure is 17.3 mg/kg bw per day for children (10 to <14 years), 12.2 mg/kg bw per day for adolescents (14 to <18 years), and 10.7 mg/kg bw per day for adults ( $\geq 18$  years) (Table 3.1-1).

**Table 3.1-1** The estimated exposure of children, adolescents and adults from collagen from fish skin in food supplements.

Group	Daily dose (mg)	Body weight (kg)	Exposure (mg/kg bw per day)
<b>Children (10 to &lt;14 years)</b>	750	43.4	17.3
<b>Adolescents (14 to &lt;18 years)</b>	750	61.3	12.2
<b>Adults (<math>\geq 18</math> years)</b>	750	70.0	10.7

## 3.1 Other sources

Collagen is the most abundant protein in vertebrates and constitutes about 25% of the total protein, and is therefore a normal constituent of foods (Ogawa et al., 2004).

EFSA reported that, based on the assumed concentrations of fish gelatin in different foods given by industry, the exposure levels are expected to be up to 1 g per day (EFSA, 2004).

Isolated collagen is used in cosmetics (CosIng, 2015); however, the source of the collagen used is not reported in the CosIng database. Gelatin is commonly used in e.g. food, pharmaceutical products and cosmetics (Ogawa et al., 2004).

## 4 Risk characterisation

NFSA requested VKM to perform a risk assessment of 750 mg/day of collagen from fish skin in food supplements for the general population, ages 10 years and above.

The value used for comparison with the estimated exposure in the risk characterization is 8.6 g/kg bw per day taken from a chronic oral toxicity study in rats (Liang et al., 2012).

From a daily dose of 750 mg collagen from fish skin, the daily exposure is 17.3 mg/kg bw for children (10 to <14 years), 12.2 mg/kg bw for adolescents (14 to <18 years) and 10.7 mg/kg bw for adults (≥18 years) (Table 3.1-1).

Using the MOE approach, for a daily intake of 750 mg/day of collagen from fish in food supplements and a NOAEL value of 8.6 g/kg bw per day (8586 mg/kg bw per day) from the rat study, the margins of exposure are 496, 704 and 802 for children (10 to <14 years), adolescents (14 to <18 years) and adults (≥18 years), respectively (Table 4.1-1).

**Table 4.1-1** The calculated margins between the NOAEL and the exposure to collagen from fish skin in food supplements (MOE values) for the various age groups.

Age groups	Margin of exposure (MOE)
Children (10 to <14 years)	496
Adolescents (14 to <18 years)	704
Adults (≥18 years)	802

For a daily intake of 750 mg/day of collagen from fish skin, the MOE values are above 100 for all age groups. VKM concludes that it is unlikely that 750 mg/day of collagen from fish skin in food supplements causes adverse health effects in children (10 to <14 years), adolescents (14 to <18 years) or adults (≥18 years).

Persons already sensitized and allergic to fish may potentially be vulnerable to the effects of collagen and gelatin from fish.

# 5 Uncertainties

## 5.1 Hazard identification and characterisation

Several limitations in the dataset adds to the uncertainty in the risk assessment:

- The NOAEL was derived from a chronic oral toxicity study in rats at the highest dose tested. Therefore, the real NOAEL for fish collagen might be higher.
- No human or animal studies on metabolism of fish collagen, gelatin or collagen/gelatin hydrolysates were found.
- No toxicity studies on fish collagen in the general human population were found.
- It is not known how hydrolysis of collagen or gelatin will affect the allergic properties of the substances.

## 5.2 Exposure

With use of the default (mean) body weight of an age (population) group, the variance in all individuals in the group will not be covered. Dividing children, adolescents and adults in different age groups minimizes the uncertainty. For individuals with a body weight below the default body weight the actual exposure will be higher than the calculated, whereas for individuals with body weight above the default body weight the actual exposure will be below the calculated. Individuals with body weights less than the default estimate in a given age group are not fully covered in the risk estimate.

## 6 Conclusions with answers to the terms of reference

The Norwegian Scientific Committee for Food Safety (VKM) has, at the request of the Norwegian Food Safety Authority, assessed the risk of collagen from fish (750 mg/day) in food supplements. The present risk assessment is based on a previous risk assessment and articles retrieved from literature searches.

The value used for comparison with the estimated exposure in the risk characterisation is the NOAEL of 8.6 g/kg bw per day taken from a chronic oral toxicity study in rats (Liang et al., 2012).

The value used for comparison with the estimated exposure in the risk characterisation is the NOAEL of 8.6 g/kg bw per day taken from the chronic oral toxicity study in rats.

From a daily dose of 750 mg collagen from fish skin, the daily exposure is 17.3 mg/kg bw per day for children (10 to <14 years), 12.2 mg/kg bw per day for adolescents (14 to <18 years), and 10.7 mg/kg bw per day for adults ( $\geq 18$  years).

The margin of exposure (MOE), the ratio of the NOAEL value to the exposure, was calculated. An acceptable MOE value based on an animal study is  $\geq 100$ . For a daily intake of 750 mg/day of collagen from fish skin, the MOE values are above 100 for all age groups.

VKM concludes that it is unlikely that 750 mg/day of collagen from fish skin in food supplements causes adverse health effects in children (10 to <14 years), adolescents (14 to <18 years) or adults ( $\geq 18$  years).

Collagen from fish has been identified as a fish allergen (Hamada et al., 2001). Persons allergic to fish are therefore vulnerable and might experience adverse effects from fish collagen. Two studies in humans indicate that individuals allergic to fish may also have allergic reactions to fish gelatin, which is processed fish collagen (Hansen et al., 2004; Sakaguchi et al., 2000).

An overview of the conclusions is presented in Table 6.1. Estimated exposures unlikely to cause adverse health effects (below the value for comparison) are shown in green.

**Table 6.1** An overview of the conclusions on collagen from fish skin in food supplements. Green: estimated exposure to collagen from fish skin is unlikely to cause adverse health effects.

Collagen from fish skin	
Food supplement	750 mg/day
Age groups	
Children (10 to <14 years)	
Adolescents (14 to <18 years)	
Adults (≥18 years)	

# 7 Data gaps

No human or animal studies on metabolism or excretion of fish collagen, gelatin or collagen/gelatin hydrolysates were found.

There were no toxicity studies found on fish collagen or gelatin or collagen/gelatin hydrolysates in the general human population.

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# 1 Appendix

Database: Embase <1974 to 2016 January 07>, Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily, Ovid MEDLINE(R) and Ovid OLDMEDLINE(R) <1946 to Present>

Search Strategy:

- 
- 1 fish skin\*.mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kw, nm, kf, px, rx, an, ui] (594)
  - 2 collagen\*.mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kw, nm, kf, px, rx, an, ui] (468557)
  - 3 1 and 2 (107)
  - 4 collagen\* (ti)
  - 5 3 and 4 (44)

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Database: Embase <1974 to 2016 January 19>, Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily, Ovid MEDLINE(R) and Ovid OLDMEDLINE(R) <1946 to Present>

Search Strategy:

- 
- 1 hydrolyse\*.mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kw, nm, kf, px, rx, an, ui] (20138)
  - 2 hydrolyze\*.mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kw, nm, kf, px, rx, an, ui] (63289)
  - 3 fish\*.mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kw, nm, kf, px, rx, an, ui] (495020)
  - 4 1 or 2 (82751)
  - 5 3 and 4 (1076)
  - 6 (risk\* or safety or adverse or side-effect\*1 or hazard\* or harm\* or eosinophil\* or negative or contraindicat\* or contra-indicat\* or interact\* or toxicity or toxic).tw. (9707016)
  - 7 5 not 6 (869)
  - 8 limit 7 to (danish or english or norwegian or swedish) (825)
  - 9 remove duplicates from 8 (541)

\*\*\*\*\*

Database: Embase <1974 to 2016 July 28>, Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) <1946 to Present>

Search Strategy:

- 1 fish skin\*.mp. (637)
  - 2 gelatin\*.mp. (129338)
  - 3 1 and 2 (99)
  - 4 remove duplicates from 3 (62)
- \*\*\*\*\*