National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Post-launch monitoring of foods and supplements with Krill oil and oil from microalgae Schizochytrium sp.

RIVM Letter report 2014-0114

M. Brosens et al.

National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Post-launch monitoring of foods and supplements with Krill oil and oil from microalgae Schizochytrium sp.

RIVM Letter report 2014-0114

M. Brosens et al.

Colophon

Marinka Brosens, RIVM
Maryse Niekerk, RIVM
Liesbeth Temme, RIVM
Joop van Raaij, RIVM

Contact:
Joop van Raaij
VPZ
joop.van.raaij@rivm.nl

This investigation has been performed by order and for the account of the Netherlands Food and Consumer Product Safety Authority (NVWA), within the framework of knowledge question 9.4.32

This is a publication of:

National Institute for Public Health and the Environment
P.O. Box 1│3720 BA Bilthoven
The Netherlands
www.rivm.nl/en

Publiekssamenvatting

Voedingsmiddelen en -supplementen met krillolie en olie uit een microalg: Monitoring na de productlancering

Sinds enkele jaren zijn er voedingssupplementen en producten op de markt die olie uit de microalg Schizochytrium sp. bevatten en krillolie, afkomstig van een kreeftachtig schaaldier. Deze oliën zijn rijk aan de vetzuren EPA en DHA, ook wel bekend als 'gezonde' visvetzuren. Uit onderzoek van het RIVM blijkt dat het gebruik van supplementen of voedingsmiddelen met deze bestanddelen geen risico's voor de gezondheid heeft. Alleen bij een extreem hoge inname zou de strengste grens die voor deze ingrediënten bestaat bij 10 procent van de oudere kinderen en volwassenen kunnen worden overschreden. Dit scenario is echter niet realistisch.

Het onderzoek betrof producten die op de Nederlandse markt beschikbaar zijn: per mei 2014 waren er 25 voedingssupplementen die hoofdzakelijk krillolie bevatten en 8 met microalgolie. Er zijn geen voedingsmiddelen met krillolie gevonden, wel drie met de DHA-rijke olie uit de genoemde microalg. Dit zijn voornamelijk maaltijdvervangers en -repen.

De grens die de EFSA (European Food Safety Authority) hanteert (5 gram per dag) voor volwassenen is in de uitgewerkte scenario's niet bereikt. Het Duitse Federal Risk Assessment Agency werkt met een strengere aanvaardbare bovengrens van 1,5 gram per dag. Die werd alleen overschreden bij het *worst case*-scenario waarbij een consument de Nederlandse richtlijn voor visconsumptie volgt (450 milligram per dag), dagelijks een supplement met EPA en DHA inneemt (645 milligram), en alle beschikbare producten gebruikt die met EPA en DHA zijn verrijkt. Mogelijke schadelijke gevolgen van de veel EPA en DHA zijn bloedingen, verminderde immuunfunctie, en verminderde vet- en glucosestofwisseling.

Voor dit onderzoek is de methode gebruikt die het RIVM heeft ontwikkeld om in kaart te brengen welke producten een bepaald ingrediënt bevatten en in welke hoeveelheden dat ingrediënt erin zit (*post-launch monitoring*). Deze procedure wordt gebruikt voor zogenoemde nieuwe voedingsmiddeleningrediënten. Dat zijn producten die na mei 1997 op de Europese markt zijn verschenen en waarvoor is beoordeeld of ze veilig zijn voordat ze op de markt mogen worden toegelaten. Met de post-launch monitoring wordt getoetst of de dagelijkse toelaatbare hoeveelheid van het toegestane ingrediënt daadwerkelijk niet wordt overschreden.

Kernwoorden: monitoring na productlancering – krillolie – microalgolie – EPA en DHA – aanvaardbare bovengrens

Abstract

Post-launch monitoring of foods and food supplements enriched with Krill oil and oil from microalgae

Since a few years foods and food supplements that contain oil from microalgae *Schizochytrium sp*. and Krill oil, have been introduced to the Dutch market. Krill are small shrimp-like crustaceans.
The oils are rich of EPA and DHA, known as 'healthy' fish fatty acids. Studies from the National Institute for Public Health and the Environment (RIVM) reveal that the consumption of foods and food supplements with these fatty acids will not do any harm to our health. Only at extreme high intakes the most conservative tolerable upper level would be surpassed by 10 percent of the older children and adults. Such a scenario is little realistic.

At least 25 food supplements with krill oil and 8 food supplements with DHA rich oil from microalgae *Schizochytrium sp.* could be identified on the Dutch market per May 2014. No foods with krill oil and 3 foods with DHA rich oil from microalgae were found, mainly meal replacers and meal bars.

The dose that EFSA (European Food Safety Authority) considers as safe to adults (5 gram per day) is not reached by children and adults. The German Federal Risk Assessment Agency deals with a more conservative tolerable upper level of 1,5 gram per day. That level is only surpassed in the worst-case scenario in which the consumer follows the Dutch guideline for fish consumption (450 milligram per day), consumes a daily supplement with EPA and DHA (at a level of 645 milligram per day), and consumes all available products enriched with EPA and DHA. Adverse effects in humans associated with high intakes of EPA and DHA comprise bleeding episodes, impaired immune function, increased lipid peroxidation, and impaired lipid and glucose metabolism.

To perform this research (RIVM) developed a procedure to monitor in what foods and to what levels an ingredient has been added (post-launch monitoring). The method can be applied to novel food ingredients, so foods that are introduced to the European market after May 1997 and for which it is required to assess whether they are safe before introduction to the market. With post-launch monitoring it might be tested whether the daily tolerable level of the novel ingredient will indeed not be exceeded.

Keywords: post-launch monitoring – Krill oil – oil microalgae – EPA and DHA – tolerable upper level

Contents

Summary

The National Institute for Public Health and the Environment (RIVM) developed a procedure to monitor in what foods and to what levels an ingredient is added (concept of post-launch monitoring). The method can be applied to foods that were introduced on the European market after May 1997 and for which it is required to assess whether they are safe for introduction on the market, so-called novel foods and novel food ingredients. Two of these novel food ingredients are krill oil and EPA and DHA rich oil from microalgae *Schizochytrium sp*. Krill are small shrimp-like crustaceans.

We monitored the presence of these oils in foods on the Dutch market in May 2014. At least 25 food supplements with krill oil and 8 food supplements with DHA rich oil from microalgae *Schizochytrium sp.* could be identified. No foods with krill oil and 3 foods with DHA rich oil from microalgae were found. Concentration levels of EPA and DHA were obtained from labels and/or websites. In order to be able to calculate the daily EPA and DHA intake, we also made an inventory of EPA and DHA food supplements and EPA and DHA enriched foods in which the fish fatty acids came from non-novel oils.

The EPA and DHA intakes were assessed by using the food consumption data from the Dutch National Food Consumption Surveys (DNFCS): the DNFCS-Young Children 2005/2006, for children aged 2-6 years, and the DNFCS 2007-2010, for children and adults in the range of 7-69 years. Median intake of EPA and DHA with the current food consumption is 8 mg/day for children 2-6 years and 33 mg/day for children and adults 7-69 years. A worst-case scenario for possibly too high intakes of EPA and DHA was designed for which it is assumed that everyone meets the recommendation for fish consumption (450 mg/day), that everyone consumes a supplement with EPA and DHA (300 mg/day in worst-case-1 and 645 mg/day in worst-case-2 scenario), and that everyone consumes the already available products enriched with EPA and DHA. Median intake of EPA and DHA in the worst-case-1 scenario is 870 mg/day (1215 mg/day in worst-case-2) for children 2-6 years, and 941 mg/day (1286 mg/day in worst-case-2) for children and adults 7-69 years.

EFSA (European Food Safety Authority) states that combined intakes of EPA and DHA at doses up to 5 g/day do not raise safety concerns for adults. In our scenarios none of the children or adults reached this level. The German Federal Risk Assessment Agency works with a more conservative tolerable upper level of 1.5 g/day. In our scenarios, less than 0.5% of the children of 2-6 years exceed this upper level. This is also true for older children and adults on the worst-case-1 scenario. Only in the very extreme worst-case-2 scenario the upper level of 1.5 g/day was exceeded by 10% of the older children and adults. In conclusion, with the present availability of EPA and DHA containing supplements and enriched foods, including those with oils from Krill and microalgae, there is no reason to fear that the increased intake levels of EPA and DHA may do any harm.

1 Introduction

Three types of omega-3 fatty acids are involved in human physiology: ALA (alpha-linolenic acid), EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid). EPA and DHA are essential components of phospholipids in cell membranes and play an important role in the functioning of hormones, proteins and enzymes (1). Unfortunately, humans only have a limited capacity to synthesize EPA from ALA (found in plant oils) and an even more limited capacity to synthesize DHA from EPA (2). Therefore, EPA and DHA need to be present in the daily diet and are considered as essential nutrients. EPA and DHA are commonly found in marine oils and are usually indicated as fish fatty acids. Important sources of EPA and DHA include fish (oil), algae (oil), and krill (oil) (3). Krill are small shrimp-like crustaceans (4).

Adequate Intake Level
The Health Council of the Netherlands recommends a consumption of 450 mg omega-3 fish fatty acids (EPA and DHA) per day for adults, which equals to two servings of fish per week, at least one of them being a portion of oily fish (100-150 g fish per serving) (5). The Health Council of the Netherlands decided to join the recommendations of the American Dietary Guidelines Advisory Committee and the English Scientific Advisory Committee on Nutrition (450 mg omega-3 fish fatty acids per day) (6). According to these committees, there are indications that such high fish consumption might lead to a reduction of mortality risk for coronary heart diseases. Based on the same background literature on cardiovascular risks, the Food and Agriculture Organization of the United Nations (FAO) and the European Food Safety Authority (EFSA) recommend a lower consumption of 250 mg of EPA and DHA per day for adults (7, 8).

Tolerable Upper Intake Level
Adverse effects in humans associated with high intakes of EPA and DHA comprise bleeding episodes, impaired immune function, increased lipid peroxidation, and impaired lipid and glucose metabolism (9). EFSA did not establish a Tolerable Upper Intake Level (UL) for EPA and DHA, because available data were not sufficient (9). However, EFSA stated that supplemental intakes of EPA and DHA combined at doses up to 5 g/day do not raise safety concerns for adults. Unlike EFSA, the German Federal Risk Assessment Agency (in the absence of EU advice) and the US Food and Drug Administration (FDA) established a tolerable upper intake level for omega-3 fatty acids EPA and DHA of respectively 1.5 g/day and 3 g/day (10, 11). The German Federal Risk Assessment Agency based this recommendation on the risk of bleeding reported in one study among children (10). The US FDA established an intake of 3 g/day as generally recognized as safe (GRAS) based upon its review of the safety of EPA and DHA omega-3 fatty acids and its review of the scientific evidence (11).

Novel foods
The European Regulation 258/97 defines novel foods as food products and food ingredients that have not been consumed to a significant degree in the European Union before May 1997. A number of novel foods or novel food ingredients have indeed been introduced to the European market since 1997 according to the European Regulation 258/97. Two of these novel food ingredients are krill oil and EPA and DHA rich oil obtained from microalgae *Schizochytrium sp*.

Krill oil is a lipid extract from Antarctic Krill *Euphausia superba* (12). Krill are at the base of a complex food chain. Krill are recognized as an emerging source of omega-3 fatty acids. Since October 2009, the lipid extract from Antarctic Krill *Euphausia superba* is authorized (12). Extension of uses of oil from Antarctic Krill (*Euphausia superba*) by other companies was authorized from 2012 onwards (13).

An alternative source of omega-3 fatty acids is EPA and DHA rich oil from micro-algae. Since June 2003, placing oil rich in DHA from the microalgae Schizochytrium sp. is authorized (14). Extension of uses of algal oil from the microalgae Schizochytrium sp. as a novel food ingredient by other companies was authorized from 2009 onwards (15-17).

Since more companies are introducing krill oil and EPA and DHA rich oil from microalgae on the Dutch market and are applying these novel food ingredients in an increasing number of food categories, it is conceivable that the daily intake of these fish fatty acids will increase, and may even exceed the tolerable upper level.

Post-launch monitoring and scenarios

Recently, we developed an approach to monitor which novel foods or novel food ingredients are introduced to the Dutch market and to what level (18). The method also allows a quantitative estimate of the daily intake of the novel food or novel food ingredient.

In the present study, we applied this methodology to krill oil and EPA and DHA rich oil. We monitored which foods with krill oil and with EPA and DHA rich oil are introduced to the Dutch market and to what level (post-launch monitoring). In addition, we estimated the expected intake of EPA and DHA in the Dutch population (based on the Dutch Food Consumption Surveys 2005-2006 and 2007-2010) for the current food consumption and for two worst-case scenarios. In the worst-case scenarios we assumed a maximum use of foods with krill oil, a maximum use of EPA and DHA rich oil, a maximum use of EPA and DHA enriched foods and we assumed that the recommendations for fish consumption are being followed. Worst-case scenarios are instruments to help to judge whether there are no health risks when consuming foods with krill oil or EPA and DHA rich oil.

We describe the methodologies as used for post-launch monitoring of and for assessing the expected habitual intakes of krill oil, and EPA and DHA rich oil in Chapter 2. The results of the monitoring and the intake calculations are reported in Chapter 3. In Chapter 4, we discuss the findings and draw conclusions.

2 Methods

2.1 Method of post-launch monitoring

The method of post-launch monitoring has been described previously (18). Briefly, a flow-chart was followed consisting of seven steps (Appendix I). In the first four steps, the foods with krill oil and with EPA and DHA rich oil as available on the Dutch market were identified. The last three steps deal with quantification of the level of krill oil and of EPA and DHA rich oil in foods and food supplements. With respect to the last three steps, we again focussed on the Dutch market.

Since we were interested in EPA and DHA intake in worst-case situations, we were also interested in other EPA and DHA enriched foods (not classified as novel foods). Therefore, we searched on the internet for all these types of foods and for their concentration levels of EPA and DHA.

2.2 Method to estimate the intake of EPA and DHA

The method to estimate the intake of EPA and DHA in the Dutch population consisted of the following steps: 1) description of the food intake and food composition data used; 2) description of the scenario under study; and 3) description of the method for intake estimation.

In the first step, data from the Dutch National Food Consumption Survey (DNFCS) were used. Data from the DNFCS-Young Children 2005/2006 (19) for children aged 2-6 years and the DNFCS 2007-2010 (20) for children and adults in the age range from 7 to 69 years were used. The DNFCS-Young Children includes 1279 children. Food consumption was collected by means of dietary records on two non-consecutive days. In addition, a written general questionnaire, asking for among others on dietary supplement use and consumption frequency of certain specific foods, was filled out. The DNFCS 2007-2010 contains information on 3819 participants. Food consumption was estimated by means of 24-hour recalls on two non-consecutive days. Furthermore, data were collected by means of a general questionnaire on paper or online. The questionnaire consisted amongst others of general characteristics of the diet, frequency of consumption of specific foods (e.g. fish) and dietary supplements. The consumed foods and recipes were entered in EPIC-Soft® software (recently presented with a new name GloboDiet). From the food consumption data we looked for food products and food supplements in which krill oil or EPA and DHA rich oil can be found. Furthermore, food products that were enriched with EPA and DHA were selected. The categorization of food groups was based on EPIC-Soft food groups and detailed food codes (NEVO) in these groups (21).

In the next step, a worst-case-1 scenario was designed and compared to the current consumption of food products and food supplements (according to DNFCS data used). In this worst-case-1 scenario, it was assumed that all consumed margarines, bread and eggs were enriched with EPA and DHA. Furthermore, it was assumed that all subjects consumed the advised daily amount of 450 mg fish fatty acids (Table 1). Therefore, we decided to exclude the current fish consumption. In addition, it was assumed that all consumed meal replacements and nutrition bars were replaced by meal replacements and by nutrition bars with DHA rich oil from microalgae Schizochytrium sp.. We assumed that all subjects consumed a food supplement with DHA rich oil from microalgae Schizochytrium sp. containing 300 mg EPA and DHA. Therefore, we decided to exclude the current consumption of supplements containing EPA and DHA. For the remaining foods, current consumption was used in the analysis. In the worst-case-2 scenario, the assumptions were similar to the worst-case-1 scenario except for the amount of EPA and DHA obtained from the food supplement with DHA rich oil from microalgae Schizochytrium sp.. We checked for available fish oil supplements in the Netherlands and 7 out of the 60 identified supplements had a daily dose of more than 300 mg (550, 500, 520, 500, 450, 645 and 380 mg EPA and DHA; highest concentration 645 mg). Therefore, in the worst-case-2 scenario the food supplement contained 645 mg EPA and DHA (instead of 300 mg) (see Table 1). The assumptions and decisions made in the selection process of food products and food supplements for the worst-case scenarios are described in Appendix II.

In the third step, all relevant consumed food products and food supplements were multiplied by the concentration levels of EPA and DHA. Consumed amounts (in grams) were multiplied by the concentration level per food. For supplements, consumed amounts (in number of tablets) were multiplied by the concentration levels in the supplements.

Table 1: Characteristics of the scenarios.

Worst-case-1 scenario	**Worst-case-2 scenario**
Current consumption of foods and supplements but excluding fish and (fish oil) supplements containing EPA and DHA*	Idem
Enriched EPA and DHA sources: margarine, bread and eggs. All margarines, (brown and multigrain) bread and eggs consumed are enriched with EPA and DHA. Highest reported concentration levels are 204 mg/100g eggs, 680mg/100g egg yolk, 750mg/100g margarine and 68 mg/100g bread.	Idem
All meal replacements and nutrition bars replaced by maximum dosage found in meal replacements (260 mg/meal replacement) and nutrition bars (106 mg/bar) with DHA rich oil from microalgae Schizochytrium sp.	Idem
Advised fish fatty acids consumption 450 mg EPA and DHA	Idem
Maximum dosage found in food supplements with DHA rich oil from microalgae Schizochytrium sp. 300 mg EPA and DHA	Maximum dosage found in food supplements with DHA rich oil from microalgae Schizochytrium sp. 645 mg EPA and DHA

*) For explanation, see text

To assess habitual dietary intake of EPA and DHA, statistical models as implemented in the Monte Carlo Risk Assessment (MCRA) program version 8.0 (22) were used. MCRA is a program for risk assessment of chemicals in the diet.

The Observed Individual Mean (OIM) approach was used to estimate a mean EPA and DHA intake over the two consumption days. These mean values were used for further risk calculations. We do realize that it would be better to base risk calculations on estimated *habitual* intakes, but the models available within MCRA for doing so (logistic-normal normal (LNN) model and the Model-Then-Add approach) could not be used because the application conditions could not be met.

Uncertainty in the assessments of intake was quantified using bootstrap analysis (generating 100 food consumption and 100 concentration bootstrap samples). The 95% confidence interval around the estimated intake at various percentiles (50, 95 and 99) indicates the degree of uncertainty. All results were weighted for small deviances in socio-demographic characteristics, days of the week and season of data collection, in order to present results representative for all days of the week, all seasons and the Dutch population.

The outcome of the intake calculations were compared with the tolerable upper level as used by the German Federal Risk Assessment Agency (1.5 g/day (10)) and with 'the upper cut off point' as given by EFSA (5.0 g/day of EPA and DHA combined (9)).

3 Results

3.1 Post-launch monitoring of krill oil and of DHA rich oil from microalgae

Per May 2014 at least 25 food supplements with krill oil were available on the Dutch market and more than 8 food supplements with DHA rich oil from microalgae *Schizochytrium sp*. We could not identify foods with krill oil but we found 3 foods with DHA rich oil from microalgae *Schizochytrium sp*. (all products claim for weight loss: soup, milkshake and chocolate bar).Identification flow is summarized in Figure 1. Detailed information about the search is described in paragraphs 3.1.1 – 3.1.4.

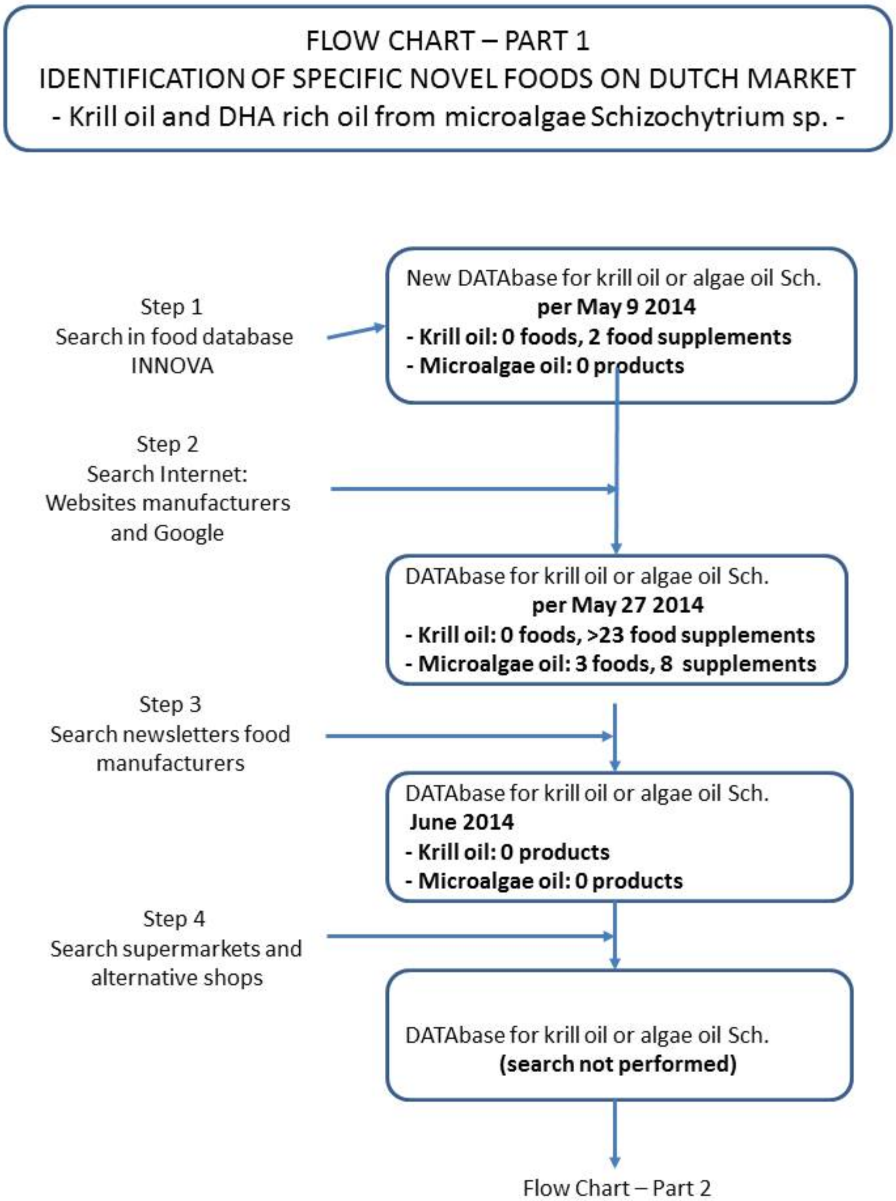

Figure 1: Identification of krill oil and DHA rich oil from microalgae Schizochytrium sp. on the Dutch market

3.1.1 *Search in food database INNOVA*

Food products containing krill oil are labelled with text 'lipid extract from the crustacean Antarctic Krill *(Euphausia superba)*'(12). Food products containing the novel food DHA rich oil from microalgae *Schizochytrium sp.* are labelled with 'oil from the microalgae *Schizochytrium sp.*'(15).

In April 2014, we searched the INNOVA database in all food categories (except for pet food and oral care) (23). We have access to information from The Netherlands and some other European countries. We used the following key words: krill, schizochytrium, algae + DHA, algae + EPA, algae oil. We also used other searching methods within INNOVA database: ingredient search (search in ingredients list: omega-3) and positioning search (position on the market, products with: DHA and omega-3).
We found several products in Europe, mostly food supplements. In the Netherlands, we found 2 supplements for krill oil and nothing for DHA rich oil from microalgae *Schizochytrium sp.*

3.1.2 *Search at internet*

Krill
We checked whether the food supplements with krill oil found in INNOVA database are still on the market by searching websites of the producers. This was indeed the case. In addition, these websites were checked for other new products with krill oil. However, no additional products from the same brands were found.

We searched the internet using the search engine Google. We used the key word 'krill'. We found food supplements with krill oil in web shops. Some were temporarily out of stock. For each item the brand's website was checked. We considered supplements that were out of stock as not applicable in our database if the product was not mentioned on the brand's website or no brand website was available.
We found 19 supplements on the Dutch market. In addition, brand websites were further checked for other new products with krill oil. With this search, we found an extra 4 supplements.

We searched in four web shops. By Google we found an additional three web shops with 10 potential krill oil supplements. Since the various web shops in general have the same brands and also have similar levels of krill oil on the label, we decided to stop with further searching for web shops and krill supplements.

Oil from microalgae Schizochytrium sp.
Using Google as search engine on the internet for the microalgae resulted in less useful hits than our krill search. We used key words 'ingredient schizochytrium' and 'oil microalg schizochytrium sp', which resulted in food supplements in web shops and one food brand website (products for weight loss). For each item the brand's website was checked. We regarded supplements that were out of stock as not applicable in our database if the product was not mentioned on the brand's website or no brand website was available. For 8 products the label/website did not mention the type of algae used and these were considered not applicable.

Thus, we found 7 food supplements and 1 food product (meal replacement for weight care). After checking brand websites for more products with oil from microalgae *Schyzochytrium sp.* we found 2 additional meal replacements and 1 food supplement.

3.1.3 *Search newsletters food manufacturers*

We searched in May 2014 for newly introduced food products at news sites VMT, FoodHolland and Nutrion insight. No specific brands were mentioned. This resulted in no extra products with krill or DHA rich oil.

3.1.4 *Search in supermarkets and shops*

We did not visit supermarkets, pharmacies or other shops to look for more information, since with the web analysis we could only trace a few foods, and we did not expect much result from such an intensive exercise. It is conceivable that pharmacies and shops supply supplements with krill oil or with EPA and DHA rich oil with a private label. However, in that case, we expect that these will contain similar amounts of krill oil and EPA/DHA as in the supplements we already had found.

3.2 Concentration levels of EPA and DHA in foods and supplements with krill oil or DHA rich oil from microalgae Schizochytrium sp.

Concentration levels of EPA and DHA were reported at the labels of the food supplements or foods and we included them in our database (Figure 2). In total, concentration levels of 3 foods and 8 food supplements with DHA rich oil and 25 food supplements with krill oil were taken into account.

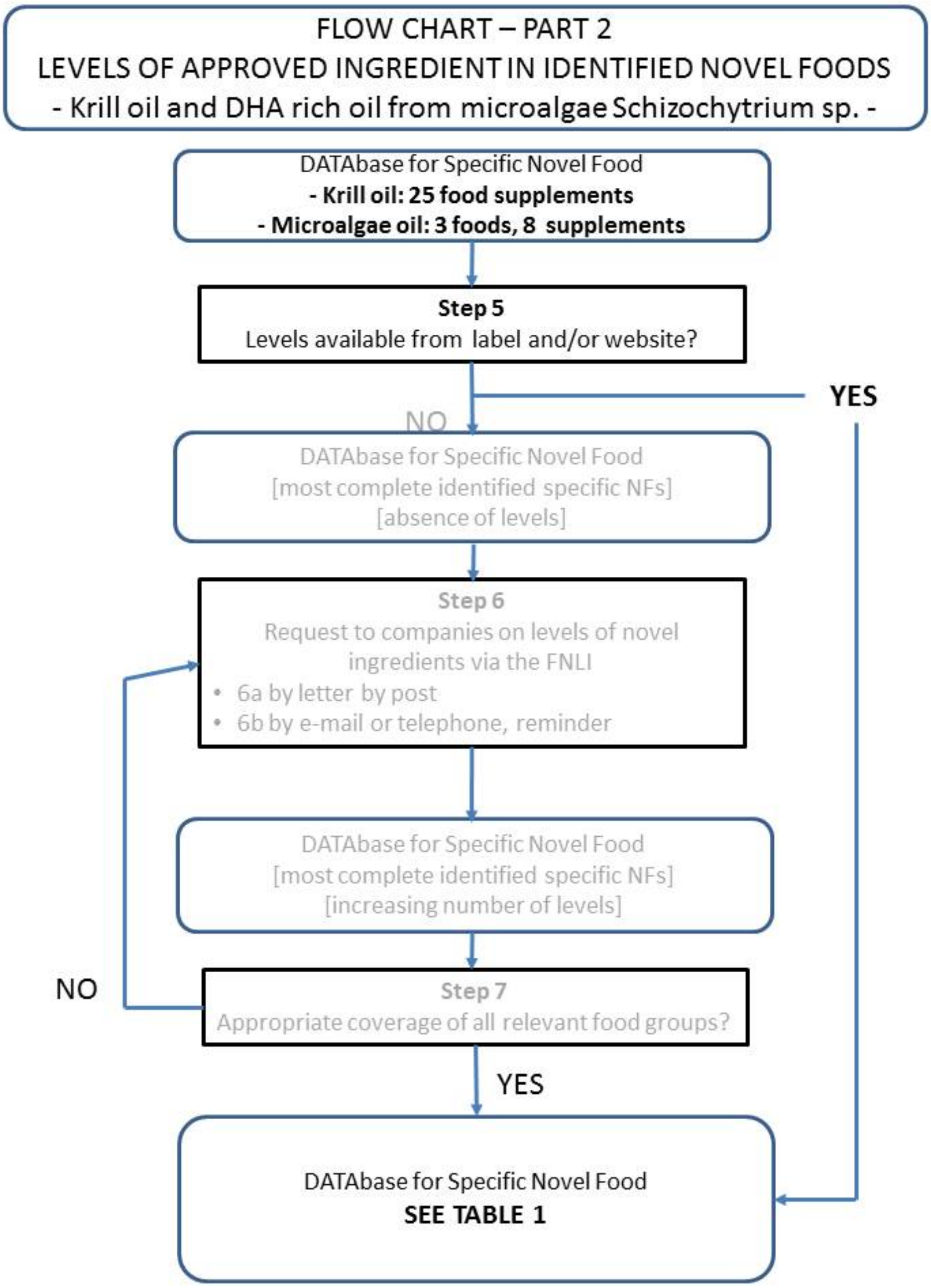

Figure 2: Concentration levels of EPA and DHA in identified novel foods.

In almost all food supplements with krill oil, the source of EPA and DHA was krill only (except for two that contained fish oil as well). The source of DHA in food supplements with microalgae schizochytrium sp. is 100% algae and did not contain fish oil. Table 2 shows the reported levels by the manufacturer in food supplements, foods intended for weight reduction and nutrition bars. Krill oil and DHA rich oil from microalgae schizochytrium sp. are authorized in more groups, however these were not found on the Dutch market. An overview of these food groups in which krill oil and DHA rich oil from microalgae schizochytrium sp. are authorized is presented in Appendix III (12-17).

Table 2: Food groups in which krill oil and DHA rich oil from microalgae is authorized, including the levels of EPA and DHA reported by the manufacturer.

	EC Classification	Reported target group	Reported level of oil *Mean (Min.-Max.)*	Reported level of EPA+DHA *Mean (Min.-Max.)*
Krill oil	Food supplements (n=25)	Children (n=2)	440 mg krill oil per daily dose (320-600 mg)	182 mg per daily dose (49-252 mg)
		No defined target group (n=23)	880 mg krill oil per daily dose (200-1000 mg; 200 mg, n=1 supplement with krill and fish oil; 1000 mg, n=11; missing daily dose n=6)	182 mg per daily dose (105-296 mg) For 1000 mg krill oil: 190 mg (155-240 mg)
DHA rich oil from microalgae schizochytrium sp.	Food supplements (n=8)	Children (n=2)	-	200 mg per daily dose (200-200 mg)
		No defined target group or adults (n=6)	-	224 mg per daily dose (150-300 mg)
	Foods intended for use in energy-restricted diets (n=2)	No defined target group	-	255 - 260 mg per meal replacement
	Cereal-/nutrition bars (n=1)	No defined target group	-	106 mg per bar

EC=European Commission

3.3 Concentration levels of EPA and DHA enriched foods

Searching on the internet for EPA and DHA enriched foods resulted in three types of foods: eggs, margarines and bread. These food products are not classified as novel foods since the EPA and DHA oil used is derived from non-novel sources. The highest reported concentration levels of EPA and DHA added to the food categories are shown in Table 3. These concentration levels have been used in the worst-case scenarios. In the calculation of the current consumption, we used the concentration levels per food as observed without adding EPA and DHA. In the second column mean concentration levels per food category are shown.

Table 3: Concentration levels of EPA and DHA in food categories without and after adding EPA and DHA to the foods

Food categories	*Current consumption measured in the DNFCS* Mean concentration levels without adding EPA and DHA	*Worst-case scenarios* Highest concentration levels after adding EPA and DHA
Eggs	38 mg/100g (n=2)	204 mg/100g (n=1)
Egg yolk	109 mg/100g (n=2)	680 mg/100g (n=1)
Margarines	17.9 mg/100g (n=28)	750 mg/100g (n=2)
Bread	1.4 mg/100g (n=19)	68 mg/100g (n=1)

DNFCS=Dutch National Food Consumption Survey

3.4 Intake estimation of EPA and DHA

Mean intake of EPA and DHA was estimated for the current consumption and the worst-case scenarios.

The median intake of EPA and DHA in the current consumption situation was 8 mg/day among children aged 2-6 years and 33 mg/day among children and adults aged 7-69 years (Table 4). The tolerable upper level (1.5 g/d) was exceeded by 0.3% among young children and by 2.3% among children and adults aged 7-69 years. Given that the P95 values are respectively 307 mg/day and 915 mg/day (Table 4), much smaller than the tolerable upper level 1.5 g/day, the obtained exceedings indicate long tails in the distribution of intakes of EPA and DHA. Nevertheless, the 'upper cut off point' (5 g/d) was not exceeded.

The worst-case-1 scenario resulted in a median of 870 mg/day EPA and DHA intake among children aged 2-6 years and a median of 941 mg/day among children and adults aged 7-69 years. The worst-case-2 scenario resulted in medians of 1215 and 1286 mg/day, respectively. Notice that the distribution for the worst-case scenarios (as reflected by the distance between the P50 and P95 values) is much narrower than the distribution for the current consumption. This is partly because in the worst-case scenarios the advised amount of fish fatty acids (450 mg/day) and the amounts provided by supplements (300 mg/day and 645 mg/day, respectively) are fixed and for everybody the same. Therefore, extreme values as observed in the current consumption situation will not be present in the worst-case scenarios. As a consequence it might happen that the exceeding rates of the tolerable upper level for the worst-case scenario are even lower than in the current consumption, although the absolute level of the distribution is substantially higher (Table 4). In the worst-case-2 scenario, the percentage of the population that exceeded the tolerable upper level of 1.5 g/day was 0.4% among young children and 10.7% among children and adults aged 7-69 years. The 'upper cut off point' (5 g/d) was not exceeded in any of the two worst-case scenarios.

Table 4: Intake of EPA and DHA (mg/day) among children and adults aged 2-69 years and the percentage that exceeds the tolerable upper level of 1500mg/day or the 'upper cut off point' of 5000mg/day.

Age group	Scenario	P50	P95	% > tolerable upper level	% > 'upper cut off point'
2-6 years (n=1279)	Current consumption	8 (7-9)	307 (287-422)	0.3	0
	Worst-case-1	870 (865-875)	1036 (1027-1047	0	0
	Worst-case-2	1215 (1210-1220)	1381 (1372-1392)	0.4	0
7-69 years (n=3819)	Current consumption	33 (30-34)	915 (846-1079)	2.3	0
	Worst-case-1	941 (934-946)	1252 (1236-1279)	0.5	0
	Worst-case-2	1286 (1281-1291)	1597 (1581-1624)	10.7	0

Data are presented as point estimate (95% CI)

Figure 3 shows the main contributors to median total dietary intake of EPA and DHA among children aged 2-6 years in the Netherlands. Fish fingers, fish oil supplements, chicken and salmon contribute the most to EPA and DHA intake in the current food consumption pattern. Among children and adults aged 7-69, the same food groups were observed as among children aged 2-6 years, but the contribution from fish fingers was substantially lower (9% versus 31%) and the contribution from 'Other fish' was substantially increased (28% versus 8%) (Figure 4).

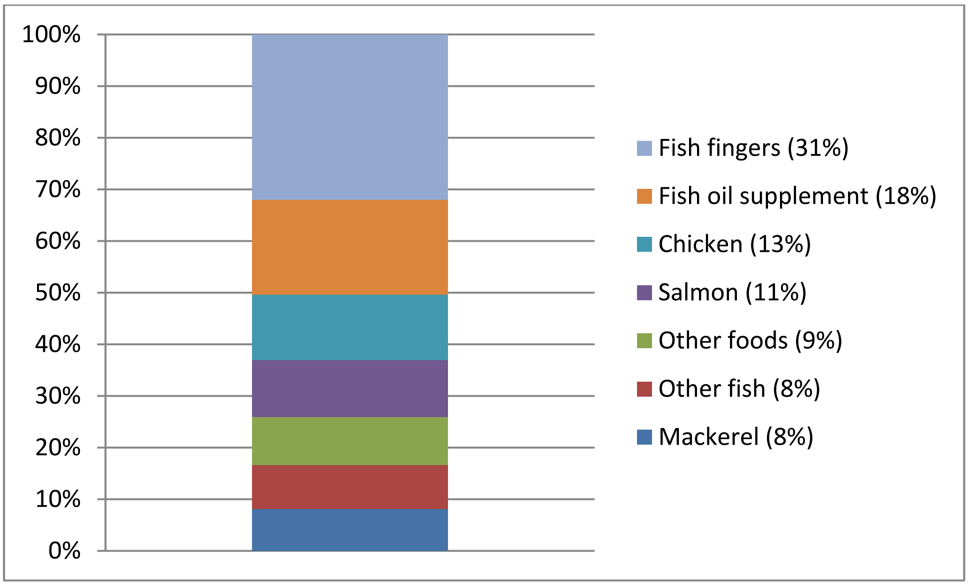

Figure 3: Main contributors to median total EPA and DHA intake among children aged 2-6 years in the Netherlands (VCP 2005-2006 current consumption).

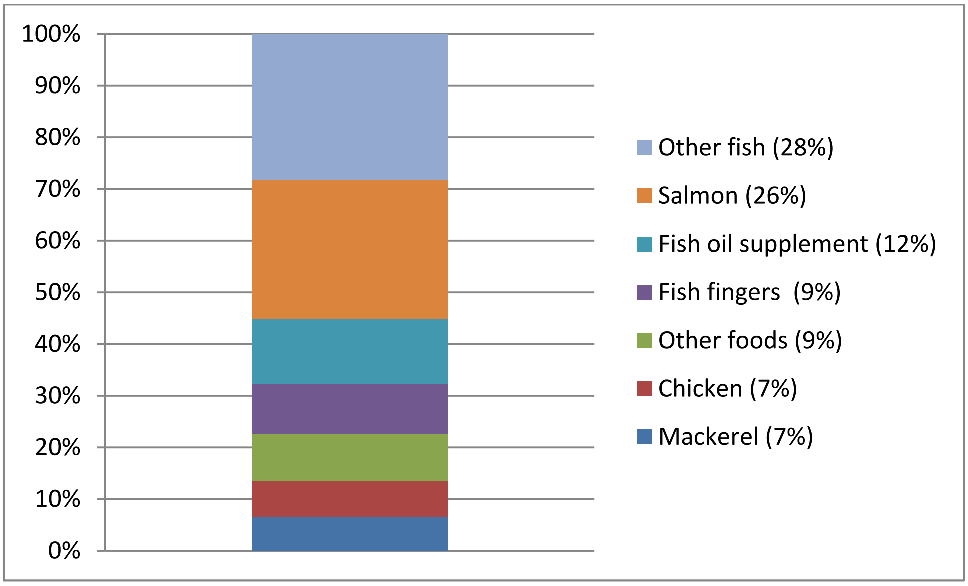

Figure 4: Main contributors to median total EPA and DHA intake among children and adults aged 7-69 years in the Netherlands (VCP 2007-2010 current consumption).

Figure 5 shows the main contributors to EPA and DHA intake in the worst-case-1 scenario for young children and Figure 6 for the older children and adults. Both population groups showed that the guideline fish consumption is the main contributor to EPA and DHA intake followed by supplement use and margarine consumption fortified with EPA and DHA. The worst-case-2 scenario showed similar patterns, but of course the relative contribution by supplements is increased. Among children aged 2-6 years, the top three food groups that contributed the most to EPA and DHA in the worst-case-2 scenario is supplement (53%), guideline fish consumption (37%) and margarine (6%). The order of food groups is similar among 7-69 year-old children and adults in the worst-case-2 scenario; supplement (50%), guideline fish consumption (34%) and margarine (9%). The contribution of food groups to total EPA and DHA intake at the 95th percentile was also analysed, however the number of dietary records at the 95th was too low to draw conclusions.

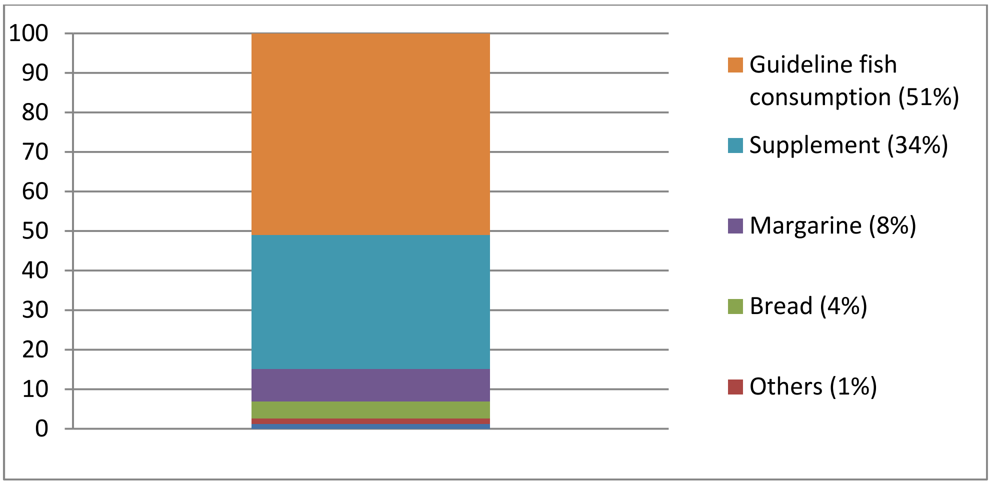

Figure 5: Main contributors to median total EPA and DHA intake among children aged 2-6 years in the Netherlands in the worst-case-1 scenario.

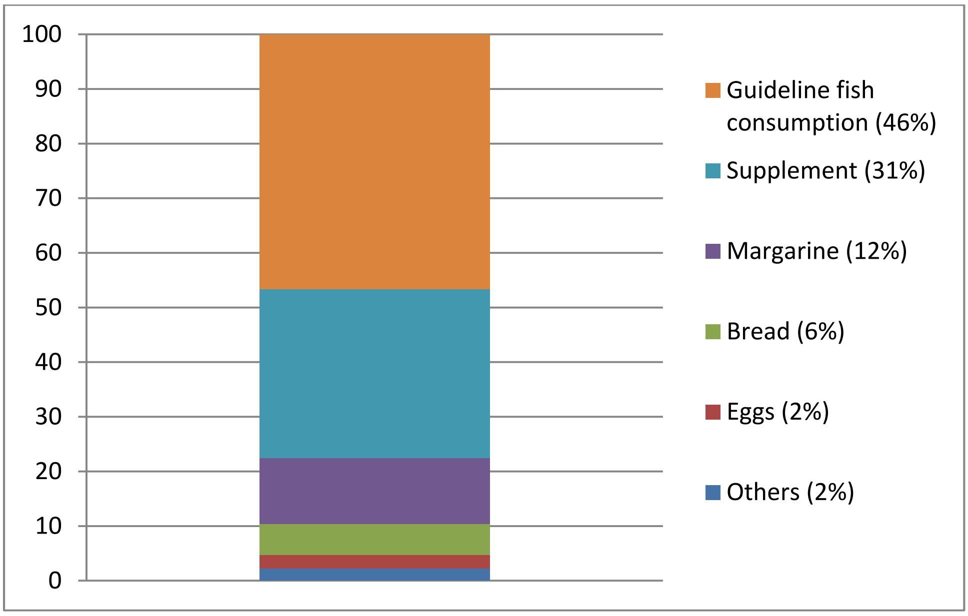

Figure 6: Main contributors to median total EPA and DHA intake among children and adults aged 7-69 years in the Netherlands (worst-case-1 scenario).

4 Discussion

The present study was performed because more and more EPA and DHA containing oils appear on the Dutch market and it was unclear whether the increases in EPA and DHA intake might result in health risks. Adverse effects in humans associated with high intakes of EPA and DHA comprise bleeding episodes, impaired immune function, increased lipid peroxidation, and impaired lipid and glucose metabolism (9). In May 2014 we monitored the use of krill oil and EPA and DHA rich oil in food supplements and food products, and we designed worst-case scenarios for the Dutch population in which the dietary guidelines for fish consumption were followed and in which it was assumed that everybody was also consuming EPA and DHA supplements (at the highest reported concentration levels) and EPA and DHA enriched foods (for those food categories reported to be on the market, and at the highest reported concentration levels). We found no risks for exceeding the upper cut off point of 5 g/day for EPA and DHA intake among children and adults aged 2-69 years. In our scenarios less than 0.5% of the children of 2-6 years exceeds the tolerable upper level of 1.5 g/day. This is also true for older children and adults on the worst-case-1 scenario. Only in the very extreme situation (worst-case-2) that all people are using a daily supplement of 645 mg per day (above the 450 mg/day from fish) then the tolerable upper level will be exceeded by 10.7%.

Methodological considerations

Per May 2014 we found on the Dutch market at least 25 food supplements with krill oil and 8 food supplements with DHA rich oil from microalgae Schizochytrium sp.. We could not identify foods with krill oil but we did find 3 foods with DHA rich oil from microalgae *Schizochytrium sp*. Obviously, both oils (novel food ingredients) are up to now mainly applied to food supplements. Fish oil (which is not a novel food) fatty acids EPA and DHA are also applied to food supplements but also to some food groups. The EPA and DHA enriched foods mainly belong to the food categories: egg and egg yolk, margarine, and bread. We feel that because of the search procedure used, the obtained overview of the applications is already quite complete. Therefore, we did not visit supermarkets, pharmacies or other shops. Some food products in which addition of krill oil and DHA rich oil from microalgae is allowed, were not found with our search strategy. Apparently, actual implementation is not yet completed for all food categories.

It should be mentioned that we assumed that the concentration levels as given on the food (supplement) labels and on the company's websites are correct. We did not validate the reported values against laboratory analyses.
In this study, we decided to use data from DNFCS Young Children 2005/2006 and DNFCS 2007-2010. These data comprise subjects within a wide age range (2-69 years). We did not use DNFCS Older adults 2010-2012 (24). Eldery people may eat fish more often. However, the energy intake of elderly people is less than the energy intake of other adults: median of 2204 kcal/day and 1888 kcal/day, respectively (24, 25). If we are modelling the EPA and DHA intake for elderly people, the intake will be lower than for adults due to a lower caloric intake. Therefore, inclusion of food consumption data of elderly would not reveal additional information on safety.

In our scenarios we assumed that everyone would follow the recommendations for fish fatty acids intake (450 mg EPA and DHA). Some people might have an EPA and DHA intake larger than 450 mg due to their higher fish consumption. For example, among children and adults aged 7-69 years fish consumption at the 95^{th} percentile was 100 g/day (20). If such a person would consume 100 g

of mackerel every day, the EPA and DHA intake would be higher than the intakes shown in our worst-case scenario.
There is a large difference between the ‘tolerable upper intake level’ of 1.5 g/day as assessed by the German Federal Risk Assessment Agency and the ‘upper cut off point’ of 5 g/day as given by EFSA. It is a pity that because of scarcity of appropriate data there is not yet consensus about a tolerable upper intake level for EPA and DHA. As mentioned before, EFSA did not establish a Tolerable Upper Intake Level (UL) for EPA and DHA, because according to their judgment available data do not justify such a step (9).

Mean intake of EPA and DHA was estimated for the current food consumption and the worst-case scenarios. The OIM (Observed Individual Mean) method was used and EPA and DHA intake was estimated over the two days of consumption. Since the mean consumption over the two days was calculated instead of the habitual intake, the mean value of an individual still contains a considerable amount of within-person variation. It is known that the distribution of OIM is wider than a distribution based on habitual intakes. This means that our presented percentages above the tolerable upper level are overestimations. Therefore we may safely assume that the actual surpasses over the Tolerable Upper Intake Level are even smaller than the already low values presented in this report.

Especially in risk assessments where the surpassings are substantial, it becomes important to perform the risk analysis on habitual (usual) intakes because then the prevalence rates of surpassing tolerable upper levels might be substantially reduced and will better reflect true prevalence. In recent years, more software to assess habitual intakes has become available and the advantages and disadvantages of the various software should be carefully compared in order to decide on what software or what combination of software would be most appropriate for assessing habitual intakes in risk assessment studies.

Interpretation of present findings

The median intake of EPA and DHA on the current food consumption pattern (8 mg/day for children 2-6 years and 33 mg/day for children and adults 7-69 years) is substantially below the recommended intake of 250 mg/day (EFSA) or 450 mg/day (Health Council of the Netherlands). Following the guidelines of fish consumption (450 mg/day) would be adequate to provide the individual with enough EPA and DHA (6). However, if people are not able to follow the fish guidelines, EPA and DHA supplements (novel food ingredients) and enriched foods may be an appropriate alternative.

In the present study, we have shown that if people are following the administration instructions of EPA and DHA supplements, following the recommendations for fish consumption and consume EPA and DHA enriched foods, they do not have to be afraid of reaching the EFSA ‘upper cut off point’ of 5 g/day for EPA and DHA intake. Even the tolerable upper level of 1.5 g/day as used by the German risk assessment agency, was exceeded by less than 0.5% of the individuals. Only in the very extreme situation (worst-case-2) that a daily supplement of 645 mg per day is used above the 450 mg/day from fish, then the tolerable upper level will be exceeded by a small part of the population (10.7 %). It should be realized that our worst-case scenarios are very conservative. We assumed that everyone consumes a supplement of 300 or 645 mg/day and that everyone met the recommendations of fish consumption (450 mg/day). Furthermore, we assumed that all margarines, bread, eggs and meal replacements within the same food category would be replaced by foods containing the highest concentration level EPA and DHA found for that category. These worst-case scenarios obviously provide an overestimation of EPA and DHA intake.

However, the situation may change if EPA and DHA will be added to more food groups and to higher concentrations. On the other hand, an interview with Unilever in VoedingNu provided information that the use of DHA rich oil in foods is rather expensive and difficult to use because of oxidation (26). An alternative option to increase the EPA and DHA level in animal products is by changing the composition of the cattle feeding.

In conclusion, with the present availability of EPA and DHA containing supplements and enriched foods, including those with oils from Krill and microalgae, there is no reason to fear that the increased intake levels of EPA and DHA may do any harm.

5 References

1. Health Council of the Netherlands. Dietary reference intakes: energy, proteins, fats, and digestible carbohydrates. The Hague: Health Council of the Netherlands, 2001 Contract No.: publication no. 2001/19ER.
2. Nordic Council. Nordic Nutrition Recommendations 2012. Integrating nutrition and physical activity. Copenhagen: 2014.
3. Deckelbaum RJ, Torrejon C. The omega-3 fatty acid nutritional landscape: health benefits and sources. The Journal of nutrition. 2012;142(3):587S-91S.
4. NKO. What are krill? 2014 [cited 2014 May 21]. Available from: http://www.neptunebiotech.com/.
5. Health Council of the Netherlands. Guidelines for a healthy diet 2006. The Hague: Health Council of the Netherlands, 2006 Contract No.: publication no. 2006/21E.
6. Netherlands HCot. Guidelines for a healthy diet 2006- background document. The Hague: Health Council of the Netherlands, 2006 Contract No.: A06/08.
7. EFSA Panel on Dietetic Products Nutrition and Allergies (NDA). Scientific Opinion on Dietary Reference Values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, *trans* fatty acids, and cholesterol. EFSA Journal. 2010;8(3):1461.
8. FAO. Fats and fatty acids in human nutrition. Report of an expert consultation. FAO, 2010.
9. EFSA Panel on Dietetic Products Nutrition and Allergies (NDA). Scientific Opinion on the Tolerable Upper Intake Level of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA). EFSA Journal. 2012;10(7):2815.
10. Bundesinstitut für Risikobewertung. Für die Anreicherung von Lebensmitteln mit Omega-3-Fettsäuren empfiehlt das BfR die Festsetzung von Höchstmengen. Stellungsnahme Nr. 030/2009 des BfR. Berlin: Bundesinstitut für Risikobewertung, 2009.
11. U.S. Food and Drug Administration. Letter Regarding Dietary Supplement Health Claim for Omega-3 Fatty Acids and Coronary Heart Disease. In: Emord & Associates P.C., editor. Silver Spring, MD: U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition.; 2000.
12. Commission Decision of 12 October 2009 authorising the placing on the market of a lipid extract from Antarctic Krill *Euphausia superba* as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council, 2009/752/EC (2009).
13. Evira. Extension of uses of oil (Neptune Krill Oil (NKO®)) manufactured from Antarctic Krill (Euphausia superba). 2012.
14. Commission Decision of 5 June 2003 authorising the placing on the market of oil rich in DHA (docosahexaenoic acid) from the micro-algae *Schizochytrium sp.* as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council, 2003/427/EC (2003).
15. Commission Decision of 22 October 2009 concerning the extension of uses of algal oil from the micro-algae *Schizochytrium sp.* as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council, 2009/778/EC (2009).
16. EC. Commission Implementing Decision of 14 July 2014 on authorising the placing on the market of oil from the micro-algae Schizochytrium sp. as a

novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council and repealing Decisions 2003/427/EC and 2009/778/EC. Official Journal of the European Union. 2014(2014/463/EU):L209/55 - L/58.
17. UK Food Standards Agency (FSA). DHA and EPA rich oil from the microalgae Schizochytrium. 2012.
18. Brosens M, Hendriksen M, Niekerk EM, Temme EHM, van Raaij JMA. Post-launch monitoring of novel foods/ingredients: Methodology applied to additive stevia (revised version). RIVM Letter Report 090432001/2014. Bilthoven: RIVM, 2014 Contract No.: 090432001/2014.
19. Ocké MC, van Rossum CTM, Fransen HP, Buurma EM, de Boer EJ, Brants HAM, et al. Dutch National Food Consumption Survey - Young Children 2005/2006. RIVM Report 350070001/2007. Bilthoven: RIVM, 2008 Contract No.: RIVM report: 350070001.
20. van Rossum CTM, Fransen HP, Verkaik-Kloosterman J, Buurma-Rethans EJM, Ocké MC. Dutch National Food Consumption Survey 2007-2010. Diet of children and adults aged 7 to 69 years. RIVM Report 350050006/2011. Bilthoven: RIVM, 2011.
21. NEVO. NEVO-tabel. Nederlands Voedingsstoffenbestand 2011. Den Haag: RIVM/Voedingscentrum, 2011.
22. MCRA. MCRA 8.0. Reference Manual. Report December 2013. Wageningen, York, Bilthoven: Biometris, Wageningen UR, The Food & Environment Research Agency (Fera) and National Institute for Public Health and the Environment (RIVM), 2013.
23. INNOVA Market Insights. INNOVA Database 2014 [cited 2014 April]. Available from: http://www.innovadatabase.com/home/index.rails.
24. Ocke MC, Buurma-Rethans EJM, de Boer EJ, Wilson-van den Hooven C, Etemad-Ghameshlou Z, Drijvers JJMM, et al. Diet of community-dwelling older adults. Dutch National Food Consumption Survey Older Adults 2010-2012. RIVM Report 050413001/2013. Bilthoven: RIVM, 2013.
25. van Rossum CT, Fransen HP, Verkaik-Kloosterman J, Buurma-Rethans EJM, Ocke MC. Dutch National Food Consumption Survey 2007-2010|Part 5 Macronutrients, Version 2, based on dataset DFC_2010_core_20120822. Bilthoven: RIVM, 2011.
26. De Jong M. Unilever wil toegevoegde waarde margarines duidelijk maken (in Dutch). VoedingNu. 2013;10.

Appendix I

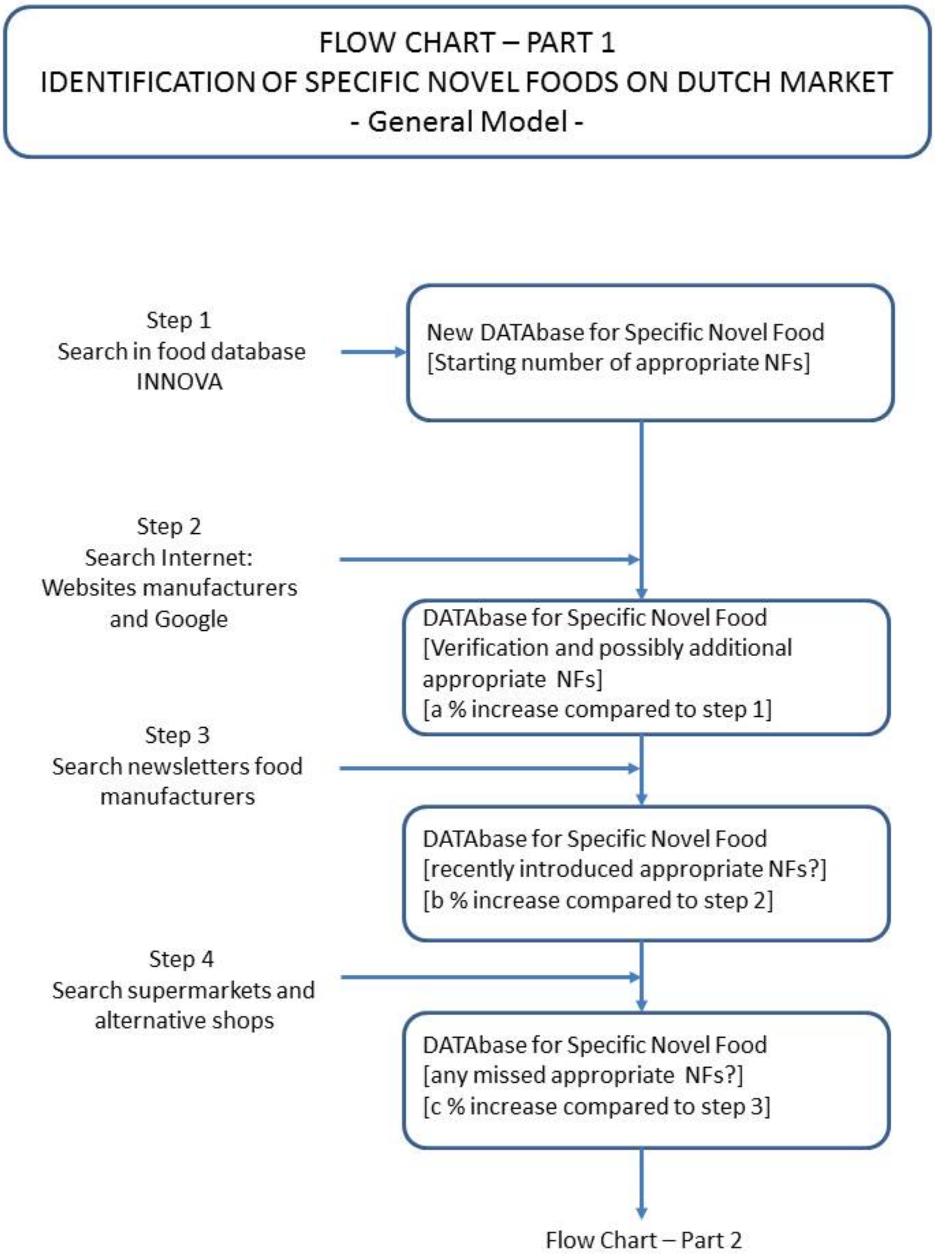

Figure I: Identification of specific novel foods on the Dutch market.

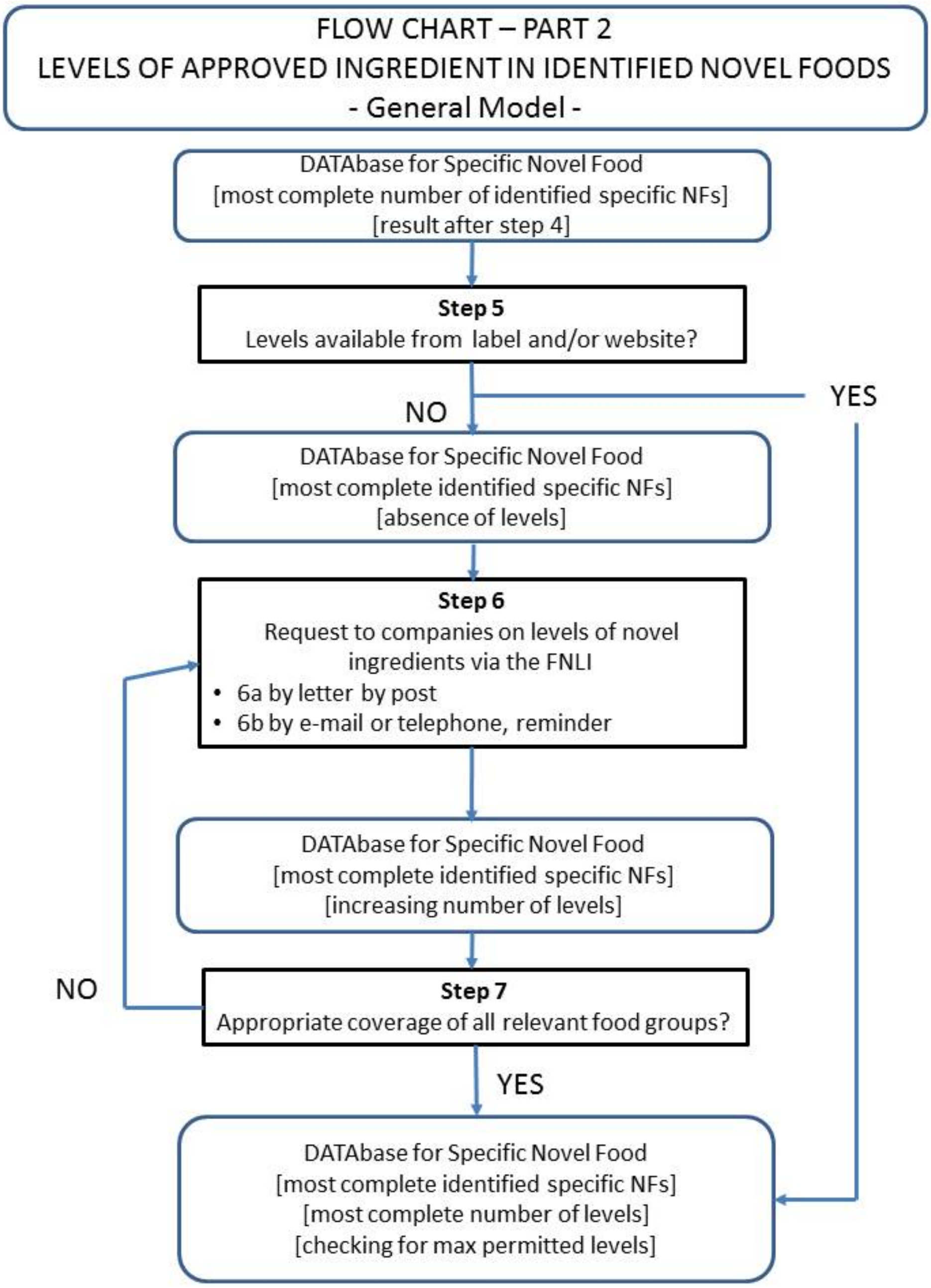

Figure II: Levels of approved ingredient in identified novel foods.

Appendix II

Decisions scenario-analysis

The categorization of food products is based on EPIC-Soft groups (version 2010). The highest reported concentrations of EPA and DHA were used in the calculations.

Meal replacements (shakes and soups)
To all meal replacements (shakes and soups) 79,7 mg/100g was added.

- The EPIC-Soft group belonging to this category is 17.2.0.
- Infant formulae and dietary food products for medical purposes were not included in analysis.

Meal replacements (powder)
To all meal replacements (powder) 980 mg/100g was added.

- The EPIC-Soft group belonging to this category is 17.2.0.
- Infant formulae and dietary food products for medical purposes were not included in analysis.

Nutriton bars
To all nutrition bars for weight care 180 mg/100g was added.

- The EPIC-Soft group belonging to this category is 17.2.0.
- Infant formulae and dietary food products for medical purposes were not included in analysis.

Eggs
To all eggs 204 mg/100g was added. This concentration was establised in omega 3 rich eggs.

- The EPIC-Soft group belonging to this category is 9.1.
- Egg white was not included in analysis, because egg white does not contain fat.

Egg yolk
To all egg yolks 680 mg/100g was added. This concentration was establised in egg yolk in omega 3 rich eggs.

- The EPIC-Soft group belonging to this category is 9.1.
- Egg white was not included in analysis.

Margarines
To all margarines (spreadable fats) 750 mg/100g was added. This concentration was found in one margarine on the Dutch market.

- The EPIC-Soft group belonging to this category is 10.3.
- Cooking fat, frying fat, margarines in liquid form and butter were not included in analysis.

Bread
To all brown and multigrain bread 67,5 mg/100g was added. This concentration was established in omega 3 rich bread.

- The EPIC-Soft group belonging to this category is 6.3.1.
- Bread currant, white, rye, raisin, gluten-free, muesli, with sugar, corn, ciabatta, pita, brioch and low in carbohydrates were not included in analysis.
- Pizza, roti, baguette, wrap/tortilla, rolls and stollen with almond/imitate paste were not included in analysis.

Food supplements
Every individual consumes one food supplement of 300 mg per daily dose in the worst-case-1 scenario and 645 mg per daily dose in the worst-case-2 scenario.

- All fish oil supplements and supplements containing EPA and DHA were removed from analysis in the worst-case scenarios. In the worst-case scenarios, we assume people will replace their current consumption of fish oil supplements and supplements containing EPA and DHA by a food supplement with DHA rich oil from microalgae.

Guideline fish
Every individual meets the guideline fish of 450 mg per daily dose.

- All fish products were removed from analysis (EPIC-Soft group 8).
- NEVO-codes 704 and 1496 do not fall into the EPIC-Soft group 8, fish and shellfish, these foods were not removed from analysis.

Remaining food products containing EPA and DHA
These products were also included in analysis.

Appendix III

Table I: Food groups in which Krill oil is authorized by the EC Decision NO 2009/752/EC of 12 October 2009.

EC Classification
Dairy products except milk-based drinks
Dairy analogues except drinks
Spreadable fat and dressings
Breakfast cereals
Food supplements
Dietary foods for special medical purposes
Foods intended for use in energy-restricted diets for weight reduction
Bakery products (breads and bread rolls)
Nutrition bars
Non-alcoholic beverages, milk-based drinks and dairy analogue drinks

EC= European Commission

Table II: Food groups in which EPA- and DHA rich oil is authorized by the EC Decision NO 2003/427/EC of 5 June 2003.

EC Classification
Dairy products except milk-based drinks
Dairy analogues except drinks
Spreadable fat and dressings
Breakfast cereals
Food supplements
Dietary foods for special medical purposes
Foods intended for use in energy-restricted diets for weight reduction
Bakery products (breads and bread rolls)
Cereal-/nutrition bars
Non-alcoholic beverages (including milk-based beverages)
Other foods for particular use (Parnuts), as described in Directive 2009/39/EC, except infant formulae and follow-on formulae
Cooking fat

EC= European Commission

RIVM
Committed to *health and sustainability*