EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA); Scientific Opinion related to the Tolerable Upper Intake Level of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA)

EFSA Publication; Tetens, Inge

Link to article, DOI:
10.2903/j.efsa.2012.2815

Publication date:
2012

Document Version
Publisher's PDF, also known as Version of record

European Food Safety Authority

EFSA Journal 2012;10(7):2815

SCIENTIFIC OPINION

Scientific Opinion on the Tolerable Upper Intake Level of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA)[1]

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Following a request from the European Commission, the Panel on Dietetic Products, Nutrition and Allergies was asked to deliver a scientific opinion on the Tolerable Upper Intake Level (UL) of the n-3 LCPUFAs eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA). Available data are insufficient to establish a UL for n-3 LCPUFA (individually or combined) for any population group. At observed intake levels, consumption of n-3 LCPUFA has not been associated with adverse effects in healthy children or adults. Long-term supplemental intakes of EPA and DHA combined up to about 5 g/day do not appear to increase the risk of spontaneous bleeding episodes or bleeding complications, or affect glucose homeostasis immune function or lipid peroxidation, provided the oxidative stability of the n-3 LCPUFAs is guaranteed. Supplemental intakes of EPA and DHA combined at doses of 2-6 g/day, and of DHA at doses of 2-4 g/day, induce an increase in LDL-cholesterol concentrations of about 3 % which may not have an adverse effect on cardiovascular disease risk, whereas EPA at doses up to 4 g/day has no significant effect on LDL cholesterol. Supplemental intakes of EPA and DHA combined at doses up to 5 g/day, and supplemental intakes of EPA alone up to 1.8 g/day, do not raise safety concerns for adults. Dietary recommendations for EPA and DHA based on cardiovascular risk considerations for European adults are between 250 and 500 mg/day. Supplemental intakes of DHA alone up to about 1 g/day do not raise safety concerns for the general population. No data are available for DPA when consumed alone. In the majority of the human studies considered, fish oils, also containing DPA in generally unknown (but relatively low) amounts, were the source of EPA and DHA.

KEY WORDS

EPA, DHA, DPA, n-3 LCPUFA, supplements, bleeding, UL, safety

[1] On request from the European Commission, Question No EFSA-Q-2011-00834, adopted on 26 June 2012.

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[3] Acknowledgement: The Panel wishes to thank the members of the Working Group on Tolerable Upper Intake Levels for nutrients: Albert Flynn, Ambroise Martin, Hildegard Przyrembel and Sean (J.J.) Strain for the preparatory work on this scientific opinion and EFSA staff: Silvia Valtueña Martínez for the support provided to this scientific opinion.

Suggested citation: EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA); Scientific Opinion related to the Tolerable Upper Intake Level of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA). EFSA Journal 2012;10(7):2815. [48 pp.] doi:10.2903/j.efsa.2012.2815. Available online: www.efsa.europa.eu/efsajournal

SUMMARY

Following a request from the European Commission, the Panel on Dietetic Products, Nutrition and Allergies was asked to deliver a scientific opinion on the Tolerable Upper Intake Level of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA).

Omega-3 (n-3) polyunsaturated fatty acids (PUFAs) contain one of the double bonds located at three carbon atoms from the methyl end. The main n-3 PUFAs in the diet are α-linoleic acid (ALA; 18:3Δ9c,12c,15c), eicosapentaenoic acid (EPA; 20:5Δ5c,8c,11c,14c,17c), docosahexaenoic acid (DHA; 22:6Δ4c,7c,10c,13c,16c,19c) and docosapentaenoic acid (DPA; 22:5Δ7c,10c,13c,16c,19c). EPA, DHA and DPA are n-3 long-chain PUFAs (n-3 LCPUFA), i.e. n-3 PUFA with 20 or more carbon atoms. The n-3 LCPUFAs are important structural components of cell membranes and contribute to various membrane functions such as fluidity, permeability, activity of membrane-bound enzymes and receptors, and signal transduction.

Fish is a uniquely rich source of n-3 LCPUFAs. Other natural sources are human milk, cultivated marine algae, marine mammals and krill. EPA, DHA and DPA may also be provided by foods and supplements enriched with n-3 LCPUFAs (e.g. fish oils, single cell oils, krill oils added to foods or consumed as food supplements). The ratios of EPA:DHA:DPA differ between the various sources of n-3 LCPUFAs, although DPA is generally a minor quantitative component compared to EPA and DHA. Food supplements containing mainly EPA, or mainly DHA (isolated from microalgae), are also available. Pure DPA is not commercialised for human consumption.

Adverse effects, which have been described in humans in association with high intakes of EPA and DHA, include bleeding episodes, impaired immune function, increased lipid peroxidation, and impaired lipid and glucose metabolism. However, no tolerable upper intake level (UL) for EPA, DHA or DPA has been set by any authoritative body.

Previous assessments on the safety of n-LCPUFAs referred to mixtures of EPA and DHA (DPA was not explicitly mentioned), and were primarily based on a large number of human studies. The Panel considers that the evaluation of the safety of n-3 LCPUFA intakes should be based on the human studies available.

The majority of human intervention studies which have investigated the effects of n-3 LCPUFAs on different health outcomes have used fish oils containing known amounts of EPA and DHA and generally unknown (but relatively low) amounts of DPA; EPA and DHA in combination as ethyl esters; or more rarely mostly EPA or mostly DHA. Very few studies are available using krill oil as a source of EPA and DHA, and no studies have been conducted with sources containing mainly DPA, or with DPA alone.

Long-term human intervention studies which have investigated the effects of supplemental intakes of EPA and DHA, either alone or in combination, at doses up to about 1 g/day on a variety of health outcomes (e.g., cardiovascular, neurological, immunological), have generally reported no adverse effects in relation to the consumption of EPA or DHA at these doses.

Long-term supplemental intakes of EPA and DHA combined up to about 5 g/day do not increase the risk of spontaneous bleeding episodes or bleeding complications even in subjects at high risk of bleeding (e.g. taking acetylsalicylic acid or anti-coagulants).

Supplemental intakes of EPA and DHA combined at doses up to 5 g/day consumed for up to 12 weeks do not significantly affect glucose homeostasis in healthy or diabetic subjects, nor do they induce changes in immune functions which might raise concern in relation to the risk of infections or inappropriate activation of inflammatory responses. The data available are insufficient to conclude on

whether the same doses administered mostly as EPA or mostly as DHA would have different effects on these outcomes.

Supplemental intakes of EPA and DHA consumed either alone or in combination at doses up to about 5 g/day for up to 16 weeks do not induce changes in lipid peroxidation which might raise concern in relation to cardiovascular disease (CVD) risk as long as the oxidative stability of these n-3 LCPUFA is guaranteed.

Supplemental intakes of EPA and DHA combined of 2-6 g/day, and supplemental intakes of mostly DHA of 2-4 g/day, increase blood concentrations of LDL cholesterol by about 3 %. Such increase is accompanied by a decrease in triglycerides with no changes in total (or non-HDL) cholesterol concentrations. Supplemental intakes of mostly EPA at doses up to 4 g/day have no significant effect on LDL-cholesterol concentrations. The Panel considers that the small increase in LDL-cholesterol concentrations associated with combined EPA and DHA supplementation or with DHA supplementation alone at the doses mentioned above may not have an adverse effect on CVD risk.

The Panel concludes that the available data are not sufficient to establish a tolerable upper intake level for n-3 LCPUFA (DHA, EPA, and DPA, individually or combined) for any population group.

At observed intake levels, consumption of n-3 LCPUFA has not been associated with adverse effects in healthy children or adults.

The Panel considers that supplemental intakes of EPA and DHA combined at doses up to 5 g/day, and supplemental intakes of EPA alone up to 1.8 g/day, do not raise safety concerns for the adult population. Limited data are available on the effects of long-term supplementation with these n-3 LCPUFAs at higher doses. The Panel also notes that observed intakes of EPA and DHA from food and food supplements in European populations are generally below these amounts. Dietary recommendations for EPA and DHA based on CVD risk considerations for European adults are between 250 and 500 mg/day. There are no specific recommendations for EPA.

The Panel also considers that supplemental intakes of DHA alone up to about 1 g/day do not raise safety concerns for the general population. Limited data are available on the effects of long-term supplementation with DHA alone at higher doses. The Panel notes that specific dietary recommendations for DHA for European adults and children are well below this amount.

No data are available for DPA when consumed alone. The Panel notes that in the majority of the human studies considered, fish oils, which also contained DPA in generally unknown (but relatively low) amounts, were the source of EPA and DHA. No dietary recommendations have been made specifically for DPA.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Several Member States have raised concerns about a potential link between the intake of omega-3 long chain polyunsaturated fatty acids (eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), docosapentaenoic acid (DPA)) and adverse health effects.

The scientific opinion of the European Food Safety Authority of 6 July 2005[4] on nutrition claims concerning omega-3 fatty acids refers to the reporting of adverse effects of high consumption of omega-3 polyunsaturated fatty acids and these effects include prolonged bleeding time and increased tendency to nasal bleeding, suppression of certain immune reactions which enable the body to attack pathogens and adverse effects on oxidation of LDL cholesterol.

Scientific opinions of the European Food Safety Authority on the substantiation of health claims related to EPA and DHA have proposed intakes of EPA and DHA of about 2-4 g/day in order to obtain the claimed effects[5].

In the absence of EU advice on a tolerable upper intake level, the German Federal Risk Assessment Agency has established a level of 1.5 g/day as the recommended upper intake level for omega-3 polyunsaturated fatty acids. The US Food and Drug Administration (FDA) has recommended not to exceed an intake of 3 g/day of omega-3 fatty acids (EPA and DHA) as a safeguard against possible adverse effects of these fatty acids.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Article 29(1)(a) of Regulation (EC) No 178/2002 of the European Parliament and of the Council of 29 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety[6], the European Commission asks the European Food Safety Authority to:

Review the existing scientific data on the possible link between the intake of omega-3 long-chain polyunsaturated fatty acids (DHA, EPA, DPA) and adverse health effects in the general population and, as appropriate in specific vulnerable subgroups of the population.

Provide advice on a tolerable upper intake level (UL) for omega-3 long-chain polyunsaturated fatty acids (DHA, EPA, DPA) individually or combined for the general population and, as appropriate, for vulnerable subgroups of the population.

In the absence of tolerable upper intake level, to provide advice on a daily intake of omega-3 long-chain polyunsaturated fatty acids (DHA, EPA, DPA) either individually or combined and which does not give rise to concerns about adverse health effects.

[4] The EFSA Journal 2005; 253, 1-29.
[5] EFSA Journal 2009; 7(9):1263; EFSA Journal 2010;8(10):1734; EFSA Journal 2010;8(10):1796
[6] OJ. L 31, 1.2.2002, p.1-24.

ASSESSMENT

1. Introduction

Omega-3 (n-3) polyunsaturated fatty acids (PUFAs) contain one of the double bonds located at three carbon atoms from the methyl end. The main n-3 PUFAs in the diet are α-linoleic acid (ALA; 18:3Δ9c,12c,15c), eicosapentaenoic acid (EPA; 20:5Δ5c,8c,11c,14c,17c), docosahexaenoic acid (DHA; 22:6Δ4c,7c,10c,13c,16c,19c) and docosapentaenoic acid (DPA; 22:5Δ7c,10c,13c,16c,19c). The n-3 PUFAs EPA, DHA and DPA are usually referred to as n-3 long-chain PUFAs (n-3 LCPUFAs), i.e. n-3 PUFA with 20 or more carbon atoms. The n-3 LCPUFAs are important structural components of cell membranes and contribute to various membrane functions such as fluidity, permeability, activity of membrane-bound enzymes and receptors, and signal transduction.

Adverse effects, which have been described in humans in association with high intakes of n-3 LCPUFA, include bleeding episodes, impaired immune function, increased lipid peroxidation, and impaired lipid and glucose metabolism. However, no tolerable upper intake level (UL) for EPA, DHA or DPA has been set by any authoritative body.

In 1997, the US Food and Drug Administration (FDA, 1997) concluded that total intakes (from diet and supplements) of EPA and DHA up to 3 g/day were generally recognised as safe (GRAS). This figure was set on the basis of increased bleeding times, increased fasting blood glucose concentrations in non-insulin dependent, type 2 diabetic subjects, and increased LDL-cholesterol concentrations, particularly in hypertriglyceridaemic or hypercholesterolaemic subjects, at higher levels of intake.

In 2004, the FDA approved a mixture of EPA and DHA in the form of ethyl esters as a registered drug for the treatment of hypertriglyceridaemia in adult patients at doses of 4 g/day. No significant adverse effects were reported for the drug *vs.* placebo in human intervention studies at this dose.

In 2005, the US Institute of Medicine (IoM, 2005) also evaluated the safety of n-3 LCPUFA and concluded that the available data were insufficient to set a UL for EPA and DHA, although subjects with impaired glucose tolerance or type 2 diabetes and subjects with familial hypercholesterolemia using anticoagulants were recommended to consume EPA and DHA supplements with caution. The bases of this recommendation are not explicitly stated.

In May 2009, the German Federal Risk Assessment Agency (BfR, 2009) recommended that 1.5 g/day of EPA and DHA from all sources should not be exceeded and this recommendation was based on the increased risk of bleeding reported in one study in children (Clarke et al., 1990).

In June 2011, the Norwegian Scientific Committee for Food Safety (VKM, 2011) conducted a safety evaluation of n-3 LCPUFA from all sources. Previous evaluations by other authoritative bodies and an up to date review of the literature available up to March 2009 were taken into account. No clear adverse effects were associated with EPA and DHA intakes up to 6.9 g/day and no UL could be established.

The Panel notes that the conclusions of all these safety assessments referred to mixtures of EPA and DHA (DPA was not explicitly mentioned) and were primarily based on a large number of human studies.

The Panel considers that the evaluation of the safety of n-3 LCPUFA intakes should be based on the human studies available.

2. Nutritional background

2.1. Food sources including dietary supplements

Fish is a uniquely rich source of n-3 LCPUFA (EFSA (European Food Safety Authority), 2005). Other natural sources are human milk, cultivated marine algae, marine mammals and krill. EPA, DHA and DPA may also be provided by foods and supplements enriched with n-3 LCPUFAs (e.g., fish oils, single cell oils, krill oils added to foods or consumed as food supplements). The ratios of EPA:DHA:DPA differ between the various sources of n-3 LCPUFAs, although DPA is generally a minor quantitative component compared to EPA and DHA. Food supplements containing mainly EPA, or mainly DHA (isolated from microalgae), are also available. Pure DPA is not commercialised for human consumption.

The n-3 LCPUFAs are present in foods and food supplements mainly as triacylglycerols (TAGs), and as free fatty acids or bound to phospholipids in smaller amounts. EPA and DHA may be found in the form of ethyl esters in synthetically produced concentrated supplements. In krill, EPA and DHA are mainly bound to phospholipids.

Lipid peroxidation may occur during the processing and storage of foods and food supplements rich in n-3 LCPUFA in the absence of appropriate amounts of antioxidants (e.g. vitamin E).

2.2. Dietary intakes

Mean intakes of n-3 LCPUFA in European countries vary according to sex, age group, and supplementation habits (Appendices A, B and C). There is a large diversity in the methodology used to assess the individual intakes of children, adolescents and adults. These differences in dietary assessment methods make direct comparisons difficult. Age classifications may not be uniform and comparability is also hindered by differences in food composition tables used for the conversion of food consumption data to nutrient intake data (Deharveng et al., 1999). Although these differences have an impact on the accuracy of between-country comparisons, the data presented give a rough overview of average intakes and intakes in high consumers of n-3 LCPUFA in a number of European countries.

References were selected in order to obtain data on intake distributions and/or high consumption of n-3 LCPUFA individually or collectively in national surveys, in large cohorts and/or in populations of fish consumers.

2.2.1. Adults

2.2.1.1. EPA

Mean daily intakes of EPA from food only were between 50 mg/day (Spain, both sexes, occasional fish consumers, 35-65 years) and 150 mg/day (France, men, ≥45 years), and median daily intakes between 14 mg/day (Belgium, women, 18-39 years) and 180 mg/day (Denmark, men, 50-64 years). Data from surveys considering food and food supplements combined reported slightly higher mean daily intakes of EPA in the general adult population (up to 330 mg/day, Norway, 16-79 years). Daily intakes of EPA from food only in the highest percentiles of consumption (P95) from the few surveys which reported on this outcome were between 308 mg/day (France, women, ≥35 years) and 428 mg/day (Belgium, women, 18-39 years). The high percentiles available for Denmark (P75) were within that range. In high seafood consumers, mean daily intakes from food only ranged from 320 mg/day (Spain, 35-65 years) to 991 mg/day (France, ≥ 18 years, fifth quintile of EPA-DHA intake). No surveys reported on EPA intakes from food and supplements combined in high seafood consumers.

2.2.1.2. DHA

Mean daily intakes of DHA from food only were between 131 mg/day (Belgium, women, 18-39 years) and 273 mg/day (France, men, ≥45 years), and median daily intakes between 42.5 mg/day (Belgium, women, 18-39 years) and 430 mg/day (Denmark, men, 50-64 years). Data from surveys considering food and food supplements combined reported higher mean daily intakes of DHA in the general adult population (up to 490 mg/day, Norway, 16-79 years). Daily intakes of DHA in the highest percentiles of consumption (P95) for the few surveys reporting on this outcome were between 574 mg/day (France, women, ≥35 years) and 668 mg/day (France, men, ≥45 years) from food only. The high percentiles available for Denmark (P75) were within that range. Mean daily intakes in high seafood consumers from food only were between 600 mg/day (Finnish, women, ≥18 years) and 1,709 mg/day (France, ≥18 years, fifth quintile of EPA-DHA intake). No surveys reported on DHA intakes from food and supplements combined in high seafood consumers.

2.2.1.3. DPA

Mean daily intakes of DPA from food only were between 25 mg/day (Belgium, women, 18-39 years) and 75 mg/day (France, men, ≥45 years), and median daily intakes between 12 mg/day (Belgium, women, 18-39 years) and 80 mg/day (Denmark, men, 50-64 years). Daily intakes of DPA from food only in the highest percentiles of consumption (P95) from the few surveys which reported on this outcome were between 100 mg/day (Belgium, women, 18-39 years) and 138 mg/day (France, men, ≥45 years). The high percentiles available for Denmark (P75) were within that range. Data from food and food supplements combined were within these ranges. Mean daily intakes in high seafood consumers from food only were up to 129 mg/day (France, men, 18-64 years).

2.2.1.4. EPA and DHA/total n-3 LCPUFA

Mean daily intakes of EPA and DHA from food only were between 127 mg/day (Germany, women, 18-24 years) and 295 mg/day (Germany, men, 45-54 years). Daily intakes of EPA and DHA in the highest percentiles of consumption (P95) were between 285 mg/day (The Netherlands, women, 19-30 years) and 1,115 mg/day (Belgium, women, 18-39 years), going up to 1,278 mg/day (Ireland, 51-64 years) when food and food supplements were considered together. Mean intakes of EPA and DHA in high fish consumers from food only were up to 2,700 mg/day (France, ≥ 18 years, fifth quintile of EPA-DHA intake). No surveys reported on EPA and DHA intakes from food and supplements combined in high seafood consumers.

Mean daily intakes of EPA, DHA and DPA were about 400-500 mg/day (France, women ≥35 years and males ≥45 years), increasing to 2,570 mg/day (Norway, males, 16-79 years, fourth quartile of n-3 LCPUFA) when food and food supplements were considered together. Data from another survey considering food and fish oil combined for total n-3 LCPUFA were within these ranges.

2.2.2. Children

No population-based data were available for infants.

2.2.2.1. Young children (1-3 years)

Intake data were available for Germany (EPA and DHA from food only excluding fortified food) and Norway (EPA, DHA and DPA considering also food supplements).

Mean daily intakes of EPA and DHA in German girls and boys, aged 2 to ≤ 4 years consuming fish were 100 to 118 mg/day. Mean intakes of EPA, DHA and DPA in Norwegian children aged 1 or 2 years were 400-600 mg/day (95th percentiles 1,400-1,700 mg/day).

2.2.2.2. Children aged 3-13 years

Data from food and food supplements combined were available for this age group for individual fatty acids (Sweden), for EPA and DHA combined (The Netherlands), and for EPA, DHA and DPA combined (Norway). Mean daily intakes in German fish consumers in a survey which considered intake of EPA and DHA from food only and excluding fortified foods have been reported to be lower than the high consumers (95th percentile) in surveys considering food and food supplements.

Mean daily intakes of EPA in Swedish children (4-12 years) were 40 mg/day, and the 95th percentiles of intake varied between 140 mg/day (4 years) and 170 mg/day (8-9 years).

Mean daily intakes of DHA in this population subgroup were between 100 mg/day (4 years) and 120 mg/day (8-12 years). The 95th percentiles of intake were between 320 mg/day (4 years) and 420 mg/day (8-12 years).

Mean daily intakes of DPA were between 30 mg/day (4 years) and 40 mg/day (8-12 years), and the 95th percentiles of intake varied from 70 mg/day (4 years) to 90 mg/day (8-12 years).

In Dutch children (7-13 years), median daily intakes of EPA and DHA were between 62 mg/day (boys 7-8 years) and 66 mg/day (girls 7-13 years). Higher percentiles of intake (P95) were between 264 mg/day (girls, 9-13 years) and 317 mg/day (boys, 9-13 years). Mean daily intakes of EPA, DHA and DPA in Norwegian children aged 4-9 years were 300-400 mg/day, and the 95th percentiles were 1,200-1,400 mg/day.

2.2.2.3. Adolescents (13-19 years)

Data for the individual fatty acids EPA, DPA and DHA from food only were available from Belgium in children of both sexes aged 13-18 years. For DHA, mean daily intakes were 111 mg/day, and the 95th percentile was 363 mg/day. Mean daily intakes for EPA was 56 mg/day, and the 95th percentile was 244 mg/day. Mean daily intakes for DPA were 18 mg/day, and the 95th percentile was 63 mg/day.

The highest mean daily intakes of EPA and DHA combined from food (excluding fortified food) were reported in German fish consumers aged 13-14 years (girls, 214 mg/day) and 15-18 years (boys, 324 mg/day) which increased up to 536 mg/day and 838 mg/day, respectively, on days of fish consumption. Data in Dutch children from food and supplements combined were lower.

In Norwegian adolescents aged 13 years, mean intakes for EPA, DHA and DPA combined from food only were 200 mg/day (95th percentiles of 700 mg/day), and 300 mg/day (95th percentile was 1,100 mg/day) when food and food supplements were considered.

2.2.3. Summary of intake data

The Panel notes that mean daily intakes of n-3 LCPUFA in adults at the highest percentiles of intake were generally <1,200 mg/day from food only, and <1,300 mg/day when food supplements were considered as well. In high fish consumers daily intakes of n-3 LCPUFA from food only were <2.7g/day. No surveys reported on EPA and DHA intakes from food and supplements combined in high seafood consumers.

In children, the highest intakes of n-3 LCPUFA were observed in children aged 1-2 years consuming food supplements (95th percentiles 1,400-1,700 mg/day).

2.3. Digestion and absorption

Triacylglycerols represent the major dietary form of n-3 LCPUFA, where three fatty acids are esterified to a glycerol backbone and represent more than 90 % by weight. Owing to the asymmetric structure of substituted glycerol, the esterified fatty acids are distinguished by their position, namely the sn-1, sn-2 and sn-3 position. Considering their metabolic fate (action of lipases) in the digestive tract, sn-1 and sn-3-esterified fatty acids are considered as esterified at "external" positions, whereas the sn-2 position is considered as "internal".

TAGs undergo lipolysis by lipases in the gastrointestinal tract prior to absorption. Although there are lipases in the saliva and gastric secretion, most lipolysis occurs in the small intestine (IoM, 2005). In the intestine, TAGs are emulsified with bile salts and phospholipids secreted into the intestine in bile, hydrolysed by pancreatic enzymes, and almost completely absorbed. Pancreatic lipase has a high specificity for the sn-1 and sn-3 position of dietary triacylglycerols so that free fatty acids from the sn-1 and sn-3 position and 2-monoacylglycerol are released for absorption. The pancreatic lipase also completely hydrolyses ethyl esters into fatty acids and the ethanol backbone, although the affinity of the lipase for the fatty acid-ethanol bond appears to be lower than for the fatty acid-glycerol bond. Dietary phospholipids are hydrolysed by pancreatic phospholipase A_2 prior to absorption.

Dietary EPA and DHA are absorbed into the enterocyte as free fatty acids or 2-monoacylglycerol, where they are incorporated into TAGs. The TAGs are then assembled together with cholesterol, phospholipids, and apoproteins into chylomicrons, which enter the circulation. Data are scarce for DPA, but there is no reason to assume that digestion and absorption of DPA might be different from EPA and DHA.

Both comparable and lower rates of absorption and incorporation of EPA and DHA into cell membranes and tissues have been reported for ethyl esters compared to TAGs. Conversely, higher rates of absorption and incorporation of EPA and DHA into cell membranes and tissues have been reported for phospholipids compared to TAGs. However, as the safety assessment of EPA, DHA and DPA refers to long-term consumption and these fatty acids are absorbed almost completely regardless of the source, the Panel considers that there is no need to undertake separate safety assessments for different sources of n-3 LCPUFA. This Opinion refers to EPA, DHA and DPA from all sources.

2.4. Metabolism

Circulating n-3 LCPUFAs are either used as a source of energy (i.e. oxidised to carbon dioxide and water), incorporated into tissue lipids, or utilised in eicosanoid synthesis. Small amounts are lost during sloughing of skin and other epithelial cells (IoM, 2005).

ALA is essential in human nutrition as a precursor for n-3 LCPUFA. EPA, DPA and to a lesser degree DHA are synthesised from ALA through the sequential action of various desaturases and elongases in animal tissues, but not in plants. Estimates for the conversion of ALA into EPA, DPA and DHA are low, and even lower when dietary intakes of these n-3 LCPUFAs are high (EFSA Panel on Dietetic Products Nutrition and Allergies (NDA), 2010). The Panel notes that endogenous production of EPA, DPA and DHA from ALA may be negligible compared to the doses used in the studies considered for the assessment of the safety of these n-3 LCPUFAs.

DHA is a component of membrane structural lipids, especially of phospholipids in nervous tissue and the retina. EPA can be transformed to eicosanoids, a group of biologically active substances including prostaglandins, prostacyclins and leukotrienes which participate in the regulation of blood pressure, renal function, blood coagulation, inflammatory and immunological reactions and other functions in tissues; EPA is also the precursor for series 3 prostanoids and series 5 leukotrienes (Kinsella et al., 1990). Other metabolites of EPA and DHA (resolvins, protectins) are thought to be involved in the resolution of the inflammatory response. Both EPA and DHA are incorporated into cell membranes, and thus may impact cellular metabolism, signal transduction, and regulation of gene expression.

Although DPA can be retro-converted to EPA and only minimally to DHA, little is known about the biological effects of DPA *in vivo* (Kaur et al., 2011). As acyl chain length and degree of saturation may affect the function of fatty acids in biological membranes, it is expected that alterations in the content of these fatty acids will differentially affect membrane structure and function. Indeed, different effects of EPA, DHA and DPA on enzyme activity, gene expression and platelet aggregation have been described *in vitro* (Kaur et al., 2011; VKM, 2011). However, the exact molecular and cellular effects of each of the n-3 LCPUFA, and their impact on disease outcomes *in vivo*, are not precisely known.

High dietary intakes of EPA and DHA result in decreased tissue concentrations of arachidonic acid (AA) and increased concentrations of EPA and DHA, respectively. Supplementation with DHA is also accompanied by an increase in EPA, which could be explained by retroconversion of DHA to EPA or by inhibition of further metabolism of the EPA formed from ALA. These effects of DHA supplementation induce changes in AA metabolism and in the balance of eicosanoids synthesized from the n-6 and n-3 fatty acids, and thus may have an impact on the functions partially regulated by eicosanoids cited above (IoM, 2005).

The Panel notes that although endogenous inter-conversion of EPA, DPA and DHA may occur *in vivo*, and particularly when either fatty acid (mainly EPA and DHA) is administered in isolation at high doses, this inter-conversion is considered to be negligible when they are administered in combination at the dose levels used in the studies considered for the assessment of their safety. The Panel also notes that different biological effects of EPA, DPA and DHA cannot be excluded, and that the effects of these n-3 LCPUFAs may depend on the mode of administration (e.g., given alone *vs*. given in combination).

2.5. Requirements and dietary reference values

ALA is an essential fatty acid required to maintain metabolic integrity and is a precursor of EPA, DPA and to a lesser degree DHA. Whereas some authoritative bodies and organisations have set dietary recommendations for total n-3 PUFAs (primarily ALA, EPA, DHA and DPA in combination) for different population subgroups, or for ALA as the essential precursor of EPA, DPA and DHA (IoM, 2005), many authorities have separate recommendations for ALA on the one hand, and for the n-3 LCPUFAs (either as total n-3 LCPUFAs or as EPA and DHA) on the other hand, owing to the different biological functions attributed to ALA and to the n-3 LCPUFAs (EFSA Panel on Dietetic Products Nutrition and Allergies (NDA), 2009, 2010).

Dietary recommendations from national and international bodies for n-3 LCPUFAs (mostly as EPA and DHA) range from 200 mg to >600 mg/day for adults (Table 1), and from 40 mg to 250 mg/day for infants older than six months and for children and adolescents (Table 2). These recommendations have been generally based on the inverse relationship observed between the consumption of these n-3 LCPUFAs (primarily from fish and fish oils) and a lower risk of coronary artery disease.

Specific recommendations have also been made for DHA for infants and young children (6 to 24 months of age) ranging from 70-100 mg/day based on its accumulation in the central nervous system and its effects on visual function during the complementary feeding period (Table 2) and for additional DHA (100-200 mg/day) for pregnant and lactating women to compensate for oxidative losses of maternal dietary DHA and accumulation of DHA in body fat of the fetus/infant (Table 1).

The Panel notes that the highest dietary recommendations for EPA and DHA (mostly as EPA and DHA or as DHA alone) for different population subgroups are 610 mg/day (Australia). The Panel also notes that the highest recommendations for EPA and DHA combined for European adults and children (250-500 mg/day) are based on the reduction of CVD risk, and that no dietary recommendations have been made specifically for DPA.

Table 1: Recommended dietary intakes for n-3 polyunsaturated fatty acids from national and international bodies (adults)[1].

National/International Body	n-3 PUFA		ALA		EPA+DHA[2]	
	% of energy	g/day	% of energy	g/day	% of energy	mg/day
(WHO/FAO, 2003)	1-2	-	-	-	-	200-1000/wk
United Kingdom, (DoH, 1991, 1994)	-	-	>0.2	-	-	**200**
(SACN, 2004)	-	-	-	-	-	**450**
(Eurodiet, 2000)	-	-	-	2	-	200
Belgium, Superior Health Council (CSS, 2009; SHC, 2004)	1.3-2.0	-	>1	-	≥0.3	
Australia, (Ministry of Health-Department of Health and Ageing - National Health and Medical Research Council, 2006)						
Adult men	-	-	-	1.3	-	**610**[3]
Adult women	-	-	-	0.8	-	**430**[3]
Pregnancy	-	-	-	1.0	-	**115**[4]
Lactation	-	-	-	1.2	-	**145**[4]
The Netherlands, (Health Council, 2001, 2006)	-	-	1	-	-	**450**
Nordic Countries, (NNR, 2004)	≥1	-	-	-	-	-
France, (ANSES, 2010)			1			500 (250 DHA)
USA, (IoM, 2005)						
Adult men	-	-	-	1.6	-	-
Adult women	-	-	-	1.1	-	-
Pregnancy	-	-	-	1.4	-	-
Lactation	-	-	-	1.3	-	-
Germany, Austria, Switzerland, (D-A-CH, 2012)	-	-	0.5	-	-	-
Pregnancy	-	-	-	-	-	200 (DHA)
Lactation	-	-	-	-	-	200 (DHA)
(EFSA Panel on Dietetic Products Nutrition and Allergies (NDA), 2010)			0.5			250
Pregnancy and lactation						+100-200 (DHA)

[1] Values for pregnancy and lactation are only indicated if different from those for adult women. [2]Values in bold refer to n-3 LCPUFA (EPA, DHA and DPA); [3]Suggested Dietary Target; [4] Adequate Intakes.

Table 2: Recommended dietary intakes for n-3 polyunsaturated fatty acids from national and international bodies (children).

National/International Body	n-3 PUFA		ALA		EPA+DHA[1]	
	% of energy	g/day	% of energy	g/day	% of energy	mg/day
United Kingdom, (DoH, 1991, 1994)	-	-	≥0.2		-	-
≥5 years	-	-	-	-	-	**200**
Belgium, Superior Health Council, (CSS, 2009)						
0-12 mo	-	-	-	0.5	-	-
>1 y	-	-	0.45-1.50	-	0.1-0.4 DHA 0.05-0.15 EPA	-
Australia, (Ministry of Health-Department of Health and Ageing - National Health and Medical Research Council, 2006)						
0-1 y	-	0.5	-	-	-	-
1-3 y	-	-	-	0.5	-	**40**
4-8 y	-	-	-	0.8	-	**55**
9-13 y boys	-	-	-	1.0	-	**70**
girls	-	-	-	0.8	-	**70**
14-18 y boys	-	-	-	1.2	-	**125**
girls	-	-	-	0.8	-	**85**
The Netherlands, (Health Council, 2001)						
0-5 mo	-	-	-	0.08/kg	-	20/kg (DHA)
6 mo-18 y	-	-	1	-	-	**150-200**
Nordic Countries, (NNR, 2004)	≥1	-	-	-	-	-
France, (ANSES, 2010)						
0-6 mo	-	-	0.45	-	-	DHA: 0.32 % of total FAs, EPA<DHA
6 mo-3y	-	-	0.45	-	-	70 (DHA)
3-9 y	-	-	1	-	-	250 (125 DHA)
10-18 y	-	-	1	-	-	500 (250 DHA)
USA, (IoM, 2005)						
0-6 mo	-	0.5				
7-12 mo	-	0.5				
1-3 y	-	-	-	0.7	-	-
4-8 y	-	-	-	0.9	-	-
9-13 y boys	-	-	-	1.2	-	-
girls	-	-	-	1.0	-	-
14-18 y boys	-	-	-	1.6	-	-
girls	-	-	-	1.1	-	-
Germany, Austria, Switzerland, (D-A-CH, 2012)	-	-	0.5	-	-	-
(EFSA Panel on Dietetic Products Nutrition and Allergies (NDA), 2010)						
7-24 mo	-	-	0.5	-	-	100 (DHA)
2-18 y	-	-	0.5	-	-	250

[1]Values in bold refer to n-3 LCPUFA (EPA, DHA and DPA).

3. Hazard identification

Adverse effects which have been described in humans in association with high intakes of n-3 LCPUFA include bleeding episodes, impaired immune function, increased lipid peroxidation, and impaired lipid and glucose metabolism.

The majority of human intervention studies which have investigated the effects of n-3 LCPUFA on different health outcomes have used fish oils containing known amounts of EPA and DHA and generally unknown (but relatively low) amounts of DPA; EPA and DHA in combination as ethyl esters; or more rarely EPA alone or DHA alone. Very few studies are available using krill oil as a source of EPA and DHA, and no studies have been conducted with sources containing mainly DPA or with DPA alone.

Long-term human intervention studies which have investigated the effects of supplemental intakes of EPA and DHA, either alone or in combination, at doses up to about 1 g/day on a variety of health outcomes (e.g. cardiovascular, neurological and immunological) have generally reported no adverse effects in relation to the consumption of EPA or DHA at these dose levels.

3.1. Bleeding complications, bleeding time and platelet function

3.1.1. Bleeding complications

An increased tendency to bleed from the nose and urinary tract, and an increased mortality from haemorrhagic stroke, have been reported in Greenlandic Inuits with high intakes of fatty fish (mean intakes of n-3 LCPUFA about 6.5 g/day), as well as increased bleeding times and reduced platelet aggregation *in vitro* (IoM, 2005). The Panel notes that these studies were uncontrolled for factors other than dietary n-3 LCPUFA which may have been responsible for the effects.

The hypothesis that n-3 LCPUFA supplementation could modify platelet function, increase bleeding time and, eventually, increase the risk of spontaneous bleeding and haemorrhagic stroke, has been addressed in several controlled human intervention studies.

Data on the effects of n-3 LCPUFA on the risk of haemorrhagic stroke are scarce. One open label human intervention study (Yokoyama et al., 2007) which investigated the effects of 1.8 g/day of EPA as ethyl esters consumed for five years in combination with statins (n=9,326) *vs*. statins alone (n=9,319) in hypercholesterolemic, high fish consumers on the primary and secondary prevention of coronary heart disease also assessed safety outcomes and the risk of stroke and its subclasses (Tanaka et al., 2008). Bleeding (cerebral and fundal bleedings, epistaxis, and subcutaneous bleeding combined) was more frequently reported in the EPA group than in controls. The Panel notes that nose or subcutaneous bleeding for example was self-reported, and that self-reported side effects are subject to high reporting bias in open label studies. The Panel also notes that no significant differences in the total incidence of stroke, or in the incidence of cerebral or subarachnoid haemorrhage, which were objectively assessed, were observed between groups. The Panel considers that intakes of EPA alone at doses up to 1.8 g/day for two years do not increase the risk of bleeding complications.

Among the several prospective cohort studies published to date on the relationship between dietary intake of n-3 LCPUFA and risk of stroke, none has reported an increased risk of haemorrhagic stroke (He et al., 2002; IoM, 2005; Skerrett and Hennekens, 2003). Mean dietary intakes of n-3 LCPUFA at the highest quintiles of intake in these studies were <1 g/day.

The Panel notes that there is no evidence for an increased risk of haemorrhagic stroke at doses of n-3 LCPUFA which are usually consumed in Western diets, or at supplemental intakes of mostly EPA up to 1.8 g/day.

Some controlled human intervention studies on the effects of n-3 LCPUFA on bleeding complications other than haemorrhagic stroke have been conducted in population subgroups at high risk of bleeding, and which include patients on antiplatelet or antithrombotic medications (acetyl salicylic acid ASA, clopidogrel, anticoagulants) undergoing invasive procedures, and pregnant women at delivery.

A Cochrane review (Hooper et al., 2004) including 48 radomised controlled trials (RCTs) conducted in subjects at high risk of cardiovascular events addressed the effects of n-3 LCPUFA at doses of 0.4 to 7 g/day (compared to placebo or to a control oil) for at least six (and up to 47) months on CVD-related outcomes. The majority of subjects were under antithrombotic medications for the prevention or treatment of CVD. Seven of the studies, which used EPA and DHA at doses of 1.8 to 6.9 g/day for 6 to 24 months, reported on bleeding episodes (Bairati et al., 1992; Eritsland et al., 1996; Franzen et al., 1993; Kaul et al., 1992; Leaf et al., 1994; Loeschke et al., 1996; Reis et al., 1991). No difference in the risk of bleeding between the intervention (n=17/949) and control (or placebo, n=13/836) groups was observed. The study which used the highest dose of n-3 LCPUFA (6.9 g/day) lasted six months (Leaf et al., 1994) and the study of longest duration (24 months) used 5.1 g/day of EPA and DHA (Loeschke et al., 1996).

Harris (2007) reviewed 19 controlled intervention studies (4,397 subjects) in patients on secondary prevention for coronary heart disease (CHD) undergoing major vascular surgery or femoral puncture for diagnostic purposes who received EPA and DHA (1.4 to 6.9 g/day) from different sources (fish oil or capsules with EPA and DHA as triglycerides or ethyl esters) for 1-28 months. This review included six of the seven studies (all except Loeschke et al., 1996) considered by Hooper et al. (2004). Except in two studies (Nye et al., 1990; Rapp et al., 1991), subjects were on antithrombotic medications (ASA, warfarin or heparin). Even if these studies were not specifically designed to address the safety of n-3 LCPUFA, they all reported on adverse events in general and on bleeding complications in particular. None of the studies observed an increased frequency or severity of bleeding complications associated with EPA and DHA supplementation. Besides Leaf et al. (1994), the study using the highest dose of n-3 LCPUFA (6 g/day) lasted 4.5 months (Cairns et al., 1996) and the study of longest duration (28 months) used 4.8 g/day of EPA and DHA (Sacks et al., 1995).

Also a recent meta-analysis of RCTs (Filion et al., 2010) on the effects of n-3 LCPUFA (EPA and DHA given at doses of 0.9-6.9 g/day for 1-55 months) on mortality (25 RCTs, mean duration 12 months) and re-stenosis following angioplasty (14 RCTs, mean duration six months) in populations with (or at high risk of) CHD addressed safety outcomes, including bleeding. The risk of bleeding was not significantly different between the intervention and control groups in the 15 RCTs which reported on this outcome and entered data analysis. Of these, only four studies had not been considered by Harris (2007), three of which used either low doses (1 g/day) of n-3 LCPUFA (Rauch et al., 2010), had a very short duration (days) of the intervention (Calo et al., 2005) or included a small sample of patients (Rossing et al., 1996).

Supplementation studies (n=31) with n-3 LCPUFA in patients (n=485) with end-stage renal disease undergoing dialysis and treated with antithrombotic medications (mostly ASA) which reported on bleeding complications were reviewed by Friedman and Moe (2006). Most studies were small (<20 subjects), uncontrolled or not randomised (n=21), lasted 4 to 24 weeks, and all except two (which provided EPA alone at doses of 1.8 and 3 g/day, respectively) used fish oil at doses of 1.4 to 7.6 g/day. The RCTs had generally bigger sample sizes, were of longer duration (≥8 weeks) and used EPA or fish oil at doses of 1.8 to 5.2 g/day. Only one case of serious gastrointestinal bleeding required hospitalisation and was reported in one small uncontrolled study (n=7) in a patient consuming 3 g/day of EPA, but the event could not be attributed to the EPA treatment (Diskin et al., 1990). There were no studies which reported on bleeding episodes using DHA in isolation at these dose levels. One single-arm intervention study was also available in children with end-stage renal disease on dialysis and at high risk of bleeding (Goren et al., 1991). A total of 16 children and adolescents (7 to 18 years) with hyperlipidaemia were given 3 to 8 g/day (weight-adjusted dose) of fish oil (0.3 to 2.4 g/day of EPA and DHA) for eight weeks, and were followed up for one month after

treatment. Platelet counts were normal in all subjects and mild side effects of treatment (abdominal cramps and diarrhoea which resolved spontaneously) did not include spontaneous bleeding.

In a single-arm intervention (Sorgi et al., 2007), nine children and adolescents (8–16 years) under treatment for attention-deficit hyperactivity disorder (ADHD) received 30 mL of a liquid EPA/DHA which provided 16.2 g of n-3 LCPUFA (10.8 g EPA and 5.4 g DHA) per day for 4 weeks. Then doses were adjusted to maintain the AA:EPA ratio in the isolated plasma phospholipids between 1.5 and 3, so that three, two and four subjects consumed 8.1, 10.8 and 16.8 g/day, respectively, for another four weeks. No bleeding episodes were reported for any of the children during the eight-week study.

No increased risk of bleeding complications at delivery was observed in pregnant women (n=533) who received 2.7 g/day n-3 LCPUFA from fish oil during the last trimester compared to olive oil or no supplement (Olsen et al., 1992).

The Panel notes that some authoritative bodies have warned about an increased risk of bleeding complications at supplemental doses of these n-3 LCPUFAs of ≥3 g/day. The concern was raised by one human intervention study in children which reported nose bleeding episodes associated with the consumption of fish oil (Clarke et al., 1990).

The study by Clarke et al. (1990) was a single-arm intervention on the effects of fish oil on blood lipids conducted in 11 children and adolescents with familial hyperlipoproteinaemia (aged 11 to 21 years) who received an increasing dose of fish oil (18 % EPA and 12 % other n-3 fatty acids) for six months (starting at 1 g/day the first month and increasing by 1 g/day monthly up to 5 g/day) after three months of pre-treatment observation. Subjects were followed after treatment for one month. Doses of EPA and DHA ranged from 0.3 to 1.5 g/day during the study. Eight of the 11 subjects reported nine episodes of epistaxis (nose bleeding) during the fish oil supplementation and none during the pre- and post-observation periods. In one case the intervention was stopped due to epistaxis with prolongation of bleeding time when doses of 1.5 g/day of EPA and DHA were being consumed. In two subjects, epistaxis was associated with modest prolongation of bleeding time (one subject was on ASA), whereas one subject who withdrew due to epistaxis had a normal bleeding time. One subject had asymptomatic occult blood in the stool on one occasion with normal bleeding time. Platelet counts, prothrombin and partial thromboplastin times were within the normal range. No information was provided in the publication about the time at which eight of the nine episodes of epistaxis occurred, nor about the dose of EPA and DHA being consumed at the time of the events. The Panel notes the uncontrolled nature and poor reporting of the study (e.g. medication use).

The Panel notes that the bleeding episodes associated with the consumption of fish oil reported by Clarke et al. (1990) have not been observed in other studies of similar design conducted with higher doses of EPA and DHA in children at low (Sorgi et al., 2007) or high (Goren et al., 1991) risk of bleeding, or in a number of controlled intervention studies in adults at high risk of bleeding.

The Panel considers that supplemental intakes of EPA and DHA combined of up to about 5 g/day for up to two years and up to about 7 g/day for up to six months, do not increase the risk of spontaneous bleeding episodes or bleeding complications, even in subjects at high risk of bleeding (e.g. taking acetylsalicylic acid or anti-coagulants). The Panel notes that the data available are insufficient to conclude on whether the same doses administered mostly as EPA or mostly as DHA would have different effects on this outcome. The Panel considers that intakes of EPA alone at doses up to 1.8 g/day for two years do not increase the risk of bleeding complications.

3.1.2. Bleeding time

An increase in bleeding time beyond the normal range and/or leading to bleeding complications is considered an adverse effect. However, changes in bleeding time which are within the normal range

and which are not associated with bleeding complications may not be considered adverse. The predictive value of changes in bleeding time within the normal range in relation to bleeding complications is low.

A number of small, short-term (4-11 weeks) controlled intervention studies have examined the effects of n-3 LCPUFA at doses of 2-15 g/day (most between 3 and 6 g/day) on bleeding time in healthy subjects and in subjects with hypercholesterolemia, hypertension, type 2 diabetes, patients with atherosclerosis undergoing coronary artery bypass graft surgery, or a combination of these, who were not under medications prolonging bleeding time, such as ASA or anti-coagulants (IoM, 2005). The majority of these studies reported a significant increase in bleeding time with n-3 LCPUFA supplementation (Cairns et al., 1996; Cobiac et al., 1991; DeCaterina et al., 1990; Emsley et al., 2008; Levinson et al., 1990; Lorenz et al., 1983; Mortensen et al., 1983; Sanders et al., 1981; Schmidt et al., 1990; Smith et al., 1989; Thorngren and Gustafson, 1981; Wojenski et al., 1991; Zucker et al., 1988) whereas other studies using doses up to 6 g/day resulted in no difference (Blonk et al., 1990; Freese and Mutanen, 1997; Nelson et al., 1997; Rogers et al., 1987). All changes in bleeding times were within the normal range and did not lead to spontaneous bleeding. The Panel also notes that only a few studies have addressed the effects of supplements containing mostly EPA (Emsley et al., 2008; Wojenski et al., 1991) or mostly DHA (Nelson et al., 1997).

Three of the studies specifically assessed dose-response relationships between n-3 LCPUFA intakes and bleeding time. Blonk et al. (1990) supplemented 45 healthy normotriglyceridaemic male volunteers with 1.5, 3 or 6 g/day of EPA and DHA as ethyl esters for 12 weeks. No significant effect on bleeding time was observed for any of the doses tested. Schmidt al. (1990) supplemented ten healthy males with increasing doses of n-3 LCPUFA (1.3 g, 4 g or 9 g/day) from fish oil daily for periods of six weeks each. Bleeding time increased significantly compared to baseline after the 4 g and the 9 g doses in a dose-dependent manner. A more recent intervention study (Cohen et al., 2011) examined the effects of increasing doses of EPA and DHA as ethyl esters (1, 2, 4 and 8 g/day for six consecutive weeks each, 24 weeks in total), either alone, in combination with ASA, or in combination with ASA plus clopidogrel, in 30 volunteers (ten subjects per group). Median bleeding times increased within the normal range in a dose-dependent manner with increasing doses of EPA and DHA given alone. No effect was reported in the ASA or the ASA plus clopidogrel groups which already had prolonged bleeding times.

Four controlled studies (reviewed in VKM, 2011) assessed bleeding time in subjects supplemented with n-3 LCPUFA who were under ASA, and/or the international normalised ratio (INR) in subjects on warfarin as antithrombotic therapy at doses from 0.9 to 6.9 g/day (Bender et al., 1998; Dehmer et al., 1988; Eritsland et al., 1996; Leaf et al., 1994). Three studies observed no significant differences in bleeding times between the intervention group and controls, whereas the fourth study (Leaf et al., 1994) did not compare the study groups directly. The Panel notes that n-3 LCPUFA supplementation did not lead to spontaneous bleeding or bleeding complications in any of the studies.

The Panel notes that supplemental intakes of EPA and DHA combined of up to about 6 g/day do not enhance the effects of anti-platelet or antithrombotic medications on bleeding time, and that the changes in bleeding times within the normal range which have been observed in some intervention studies are not considered to be adverse as they were not associated with an increased risk of clinical complications (e.g. spontaneous bleeding).

3.1.3. Platelet function

Platelet dysfunction leading to bleeding complications is considered an adverse effect. However, changes in platelet function which are not associated with bleeding complications may not be considered adverse.

Several, mostly short-term, intervention studies have investigated the effects of n-3 LCPUFA on platelet function assessed by different methods and using a variety of outcome measures.

Violi et al., (2010) recently reviewed published studies on the effects of EPA and DHA supplementation on platelet function. Among the 21 studies identified, only seven were controlled. Of these, three were conducted in healthy subjects and four in subjects with hypercholesteroaemia, hypertension, type 2 diabetes, or a combination of these. Doses of n-3 LCPUFA ranged from 1 to 4 g/day and study duration from 30 days to one year. No effect of n-3 LCPUFA intake on platelet aggregation was observed in the two studies of shorter and longer duration, respectively, whereas five studies observed inhibition of platelet function or prolongation of platelet survival (study duration 4-16 weeks). The effect on platelet function did not appear to be dose-dependent. Dose-response relationships between the intake of EPA and DHA and platelet aggregation, vWF, coagulation factors VII and VIII, AT III activity, protein C activity, plasma fibrinogen, fibronectin and fibrinolysis (PAI and t-PA ag) were specifically assessed in one study on ten healthy males supplemented with 1.3 g, 4 g or 9 g of n-3 LCPUFA daily for periods of six weeks each (Schmidt et al., 1990). No significant effect of EPA and DHA was observed on platelet aggregation. Plasma fibrinogen decreased in a dose-dependent manner after intake of 1.3 g and 9 g of n-3 LCPUFA. The vWF decreased after the high dose, while plasma concentrations of factor VII, factor VIII, and AT III activity, protein C activity and fibronectin were unaltered by n-3 LCPUFA. At rest, PAI and t-PA ag. increased after intake of 9 g of n-3 LCPUFA, and PAI increased after n-3 LCPUFA ingestion in a dose-dependent fashion. The Panel notes that no effect on platelet aggregation was observed and that no dose-response relationship was reported between the intake of EPA and DHA and most of the variables related to blood coagulation.

One of the studies specifically assessed whether DHA and EPA could have differential effects on platelet aggregation. In a double-blind placebo-controlled trial of parallel design, Woodman et al. (2003) randomised 59 treated hypertensive Type 2 diabetic men and postmenopausal women to 4 g/day of EPA, DHA or olive oil (placebo) for six weeks. DHA but not EPA supplementation significantly reduced collagen aggregation (by 16.9 %) and TXB_2 (by 18.8 %), whereas no significant changes were reported in either platelet activating factor (PAF)-stimulated platelet aggregation, fibrinolytic function or vascular function in either the EPA or DHA groups relative to placebo. However, another study comparing 4 g/day of EPA to the same amounts of n-3 LCPUFA (mostly EPA and DHA) from a fish oil concentrate given for four weeks found EPA to being more effective in decreasing platelet aggregation than fish oil concentrate (Wojenski et al., 1991). The Panel notes that available data on differential effects of EPA and DHA on platelet aggregation are scarce and inconsistent.

The Panel notes that the changes on platelet function which are observed at supplemental intakes of EPA and DHA (either alone or in combination) up to about 4 g/day are not considered to be adverse as they are not associated with an increased risk of clinical complications (e.g. spontaneous bleeding).

3.2. Glucose homeostasis

Human intervention studies, mostly uncontrolled, have described adverse effects of supplemental n-3 LCPUFA (≥10 g/day) on glucose homeostasis, such as increased insulin requirements, an increase in glycated haemoglobin (HbA1c), and an increase in fasting and postprandial glycaemia, in patients with type 1 and type 2 diabetes (see De Caterina et al., 2007 for review). In 2005, the IoM advised that subjects with "impaired glucose tolerance or diabetic conditions requiring increased doses of hypoglycaemic agents" should take EPA and DHA supplements with caution (IoM, 2005).

Data from (mostly controlled) human intervention studies with respect to the effects of n-3 LCPUFA supplementation on insulin requirements in type 1 diabetics, and on HbA1c and fasting/postprandial glycaemia/insulinaemia in type 2 diabetic subjects, have been recently reviewed in a number of

systematic reviews and meta-analyses (Balk et al., 2004; De Caterina et al., 2007; Farmer et al., 2001; Friedberg et al., 1998; Hartweg et al., 2008; 2009; Hendrich, 2010; MacLean et al., 2004; Montori et al., 2000).

Doses of up to 5 g/day of n-3 LCPUFA given as triglycerides for 2-12 weeks do not appear to increase insulin requirements in subjects with type 1 diabetes, although the studies are small (De Caterina et al., 2007; Friedberg et al., 1998).

With respect to subjects with type 2 diabetes, a Cochrane systematic review and meta-analysis performed in 2001 (Farmer et al., 2001) on the effects of fish oil in subjects with type 2 diabetes on different outcomes, including those related to glucose homeostasis, was updated in 2008 and 2009 (Hartweg et al., 2008; 2009). The meta-analysis by Hartweg et al. (2008) included 23 trials (1,075 subjects, sample size range 8 to 418) where the majority of participants were male (age range 21-85 years) with type 2 diabetes of 5-10 years duration under treatment with diet or oral hypoglycaemic agents, and generally with no diabetes-related complications. The mean dose of n-3 LCPUFA was 3.5 g/day, ranged from 1.7 to 10 g/day (from 1.08 to 5.2 g/day of EPA and from 0.3 to 4.8 g/day of DHA), and the mean duration of treatment was 8.9 weeks. The EPA and DHA were given mostly in combination (two intervention arms gave EPA only and one DHA only) and in capsules. In most cases, controls received similar amounts of fat from vegetable oils (olive, sunflower, linseed, corn, safflower, and flaxseed). Linoleic acid, non-fat placebo, a saline solution, or usual diet served as control in the remaining studies (n=5). A total of 15 trials (n=848 subjects) reported on HbA1c, of which only four lasted at least 12 weeks, which is the time normally required to detect differences in HbA1c, and 11 lasted at least eight weeks, which may only allow detection of major changes in blood glucose control. A total of 21 trials reported fasting glucose concentrations, and the results could be pooled for 16 studies (n=930 subjects). Results from six of the eight studies reporting on fasting insulin concentrations could also be pooled. Supplementation with n-3 LCPUFA did not significantly affect HbA1c, fasting glucose or insulin concentrations. Similar results were obtained when seven new trials with a mean dose of 2.4 g/day (range 0.8 to 4.8 g/day) and mean duration of 24 weeks were added to the analyses (Hartweg et al., 2009). More recent studies are in line with these results (Hendrich, 2010).

Galgani et al. (2008) reviewed the effects of n-3 LCPUFA on insulin sensitivity in high-quality randomised intervention studies which used the euglycemic hyperinsulinemic clamp or the frequently sampled intravascular glucose tolerance test while controlling for the energy and macronutrient composition of the intervention and control diets. One study which met these requirements was published thereafter (Giacco et al., 2007). All studies used fish oil as a source of EPA and DHA and vegetable oils as control (olive oil, corn oil, safflower oil). Four studies (n=32 to 162) were conducted in healthy subjects using 2.4 to 3.6 g/day of EPA and DHA for 12-16 weeks. No effect of n-3 LCPUFA on insulin sensitivity was observed compared to the control oils (olive oil, corn oil). Five studies (n= 10 to 26) recruited subjects with type 2 diabetes and used EPA and DHA at doses of 1.8 to 5 g/day and vegetable oils (olive oil, corn oil, safflower oil) as controls for 3-24 weeks. Only the study providing the highest dose of n-3 LCPUFA (5 g/day containing about 2.1 g EPA, 3.5 g DHA and 0.3 g DPA) for nine weeks (Mostad et al., 2006) reported a marginal decrease in glucose utilisation during the euglycemic hyperinsulinaemic clamp in the fish oil group (n=13) compared to the maize oil group (n=14; p=0.049) in a small sample of subjects. No significant changes in HbA1c were observed in this and other longer-term studies described above.

The Panel notes that human intervention studies which have controlled for fat intake generally do not show a differential effect of vegetable oils and supplemental fish oil at doses up to 5 g/day of EPA and DHA consumed for 12 weeks on blood glucose control in diabetic subjects, or on insulin sensitivity in healthy or diabetic subjects.

The Panel considers that supplemental intakes of EPA and DHA combined of up to 5 g/day consumed for up to 12 weeks do not significantly affect glucose homeostasis in healthy or diabetic subjects. The

Panel notes that the data available are insufficient to conclude on whether the same doses administered mostly as EPA or mostly as DHA would have different effects on this outcome.

3.3. LDL-cholesterol concentrations in blood

Several human intervention studies have addressed the effects of supplementation with n-3 LCPUFA on blood LDL-cholesterol concentrations.

A meta-analysis of RCTs (Balk et al., 2006) pooled the results from 21 RCTs (about 8,000 subjects, 37 intervention arms) conducted in healthy subjects; in subjects with diabetes, hypertension, or dyslipidemia; or in subjects with cardiovascular disease. Studies using >6 g/day of EPA + DHA or lasting <4 weeks were excluded. Due to the high number of studies found in the literature, a minimum sample size of 12 subjects per each n-3 LCPUFA intervention arm was required for inclusion. Doses of EPA and DHA ranged from 0.9 to 5.9 g/day (from fish oil or food) and the duration of the intervention was between 4 weeks and 2 years (17 studies lasted ≥6 months and 8 studies lasted ≥1 year at doses of about 3.4 g/day). Study design was considerably heterogeneous. Random effects model meta-analyses found a significant increase in LDL cholesterol of +0.155 mmol/L (95 % CI +0.078 mmol/L, +0.207 mmol/L) compared to the control oils. In the majority of the studies, changes in LDL cholesterol associated with EPA and DHA intakes were <5 %. Earlier studies reported the highest increases in LDL-cholesterol. The Panel notes that these changes in LDL-cholesterol were accompanied by a significant decrease in TG (−0.31 mmol/L; 95 % CI −0.37 mmol/L, −0.23 mmol/L) and by a significant increase in HDL-cholesterol (+0.041 mmol/L; 95 % CI +0.021 mmol/L, +0.060 mmol/L), and that total blood cholesterol did not change significantly. Sensitivity analyses showed that dose of EPA and DHA and baseline concentrations of TG had a cumulative impact on the magnitude of changes in TG, but no influence on changes in HDL or LDL cholesterol, which appeared to be dose-independent.

Since hypertriglyceridaemia is often observed in type 2 diabetes and this population subgroup is already at higher risk for CVD, an increase in LDL-cholesterol concentrations resulting from the treatment of elevated TG concentrations could be of particular concern in diabetic subjects. In the meta-analyses by Hartweg et al., (2008; 2009) that are described in Section 3.2, the effects of EPA and DHA supplementation on LDL-cholesterol concentrations were investigated in subjects with type 2 diabetes. Most studies provided EPA and DHA in combination at doses up to 6 g/day. Out of the 30 RCTs considered, 27 reported on LDL-cholesterol, 24 (n=1,530) on TG, 23 (n=1,533) on total cholesterol, 22 (n=1,443) on HLD-cholesterol, nine (n=637) on VLDL, five (n=476) on apoproteins A1 and B, and four (n=443) assessed LDL particle size. Compared to placebo (mostly vegetable oils), n-3 LCPUFA supplementation significantly increased LDL-cholesterol concentrations by 3 % (mean increase=+0.08 mmol/L). This effect was only observed at doses of >2 g/day of n-3 LCPUFA and was accompanied by a significant reduction in blood concentrations of TG of about 7 % (mean reduction=0.17 mmol/L), whereas no significant effect was reported on the remaining outcomes related to blood lipids, including total cholesterol concentrations.

A recently published systematic review and meta-analysis of RCTs lasting four weeks or longer investigated whether these n-3 LCPUFA had different effects on blood lipids (Jacobson et al., 2012; Wei and Jacobson, 2011). Twelve studies used mostly DHA and four studies used mostly EPA. The control intervention in the studies using mostly DHA (from algal oils, 38 % DHA and 30 % saturated fatty acids, doses 0.7-3.0 g/day, mean=1.7 g/day) was either a control fat (olive oil, two studies) or a control diet, and lasted six weeks to three months (average seven weeks). In the studies comparing EPA (ethyl esters) to placebo or to a control intervention, the dose was always 1.8 g/day and EPA was given for three months to five years (average 12 weeks excluding the five-year study). The Panel notes that these studies may not have been appropriately controlled for other dietary components known to increase LDL-cholesterol concentrations (e.g. saturated fatty acids) and for the different

dose range of DHA and EPA used. The Panel considers that these studies do not allow conclusions to be drawn on the effects of EPA or DHA, or on the effects of EPA *vs*. DHA, on LDL cholesterol.

In the same systematic review and meta-analysis (Jacobson et al., 2012; Wei and Jacobson, 2011), six studies which directly compared EPA (ethyl esters, >90 % EPA) with DHA (ethyl esters, >90 % DHA) used olive oil, safflower oil, corn oil or ALA as control fat, lasted 4-7 weeks, and administered EPA and DHA at doses between 2.3 and 4 g/day each. Control-adjusted changes in blood lipids were calculated for the EPA and DHA groups. DHA significantly increased LDL-cholesterol by 2.6 % compared to the control fat and by 3.3 % compared to EPA, which did not induce significant changes in LDL cholesterol (-0.7 %). The Panel notes that the observed increase in LDL cholesterol induced by DHA supplementation compared to the control fats was associated with a significant decrease in TG (-22.4 %) and with a significant increase in HDL-cholesterol (+7.3 %), and that non-HDL cholesterol was virtually not affected (-1.2 %). The Panel also notes that EPA supplementation did not have a significant effect on LDL- (-0.7 %) or HDL- (+1.4 %) cholesterol concentrations, and that these n-3 LCPUFAs appear to exert different effects on blood lipids.

It has been suggested that n-3 LCPUFA may enhance transformation of TG-rich VLDL lipoproteins to cholesterol-rich LDL lipoproteins leading to a decrease in fasting TGs and to an increase in LDL-cholesterol by increasing particle size rather than particle number. These changes do not appear to be associated with an increase in total cholesterol or apolipoprotein B (VKM, 2011).

The Panel notes that supplemental intakes of EPA and DHA combined of 2-6 g/day, and supplemental intakes of mostly DHA of 2-4 g/day, increase blood concentrations of LDL-cholesterol by about 3 %, and that such increase is accompanied by a decrease in TG with no changes in total (or non-HDL) cholesterol concentrations. The Panel also notes that supplemental intakes of mostly EPA at doses up to 4 g/day have no significant effect on LDL cholesterol concentrations. The Panel considers that the small increase in LDL-cholesterol concentrations associated with combined EPA and DHA supplementation or with DHA supplementation alone at the doses mentioned above may not be adverse in relation to CVD risk.

3.4. Markers of lipid peroxidation

Enhanced oxidative stress and increased lipid peroxidation occurring either locally in the vessel wall or systemically have been implicated in the pathogenesis of atherosclerosis in humans, although it is uncertain and poorly characterised whether, and the extent to which, changes in different markers of lipid peroxidation may modulate the risk of cardiovascular diseases independently of traditional risk factors.

Early observations linking DHA intake with increased lipid peroxidation and oxidative damage to cells and molecules in laboratory animals may have been confounded by the presence of primary and secondary oxidation products in supplements lacking antioxidants. This effect was indeed reversed when DHA was administered with supplemental vitamin E (IoM, 2005; VKM, 2011).

The majority of the human intervention studies considered below used fish oil stabilised with antioxidants, but some studies did not report whether sources of EPA, DHA, or both, contained antioxidants or not, whereas only a few studies reported on the concentration of primary and secondary oxidation products in the supplements administered. The Panel notes that the addition of antioxidants to food supplements containing n-3 LCPUFA to ensure product stability appears to be optional (GOED (Global Organisation for EPA and DHA Omega-3s), 2012).

3.4.1. F_2-isoprostanes

Some F_2-isoprostanes assessed in urine or plasma (i.e. by immunometric assays or by mass-spectrometry) are reliable measures of *in vivo* lipid peroxidation. F_2-isoprostanes are increased in association with a number of atherosclerotic risk factors, including cigarette smoking, hypercholesterolaemia, diabetes mellitus and obesity, among others. Also a reduction in cardiovascular risk factors is associated with a decrease in F_2-isoprostanes formation in humans. However, the potential contribution of these compounds to the pathophysiology of vascular damage and atherosclerosis has not yet been defined (Minuz et al., 2006; Morrow, 2005; Patrignani and Tacconelli, 2005).

A recent review identified nine controlled human intervention studies which used n-3 LCPUFA-rich oils stabilised with antioxidants, and mostly vegetable oils as control (olive, maize, sunflower, safflower or soy oil), and reported on plasma or urinary F_2-isoprostanes (VKM, 2011). An additional study of more recent publications was identified by the Panel (Mas et al., 2010). Three studies were conducted in newborns (following maternal supplementation from 20 weeks of gestation until delivery with 4 g/day EPA and DHA from fish oil) (Barden et al., 2004), pre-term infants (EPA and DHA were incorporated to the pre-term formula; 5.25-8.75 mg/100 mL of formula) (Stier et al., 2001) or children with familial hypercholesterolaemia (9-19 years, 1.2 g/day DHA) (Engler et al., 2004). The remaining studies had recruited a variety of adults who were either healthy (e.g. young men, post-menopausal women) or with various disease conditions (e.g. obesity, non insulin-dependent diabetes mellitus, hypertension, end-stage renal disease), and used either DHA alone (800 mg-4 g/day), EPA alone (1.6-4 g/day) or EPA and DHA in combination as fish oil (2-4 g/day) for three to six weeks. The studies of longer duration (six weeks) used the highest doses of EPA and DHA, both alone and in combination. Half of the studies reported a significant decrease in plasma or urinary concentrations of F_2-isoprostanes in the n-3 LCPUFA group compared to controls (Barden et al., 2004; Higdon et al., 2000; Mas et al., 2010; Mori et al., 2000; 2003), whereas the remaining studies did not observe significant changes between groups (Engler et al., 2004; Himmelfarb et al., 2007; Stier et al., 2001; Tholstrup et al., 2004; Wu et al., 2006). The Panel notes that the concentration of primary and secondary oxidation products in the oils used measured as peroxide value (PV) and anisidine value (AV) were reported only in a few studies.

The Panel considers that supplemental intakes of EPA and DHA consumed either alone or in combination at doses up to about 4 g/day for six weeks do not induce lipid peroxidation as assessed by F_2-isoprostanes.

3.4.2. Oxidation of LDL particles

As for F_2-isoprostanes, oxidation of LDL particles has been associated with an increased risk of CVD in some studies, but the causality of such association has not been established. Oxidised LDL particles can be measured in blood directly by immunological methods, and their susceptibility to oxidation may be measured *ex vivo* after challenge with different pro-oxidant agents. The Panel notes that the latter is not an appropriate method to assess *in vivo* LDL peroxidation.

Susceptibility of LDL to oxidation has been reported to be increased, decreased or unchanged during consumption of EPA and DHA from either fish oil or as ethyl esters, in a number of studies. Whereas an increased susceptibility of LDL to oxidation has been reported in some short-term studies (4-6 weeks), longer-term interventions (6-16 weeks) show no effect compared to control (mostly vegetable) oils at doses up to about 5 g/day (VKM, 2011).

Two studies in which the diet was supplemented with salmon containing EPA and DHA 1.5 g/day and 2.9 g/day (Seierstad et al., 2005) or herring containing EPA and DHA 1.2 g/day (Lindqvist et al., 2009) did not show an effect of the intervention on plasma oxidised LDL concentrations compared to controls.

The Panel considers that supplemental intakes of EPA and DHA combined at doses up to about 5 g/day consumed for up to 16 weeks do not induce sustained oxidative changes in circulating LDL particles.

3.4.3. Other markers of lipid peroxidation

Supplementation with EPA and DHA at doses up to 4.5 g/day has not been shown to affect other measures traditionally used to assess lipid peroxidation, such as thiobarbituric acid reactive substances (TBARS), malondialdehyde (MDA), conjugated diens or lipid hydroperoxides (VKM, 2011). The Panel notes that these are not reliable markers of *in vivo* lipid peroxidation (EFSA Panel on Dietetic Products Nutrition and Allergies (NDA), 2011).

3.4.4. Conclusion

The Panel considers that supplemental intakes of EPA and DHA consumed either alone or in combination at doses up to about 5 g/day for up to 16 weeks do not induce changes in lipid peroxidation which might raise concern in relation to CVD risk as long as the oxidative stability of these n-3 LCPUFAs is guaranteed.

3.5. Immune function

Immunosuppression, if sustained, may increase the risk of infections. There are no human intervention studies available which have investigated the effects of n-3 LCPUFA supplementation on the risk of infections *in vivo*. There is some indication, from *ex vivo* and *in vitro* studies performed in peripheral white blood cells of human subjects consuming n-3 LCPUFA, that EPA and DHA may decrease the expression of cytokines and the proliferation of peripheral white blood cells at doses as low as 0.9 g/day EPA and 0.6 g/day DHA consumed as fish oil for 6-8 weeks (reviewed in IoM, 2005). However, the clinical relevance of these changes *in vivo* is unknown.

Chronic and/or inappropriate activation of inflammatory responses (innate immunity) can also lead to disease. However, there is no information available on the effect of high intakes of n-3 LCPUFA on the risk of chronic diseases of inflammatory origin. Some markers of the so-called low-grade systemic (e.g. high-sensitivity C-reactive protein, and some cytokines) and vascular (e.g., sICAM-1, VCAM-1, and E-selectin) inflammation have been associated with an increased risk of cardiovascular events in healthy and high-risk subjects. However, there is no evidence that changes induced by diet or drugs in any of these markers modify the risk of disease *per se*. Most of the intervention studies available (reviewed in VKM, 2011) which report on the effects of EPA and DHA on markers of systemic and vascular inflammation are small and generally not designed for that purpose. Although an increase in E-selectin and/or in sVCAM-1 has been reported in some studies at doses of EPA and DHA of about 5 g/day, a recent meta-analysis of 18 randomised controlled trials found no effect of n-3 LCPUFA supplementation (dose 0.272 to 6.6 g/day) on these markers of vascular inflammation and a significant decrease in sICAM-1 (Yang et al., 2012). The majority of the studies report either no effect or a decrease in systemic markers of inflammation, including hs-CRP and TNF-alpha (Bloomer et al., 2009; VKM, 2011).

The Panel considers that supplemental intakes of EPA and DHA up to about 5 g/day are unlikely to induce changes in immune functions which might raise concern in relation to the risk of infections or inappropriate activation of inflammatory responses. The Panel notes that the data available are insufficient to conclude on whether the same doses administered mostly as EPA or mostly as DHA would have different effects on this outcome.

4. Derivation of a tolerable upper intake level (UL)

The available data are not sufficient to establish a tolerable upper intake level for n-3 LCPUFA (DHA, EPA, and DPA, individually or combined) for any population group.

5. Characterisation of the risk

Mean dietary intake estimates of n-3 LCPUFA (EPA, DHA ± DPA) from foods in European populations are up to 400-500 mg/day in adults and up to 324 mg/day in children. When supplements were included, or when only high consumers of fatty fish were considered, reported intakes in EU populations can be much higher, for example up to 2,570-2,700 mg/day in adults and up to 400-600 mg/day in children (95 % percentile, 1,400-1,700 mg/day). The Panel notes that the studies reporting on high consumers of fish did not consider n-3 LCPUFA intakes from food supplements.

The Panel notes that at observed intake levels, consumption of n-3 LCPUFA has not been associated with adverse effects in healthy children or adults.

The Panel considers that supplemental intakes of EPA and DHA combined at doses up to 5 g/day, and supplemental intakes of EPA alone up to 1.8 g/day, do not raise safety concerns for the adult population. Limited data are available on the effects of long-term supplementation with these n-3 LCPUFAs at higher doses. The Panel also notes that observed intakes of EPA and DHA from food and food supplements in European populations are generally below these amounts. Dietary recommendations for EPA and DHA based on CVD risk considerations for European adults are between 250 and 500 mg/day. There are no specific recommendations for EPA.

The Panel also considers that supplemental intakes of DHA alone up to about 1 g/day do not raise safety concerns for the general population. Limited data are available on the effects of long-term supplementation with DHA alone at higher doses. The Panel notes that specific dietary recommendations for DHA for European adults and children are well below this amount.

No data are available for DPA when consumed alone. The Panel notes that in the majority of the human studies considered, fish oils, which also contained DPA in generally unknown (but relatively low) amounts, were the source of EPA and DHA. No dietary recommendations have been made specifically for DPA.

CONCLUSIONS

The Panel concludes that the available data are not sufficient to establish a tolerable upper intake level for n-3 LCPUFA (DHA, EPA, and DPA, individually or combined) for any population group.

The Panel considers that supplemental intakes of EPA and DHA combined at doses up to 5 g/day, and supplemental intakes of EPA alone up to 1.8 g/day, do not raise safety concerns for the adult population. The Panel also considers supplemental intakes of DHA alone up to about 1 g/day do not raise safety concerns for the general population. No data are available for DPA when consumed alone. The Panel notes that in the majority of the human studies considered, fish oils, which also contained DPA in generally unknown (but relatively low) amounts, were the source of EPA and DHA.

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APPENDICES

A. INTAKE OF LONG-CHAIN N-3 FATTY ACIDS (MG/DAY) AMONG ADULTS IN EUROPEAN COUNTRIES

LCn-3 PUFA	Nutrient source	Sex	Country	Reference	Dietary method	n	Age min		Age max	Population / Fortified foods included or excluded	mean	median	P75	P95
EPA	**Food**	**Women**	Belgium	(Sioen et al., 2006)	2-day record	641	18		39	Data collected only on women in Ghent, Flanders	77.8	14.0		427.7
			Denmark	(Joensen et al., 2010)	food frequency questionnaire	29,017	50		64	Data of the Danish Diet, Cancer and Health cohort study, collected on volunteers living in Copenhagen or Aarhus. Recruitment 1993-1997		150.0	230.0	
			France	(Bemrah et al., 2009)	food frequency questionnaire	344	18		44	French high seafood consumers (CALIPSO study). N-3 consumption from seafood (not from the total diet)	432.0			
						630	18		64	French high seafood consumers (CALIPSO study). N-3 consumption from seafood (not from the total diet)	448.0			
EPA	**Food**	**Men**	Denmark	(Joensen et al., 2010)	food frequency questionnaire	24,786	50		64	Data of the Danish Diet, Cancer and Health cohort study, collected on volunteers living in Copenhagen or Aarhus. Recruitment 1993-1997		180.0	270.0	
			France	(Bemrah et al., 2009)	food frequency questionnaire	243	18		64	French high seafood consumers (CALIPSO study). N-3 consumption from seafood (not from the total diet)	456.0			
EPA	**Food**	**Men and women**	France	(Guevel et al., 2008)	food frequency questionnaire	n.a.	18	>	65	French high seafood consumers (CALIPSO study). First quintile of EPA-DHA intake	141.0	140.0		
				(Guevel et al., 2008)	food frequency questionnaire	n.a.	18	>	65	French high seafood consumers (CALIPSO study).Fifth quintile of EPA-DHA intake	991.0	858.0		
				(Bemrah et al., 2009)	food frequency questionnaire	126	65	>	65	French high seafood consumers (CALIPSO study). N-3 consumption from seafood (not from the total diet)	467.0			
			Spain	(Amiano et al., 2001)	Diet history questionnaire (previous year)	26	35		65	Occasional fish consumers (<31 g/d). Adjusted for energy intake. Data on n-3 fatty acid intake collected in the EPIC cohort of Gipuzkoa (Basque Country)	50.0			
						24	35		65	Low fish consumers (32-64 g/d). Adjusted for energy intake. Data on n-3 fatty acid intake collected in the EPIC cohort of Gipuzkoa (Basque Country)	130.0			

LCn-3 PUFA	Nutrient source	Sex	Country	Reference	Dietary method	n	Age min		Age max	Population / Fortified foods included or excluded	mean	median	P75	P95
EPA	**Food**	**Men and women**	Spain	(Amiano et al., 2001)	Diet history questionnaire (previous year)	27	35		65	Moderate fish consumers (65-115 g/d). Adjusted for energy intake. Data on n-3 fatty acid intake collected in the EPIC cohort of Gipuzkoa (Basque Country)	210.0			
						25	35		65	High fish consumers (>115 g/d). Adjusted for energy intake. Data on n-3 fatty acid intake collected in the EPIC cohort of Gipuzkoa (Basque Country)	320.0			
EPA	**n.a.**	**Women**	Finland	(Suominen-Taipale et al., 2010)	food frequency questionnaire	166	n.a.		n.a.	Finnish maritime and freshwater area fishermen, their wives and other family members of a sub-group (living near Helsinki) of the Fishermen Study population. Mean age of the full study (n=1410): 47 y (SE: 0,5) for men, 46 (SE: 0,5) for women.	200.0			
			France	(Astorg et al., 2004)	at least ten 24-hour recalls	2,785	35	>	35	Data from the Su.Vi.Max cohort Study (inclusion in 1994-19695, follow-up of 8 years), collected from at least ten 24-hour recalls over a period of 2.5 years, between the inclusion and 1998. Age at inclusion: 45-63 y (men), 35-63 y (women)	117.8	91.4		308.5
EPA	**n.a.**	**Men**	Finland	(Suominen-Taipale et al., 2010)	food frequency questionnaire	142	n.a.		n.a.	Finnish maritime and freshwater area fishermen, their wives and other family members of a sub-group (living near Helsinki) of the Fishermen Study population. Mean age of the full study (n=1410): 47 y (SE: 0,5) for men, 46 (SE: 0,5) for women.	300.0			
			France	(Astorg et al., 2004)	at least ten 24-hour recalls	2,099	45	>	45	Data from the Su.Vi.Max cohort Study (inclusion in 1994-19695, follow-up of 8 years), collected from at least ten 24-hour recalls over a period of 2.5 years, between the inclusion and 1998. Age at inclusion: 45-63 y (men), 35-63 y (women)	149.9	119.6		375.1
EPA	**Food and supplements**	**Women**	United-Kingdom	(Welch et al., 2010)	7-day record (24-h recall and 6-day diary)	6258	39		78	Fish-eaters in the EPIC-Norfolk cohort	110.0			
EPA	**Food and supplements**	**Men**	United-Kingdom	(Welch et al., 2010)	7-day record (24-h recall and 6-day diary)	5,952	39		78	Fish-eaters in the EPIC-Norfolk cohort	130.0			

LCn-3 PUFA	Nutrient source	Sex	Country	Reference	Dietary method	n	Age min		Age max	Population / Fortified foods included or excluded	mean	median	P75	P95
EPA	**Food and supplements**	**Men and women**	Norway	(Johansson et al., 1998)	food frequency questionnaire	3,144	16		79	National survey NORKOST 1997	330.0			
DPA	**Food**	**Women**	Belgium	(Sioen et al., 2006)	2-day record	641	18		39	Data collected only on women in Ghent, Flanders	25.3	11.7		100.2
			Denmark	(Joensen et al., 2010)	food frequency questionnaire	29,017	50		64	Data of the Danish Diet, Cancer and Health cohort study, collected on volunteers living in Copenhagen or Aarhus. Recruitment 1993-1997		60.0	90.0	
			France	(Bemrah et al., 2009)	food frequency questionnaire	344	18		44	French high seafood consumers (CALIPSO study). N-3 consumption from seafood (not from the total diet)	126.0			
						630	18		64	French high seafood consumers (CALIPSO study). N-3 consumption from seafood (not from the total diet)	127.0			
DPA	**Food**	**Men**	Denmark	(Joensen et al., 2010)	food frequency questionnaire	24,786	50		64	Data of the Danish Diet, Cancer and Health cohort study, collected on volunteers living in Copenhagen or Aarhus. Recruitment 1993-1997		80.0	100.0	
			France	(Bemrah et al., 2009)	food frequency questionnaire	243	18		64	French high seafood consumers (CALIPSO study). N-3 consumption from seafood (not from the total diet)	129.0			
DPA	**Food**	**Men and Women**	France	(Bemrah et al., 2009)	food frequency questionnaire	126	65	>	65	French high seafood consumers (CALIPSO study). N-3 consumption from seafood (not from the total diet)	125.0			
DPA	**n.a.**	**Women**	France	(Astorg et al., 2004)	at least ten 24-hour recalls	2,785	35	>	35	Data from the Su.Vi.Max cohort Study (inclusion in 1994-19695, follow-up of 8 years), collected from at least ten 24-hour recalls over a period of 2.5 years, between the inclusion and 1998. Age at inclusion: 45-63 y (men), 35-63 y (women)	55.9	50.2		109.1
DPA	**n.a.**	**Men**	France	(Astorg et al., 2004)	at least ten 24-hour recalls	2,099	45	>	45	Data from the Su.Vi.Max cohort Study (inclusion in 1994-19695, follow-up of 8 years), collected from at least ten 24-hour recalls over a period of 2.5 years, between the inclusion and 1998. Age at inclusion: 45-63 y (men), 35-63 y (women)	74.8	68.2		138.4
DPA	**Food and supplements**	**Men and Women**	Norway	(Johansson et al., 1998)	food frequency questionnaire	3,144	16		79	National survey NORKOST 1997	70.0			

LCn-3 PUFA	Nutrient source	Sex	Country	Reference	Dietary method	n	Age min		Age max	Population / Fortified foods included or excluded	mean	median	P75	P95
DHA	**Food**	**Women**	Belgium	(Sioen et al., 2006)	2-day record	641	18		39	Data collected only on women in Ghent, Flanders	131.2	42.5		647.1
			Denmark	(Joensen et al., 2010)	food frequency questionnaire	29,017	50		64	Data of the Danish Diet, Cancer and Health cohort study, collected on volunteers living in Copenhagen or Aarhus. Recruitment 1993-1997		360.0	520.0	
			France	(Bemrah et al., 2009)	food frequency questionnaire	344	18		44	French high seafood consumers (CALIPSO study). N-3 consumption from seafood (not from the total diet)	757.0			
						630	18		64	French high seafood consumers (CALIPSO study). N-3 consumption from seafood (not from the total diet)	776.0			
DHA	**Food**	**Men**	Denmark	(Joensen et al., 2010)	food frequency questionnaire	24,786	50		64	Data of the Danish Diet, Cancer and Health cohort study, collected on volunteers living in Copenhagen or Aarhus. Recruitment 1993-1997		430.0	630.0	
			France	(Bemrah et al., 2009)	food frequency questionnaire	243	18		64	French high seafood consumers (CALIPSO study). N-3 consumption from seafood (not from the total diet)	797.0			
DHA	**Food**	**Men and women**	France	(Guevel et al., 2008)	food frequency questionnaire	n.a.	18	>	65	French high seafood consumers (CALIPSO study). First quintile of EPA-DHA intake	251.0	260.0		
			France	(Guevel et al., 2008)	food frequency questionnaire	n.a.	18	>	65	French high seafood consumers (CALIPSO study). Fifth quintile of EPA-DHA intake	1,709.0	1,434.0		
				(Bemrah et al., 2009)	food frequency questionnaire	126	65	>	65	French high seafood consumers (CALIPSO study). N-3 consumption from seafood (not from the total diet)	819.0			
			Spain	(Amiano et al., 2001)	Diet history questionnaire (previous year)	26	35		65	Occasional fish consumers (<31 g/d). Adjusted for energy intake. Data on n-3 fatty acid intake collected in the EPIC cohort of Gipuzkoa (Basque Country)	190.0			
						24	35		65	Low fish consumers (32-64 g/d). Adjusted for energy intake. Data on n-3 fatty acid intake collected in the EPIC cohort of Gipuzkoa (Basque Country)	440.0			
						27	35		65	Moderate fish consumers (65-115 g/d). Adjusted for energy intake. Data on n-3 fatty acid intake collected in the EPIC cohort of Gipuzkoa (Basque Country)	580.0			

LCn-3 PUFA	Nutrient source	Sex	Country	Reference	Dietary method	n	Age min		Age max	Population / Fortified foods included or excluded	mean	median	P75	P95
DHA	**Food**	**Men and women**	Spain	(Amiano et al., 2001)	Diet history questionnaire (previous year)	25	35		65	High fish consumers (>115 g/d). Adjusted for energy intake. Data on n-3 fatty acid intake collected in the EPIC cohort of Gipuzkoa (Basque Country)	850.0			
DHA	**n.a.**	**Women**	Finland	(Suominen-Taipale et al., 2010)	food frequency questionnaire	166	n.a.		n.a.	Finnish maritime and freshwater area fishermen, their wives and other family members of a sub-group (living near Helsinki) of the Fishermen Study population. Mean age of the full study (n=1410): 47 y (SE: 0,5) for men, 46 (SE: 0,5) for women.	600.0			
			France	(Astorg et al., 2004)	at least ten 24-hour recalls	2,785	35	>	35	Data from the Su.Vi.Max cohort Study (inclusion in 1994-19695, follow-up of 8 years), collected from at least ten 24-hour recalls over a period of 2.5 years, between the inclusion and 1998. Age at inclusion: 45-63 y (men), 35-63 y (women)	225.9	177.0		574.2
DHA	**n.a.**	**Men**	Finland	(Suominen-Taipale et al., 2010)	food frequency questionnaire	142	n.a.		n.a.	Finnish maritime and freshwater area fishermen, their wives and other family members of a sub-group (living near Helsinki) of the Fishermen Study population. Mean age of the full study (n=1410): 47 y (SE: 0,5) for men, 46 (SE: 0,5) for women.	700.0			
			France	(Astorg et al., 2004)	at least ten 24-hour recalls	2,099	45	>	45	Data from the Su.Vi.Max cohort Study (inclusion in 1994-19695, follow-up of 8 years), collected from at least ten 24-hour recalls over a period of 2.5 years, between the inclusion and 1998. Age at inclusion: 45-63 y (men), 35-63 y (women)	272.6	221.3		668.4
DHA	**Food and supplements**	**Women**	United-Kingdom	(Welch et al., 2010)	7-day record (24-h recall and 6-day diary)	6258	39		78	Fish-eaters in the EPIC-Norfolk cohort	150.0			
DHA	**Food and supplements**	**Men**	United-Kingdom	(Welch et al., 2010)	7-day record (24-h recall and 6-day diary)	5,952	39		78	Fish-eaters in the EPIC-Norfolk cohort	190.0			
DHA	**Food and supplements**	**Men and women**	Norway	(Johansson et al., 1998)	food frequency questionnaire	3,144	16		79	National survey NORKOST 1997	490.0			

LCn-3 PUFA	Nutrient source	Sex	Country	Reference	Dietary method	n	Age min		Age max	Population / Fortified foods included or excluded	mean	median	P75	P95
EPA+ DHA	**Food**	**Women**	Belgium	(Sioen et al., 2010)	food frequency questionnaire	19	18		39	Non-consumers of n-3 foods and supplements. Data collected from a convenience sample (relatives, friends and colleagues) of women living in Flanders	181.0	118.0		
				(Sioen et al., 2006)	2-day record	641	18		39	Data collected only on women in Ghent, Flanders	208.9	54.1		1,115.4
			The Netherlands	(van Rossum et al., 2011)	two non-consecutive 24-hour dietary recalls	347	19		30	National Dutch survey 2007-2010		75.0	133.0	285.0
						351	31		50	National Dutch survey 2007-2010		89.0	155.0	330.0
						353	51		69	National Dutch survey 2007-2010		107.0	185.0	388.0
EPA+ DHA	**Food**	**Men**	The Netherlands	(van Rossum et al., 2011)	two non-consecutive 24-hour dietary recalls	356	19		30	National Dutch survey 2007-2010		77.0	139.0	305.0
						348	31		50	National Dutch survey 2007-2010		95.0	169.0	364.0
						351	51		69	National Dutch survey 2007-2010		110.0	194.0	414.0
EPA+ DHA	**Food**	**Men and women**	France	(Guevel et al., 2008)	food frequency questionnaire	n.a.	18	>	65	French high seafood consumers (CALIPSO study). First quintile of EPA-DHA intake	392.0	405.0		
						n.a.	18	>	65	French high seafood consumers (CALIPSO study). Fifth quintile of EPA-DHA intake	2,700.0	2,324.0		
EPA+ DHA	**Food (without fortified food)**	**Women**	Germany	(Bauch et al., 2006)	Dietary history (last 4 weeks)	181	18		24	National German survey 1998	126.6	83.9		367.8
						396	25		34	National German survey 1998	167.4	121.2		501.0
						399	35		44	National German survey 1998	196.6	147.2		471.8
						319	45		54	National German survey 1998	207.1	160.3		578.3
						369	55		64	National German survey 1998	218.9	172.4		560.1
						408	65		79	National German survey 1998	199.9	158.4		556.3
EPA+ DHA	**Food (without fortified food)**	**Men**	Germany	(Bauch et al., 2006)	Dietary history (last 4 weeks)	189	18		24	National German survey 1998	232.1	153.9		790.3
						412	25		34	National German survey 1998	212.0	161.7		553.2
						411	35		44	National German survey 1998	238.3	181.8		643.5
						321	45		54	National German survey 1998	295.0	216.5		826.6
						353	55		64	National German survey 1998	274.4	195.1		794.5

LCn-3 PUFA	Nutrient source	Sex	Country	Reference	Dietary method	n	Age min	Age max	Population / Fortified foods included or excluded	mean	median	P75	P95
EPA+ DHA	**Food (without fortified food)**	**Men**	Germany	(Bauch et al., 2006)	Dietary history (last 4 weeks)	271	65	79	National German survey 1998	277.7	204.1		668.3
EPA+ DHA	**Food and supplements**	**Women**	Belgium	(Sioen et al., 2010)	food frequency questionnaire	5	18	39	Breasfeeding women. Data collected from a convenience sample (relatives, friends and colleagues) of women living in Flanders	299.0	205.0		
						18	18	39	Pregnant women. Convenience sample, in Flanders	328.0	232.0		
						19	18	39	Supplement users. Convenience sample, in Flanders	1,067.0	998.0		
						395	18	39	Consumers of n-3 foods. Convenience sample, in Flanders	281.0	209.0		
						414	18	39	Total population. Convenience sample, in Flanders	276.0	199.0		
			The Netherlands	(van Rossum et al., 2011)	two non-consecutive 24-hour dietary recalls	347	19	30	National Dutch survey 2007-2010		76.0	133.0	296.0
						351	31	50	National Dutch survey 2007-2010		100.0	189.0	488.0
						353	51	69	National Dutch survey 2007-2010		133.0	264.0	611.0
EPA+ DHA	**Food and supplements**	**Men**	The Netherlands	(van Rossum et al., 2011)	two non-consecutive 24-hour dietary recalls	356	19	30	National Dutch survey 2007-2010		75.0	137.0	312.0
						348	31	50	National Dutch survey 2007-2010		97.0	179.0	416.0
						351	51	69	National Dutch survey 2007-2010		131.0	239.0	513.0
EPA+ DHA	**Food and supplements**	**Men and women**	Ireland	(Leite et al., 2010)	7-day record	1097	18	64	Non under-reporters. North/South Ireland food consumption survey	275.0	124.0		1,147.0
						424	18	35	Non under-reporters. North/South Ireland food consumption survey	187.0	99.0		825.0
						422	36	50	Non under-reporters. North/South Ireland food consumption survey	297.0	133.0		1,160.0
						251	51	64	Non under-reporters. North/South Ireland food consumption survey	386.0	179.0		1,278.0

LCn-3 PUFA	Nutrient source	Sex	Country	Reference	Dietary method	n	Age min		Age max	Population / Fortified foods included or excluded	mean	median	P75	P95
EPA+ DHA	**Supplements**	**Women**	Austria	(Elmadfa et al., 2009)	Quantitative consumption frequency questionnaire	6	18		65	Data collected on consumption of dietary supplements among 282 adults in all Austria (77 supplements users)		268.0	738.0	
EPA+ DHA	**Supplements**	**Men**	Austria	(Elmadfa et al., 2009)	Quantitative consumption frequency questionnaire	5	18		65	Data collected on consumption of dietary supplements among 282 adults in all Austria (77 supplements users)		557.0	1,000.0	
EPA+ DPA+ DHA	**n.a.**	**Women**	France	(Astorg et al., 2004)	at least ten 24-hour recalls	2785	35	>	35	Data from the Su.Vi.Max cohort Study (inclusion in 1994-19695, follow-up of 8 years), collected from at least ten 24-hour recalls over a period of 2.5 years, between the inclusion and 1998. Age at inclusion: 45-63 y (men), 35-63 y (women)	399.6	320.6		980.2
EPA+ DPA+ DHA	**n.a.**	**Men**	France	(Astorg et al., 2004)	at least ten 24-hour recalls	2785	45	>	45	Data from the Su.Vi.Max cohort Study (inclusion in 1994-19695, follow-up of 8 years), collected from at least ten 24-hour recalls over a period of 2.5 years, between the inclusion and 1998. Age at inclusion: 45-63 y (men), 35-63 y (women)	497.3	408.3		1,159.3
EPA+ DPA+ DHA	**Food and supplements**	**Women**	Norway	(Johansson et al., 1998)	food frequency questionnaire	406	16		79	First quartile of long chain n-3 fatty acids. National survey NORKOST 1997	130.0			
						406	16		79	Fourth quartile of long chain n-3 fatty acid intake. National survey NORKOST 1997	1,730.0			
EPA+ DPA+ DHA	**Food and supplements**	**Men**	Norway	(Johansson et al., 1998)	food frequency questionnaire	379	16		79	First quartile of long chain n-3 fatty acids. National survey NORKOST 1997	190.0			
						378	16		79	Fourth quartile of long chain n-3 fatty acid intake. National survey NORKOST 1997	2,570.0			
EPA+ DPA+ DHA	**Food and supplements**	**Men and women**	Norway	(Johansson et al., 1998)	food frequency questionnaire	3144	16		79	National survey NORKOST 1997	890.0			

LCn-3 PUFA	Nutrient source	Sex	Country	Reference	Dietary method	n	Age min	Age max	Population / Fortified foods included or excluded	mean	median	P75	P95
LC n-3 PUFA	Food (including fish oil)	Women	Iceland	(Steingrímsdóttir et al., 2003)	24-hour recall	n.a. (total female sample 15-80 y: 662)	20	39	National Icelandic survey	300.0			
						n.a. (total female sample 15-80 y: 662)	40	59	National Icelandic survey	600.0			
						n.a. (total female sample 15-80 y: 662)	60	80	National Icelandic survey	900.0			
LC n-3 PUFA	food (including fish oil)	Men	Iceland	(Steingrímsdóttir et al., 2003)	24-hour recall	n.a. (total male sample 15-80 y: 580)	20	39	National Icelandic survey	700.0			
						n.a. (total male sample 15-80 y: 580)	40	59	National Icelandic survey	1,100.0			
						n.a. (total male sample 15-80 y: 580)	60	80	National Icelandic survey	1,300.0			
LC n-3 PUFA	food (including fish oil)	Men and women	Iceland	(Steingrímsdóttir et al., 2003)	24-hour recall	1242	15	80	National Icelandic survey	700.0			

n.a.: not available

B. INTAKE OF LONG-CHAIN N-3 FATTY ACIDS (MG/DAY) AMONG CHILDREN IN EUROPEAN COUNTRIES

LCn-3PUFA	Nutrient source	Sex	Country	Reference	Dietary method	n	Age min	Age max	Population / Fortified foods included or excluded	mean	median	P75	P95
EPA	**Food**	Boys and girls	Belgium	(Sioen et al., 2007a)	3-day record (food)	661	2.5	6.5	No consumption of supplements containing PUFA. Data collected in Flanders.	25.0		21.0	
EPA	**n.a.**	Boys and girls	Belgium	(Sioen et al., 2007b)	7-day record	341	13	18	Data collected in the region of Ghent in Flanders	55.9	25.4		244.2
EPA	**Food and supplements**	Boys and girls	Sweden	(Enghardt Barbieri et al., 2006)	4-day record	590	4	4	National survey	40.0	10.0		140.0
						889	8	9	National survey	40.0	20.0		170.0
						1,016	11	12	National survey	40.0	20.0		160.0
DPA	**Food**	Boys and girls	Belgium	(Sioen et al., 2007a)	3-day record (food)	661	2.5	6.5	No consumption of supplements containing PUFA. Data collected in Flanders.	10.0		10.0	
DPA	**n.a.**	Boys and girls	Belgium	(Sioen et al., 2007b)	7-day record	341	13	18	Data collected in the region of Ghent in Flanders	18.4	9.6		62.5
DPA	**Food and supplements**	Boys and girls	Sweden	(Enghardt Barbieri et al., 2006)	4-day record	590	4	4	National survey	30.0	20.0		70.0
						889	8	9	National survey	40.0	30.0		90.0
						1,016	11	12	National survey	40.0	30.0		90.0
DHA	**Food**	Boys and girls	Belgium	(Sioen et al., 2007a)	3-day record (food)	661	2.5	6.5	No consumption of supplements containing PUFA. Data collected in Flanders.	47.0		46.0	
DHA	**n.a.**	Boys and girls	Belgium	(Sioen et al., 2007b)	7-day record	341	13	18	Data collected in the region of Ghent in Flanders	111.4	72.4		363.2
DHA	**Food and supplements**	Boys and girls	Sweden	(Enghardt Barbieri et al., 2006)	4-day record	590	4	4	National survey	100.0	60.0		320.0
						889	8	9	National survey	120.0	80.0		420.0
						1,016	11	12	National survey	120.0	70.0		420.0

LCn-3PUFA	Nutrient source	Sex	Country	Reference	Dietary method	n	Age min		Age max	Population / Fortified foods included or excluded	mean	median	P75	P95
EPA+ DHA	**Food**	Girls	The Netherlands	(van Rossum et al., 2011)	2 non-consecutive 24-hour dietary recalls	151	7		8	National survey		63.0	112.0	243.0
						352	9		13	National survey		65.0	119.0	251.0
						354	14		18	National survey		69.0	122.0	263.0
		Boys	The Netherlands	(van Rossum et al., 2011)	2 non-consecutive 24-hour dietary recalls	153	7		8	National survey		48.0	88.0	200.0
						351	9		13	National survey		56.0	102.0	230.0
						352	14		18	National survey		65.0	118.0	263.0
EPA+ DHA	**n.a.**	Boys and girls	Belgium	(Sioen et al., 2007b)	7-day record	341	13		18	Data collected in the region of Ghent in Flanders	167.3	96.9		603.0
EPA+ DHA	**Food (without fortified food)**	Girls	Germany	(Sichert-Hellert et al., 2009)	yearly 3-day dietary records	241	2	<	4	fish consumers. Data from the DONALD cohort, from 7152 records of 1024 subjects living in/near Dortmund. Mean number of repeated 3-day records: 7. In 2717 3-day records, i.e. on 3018 single days, fish consumption was documented.	100.0			
						241	2	<	4	on days with fish consumption. Data from the DONALD cohort	245.0			
						330	4		6	fish consumers.	135.0			
						330	4		6	on days with fish consumption.	335.0			
						294	7		9	fish consumers	181.0			
						294	7		9	on days with fish consumption	438.0			
						213	10		12	fish consumers	188.0			
						213	10		12	on days with fish consumption	473.0			
						113	13		14	fish consumers	214.0			
						113	13		14	on days with fish consumption	536.0			
						132	15		18	fish consumers	264.0			
						132	15		18	on days with fish consumption	685.0			

LCn-3PUFA	Nutrient source	Sex	Country	Reference	Dietary method	n	Age min		Age max	Population / Fortified foods included or excluded	mean	median	P75	P95
EPA+ DHA	**Food (without fortified food)**	Boys	Germany	(Sichert-Hellert et al., 2009)	yearly 3-day dietary records	236	2	<	4	fish consumers. Data from the DONALD cohort, from 7152 records of 1024 subjects living in/near Dortmund. Mean number of repeated 3-day records: 7. In 2717 3-day records, i.e. on 3018 single days, fish consumption was documented.	118.0			
						236	2	<	4	on days with fish consumption	289.0			
						352	4		6	fish consumers	142.0			
						352	4		6	on days with fish consumption	359.0			
						306	7		9	fish consumers	168.0			
						306	7		9	on days with fish consumption	433.0			
						220	10		12	fish consumers	206.0			
						220	10		12	on days with fish consumption	528.0			
						128	13		14	fish consumers	324.0			
						128	13		14	on days with fish consumption	838.0			
						152	15		18	fish consumers	301.0			
						152	15		18	on days with fish consumption	763.0			
EPA+ DHA	**Food and supplements**	Girls	The Netherlands	(van Rossum et al., 2011)	2 non-consecutive 24-hour dietary recalls	151	7		8	National survey		66.0	120.0	294.0
						352	9		13	National survey		66.0	117.0	264.0
						354	14		18	National survey		71.0	126.0	282.0
EPA+ DHA	**Food and supplements**	Boys	The Netherlands	(van Rossum et al., 2011)	2 non-consecutive 24-hour dietary recalls	153	7		8	National survey		62.0	122.0	314.0
						351	9		13	National survey		65.0	126.0	317.0
						352	14		18	National survey		67.0	124.0	295.0

LCn-3PUFA	Nutrient source	Sex	Country	Reference	Dietary method	n	Age min	Age max	Population / Fortified foods included or excluded	mean	median	P75	P95
EPA+ DPA+ DHA	**Food (without fortified food)**	Boys and girls	Norway	(VKM, 2011)	semi-quantitative FFQ	1,231	1	1	National survey. Non breast-fed children.	100.0			400.0
					semi-quantitative FFQ	1,720	2	2	National survey	200.0			700.0
					4-day record	391	4	4	National survey	200.0			600.0
					4-day record	815	9	9	National survey	200.0			700.0
					4-day record	1,009	13	13	National survey	200.0			700.0
EPA+ DPA+ DHA	**Food and supplements**	Boys and girls	Norway	(VKM, 2011)	semi-quantitative FFQ	1,231	1	1	National survey. Non breast-fed children.	400.0			1,400.0
					semi-quantitative FFQ	1,720	2	2	National survey	600.0			1,700.0
					4-day record	391	4	4	National survey	400.0			1,400.0
					4-day record	815	9	9	National survey	300.0			1,200.0
					4-day record	1,009	13	13	National survey	300.0			1,100.0
LC n-3 PUFA	**Food (including fish oil)**	Girls	Iceland	(Steingrímsdóttir et al., 2003)	24-hour recall	n.a.	15	19		200.0			
		Boys	Iceland	(Steingrímsdóttir et al., 2003)	24-hour recall	n.a.	15	19		400.0			

C. INTAKE OF LONG-CHAIN n-3 FATTY ACIDS (% E) AMONG CHILDREN

LCn-3PUFA	Nutrient source	Sex	Country	Reference	Dietary method	n	Age min	Age max	Population / Fortified foods included or excluded	mean	median	P75	P95
EPA	**Food**	**Boys and girls**	Belgium	(Sioen et al., 2007a)	3-day record (food)	661	2.5	6.5	No consumption of supplements containing PUFA. Data collected in Flanders.	0.02		0.01	
	n.a.	**Boys and girls**	Belgium	(Sioen et al., 2007b)	7-day record	341	13	18	Data collected in the region of Ghent in Flanders	0.02	0.01		0.09
DPA	**Food**	**Boys and girls**	Belgium	(Sioen et al., 2007a)	3-day record (food)	661	2.5	6.5	No consumption of supplements containing PUFA. Data collected in Flanders.	0.01		0.01	
	n.a.	**Boys and girls**	Belgium	(Sioen et al., 2007b)	7-day record	341	13	18	Data collected in the region of Ghent in Flanders	0.01	0		0.03
DHA	**Food**	**Boys and girls**	Belgium	(Sioen et al., 2007a)	3-day record (food)	661	2.5	6.5	No consumption of supplements containing PUFA. Data collected in Flanders.	0.03		0.03	
	n.a.	**Boys and girls**	Belgium	(Sioen et al., 2007b)	7-day record	341	13	18	Data collected in the region of Ghent in Flanders	0.05	0.03		0.15
EPA+ DHA	**n.a.**	**Boys and girls**	Belgium	(Sioen et al., 2007b)	7-day record	341	13	18	Data collected in the region of Ghent in Flanders	0.07	0.04		0.26

GLOSSARY AND ABBREVIATIONS

AA	Arachidonic acid
ADHD	Attention-deficit hyperactivity disorder
ALA	α-linoleic acid
ANSES	Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail
ASA	Acetylsalicylic acid
AT	Antithrombin
AV	Anisidine value
BfR	Bundesinstitut fur Risikobewertung
CHD	Coronary heart disease
CRP	C-reactive protein
CSS	Conseil Supérieur de la Santé
CVD	Cardiovascular disease
D-A-CH	Deutschland-Austria-Confoederatio Helvetica
DHA	Docosahexaenoic acid
DoH	Department of Health
DPA	Docosapentaenoic acid
EPA	Eicosapentaenoic acid
FA	Fatty Acid
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
GOED	Global Organisation for EPA and DHA Omega-3s
GRAS	Generally recognised as safe
HbA1c	Glycated haemoglobin
HDL	High density lipoprotein
hs-CRP	High-sensitivity C-reactive Protein
INR	International normalised ratio

IoM	Institute of Medecine
LCPUFA	Long chain polyunsaturated fatty acids
LDL	Low density lipoprotein
MD	Malondialdehyde
NNR	Nordic Nutrition Recommendations
PAI	Plasminogen activator inhibitor
PAF	Platelet activating factor
PV	Peroxide value
PUFA	Polyunsaturated fatty acids
RCTs	Randomised control trials
SACN	Scientific Advisory Committee on Nutrition
SHC	Superior Health Council
sICAM-1	Soluble intercellular adhesion molecule-1
TAGs	Triacylglycerols
TBARS	Thiobarbituric acid reactive substances
TG	Triglycerides
TXB2	Thromboxane B2
TNF-alpha	Tumor necrosis factor-alpha
t-PA ag	Tissue plasminogen activator antigen
UL	Tolerable Upper Intake Level
VCAM-1	Vascular cell adhesion molecule-1
VKM	Norwegian Scientific Committee for Food Safety
vWF	von Willebrand factor
WHO	World Health Organization