Vitenskapskomiteen for mattrygghet
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VKM Report 2015: 27

Risk assessment of "other substances" – eicosapentaenoic acid, docosapentaenoic acid and docosahexaenoic acid

Opinion of the Panel on Nutrition, Dietetic Products, Novel Food and Allergy of the Norwegian Scientific Committee for Food Safety

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Assessed and approved

The opinion has been assessed by the Panel on Nutrition, Dietetic Products, Novel Food and Allergy of the Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM). Members of the panel are: Per Ole Iversen (chair), Livar Frøyland, Margaretha Haugen, Kristin Holvik, Martinus Løvik, Tor A Strand, Grethe S Tell and Arild Vaktskjold.

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

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Competence of VKM experts

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

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Summary

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

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

The present report is a risk assessment of eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) in food supplements, and is based on previous risk assessments and a literature search.

It is emphasised that this risk assessment concerns the single fatty acids EPA, DPA or DHA separately and not mixtures of these as found in e.g. fish oil/cod liver oil. For risk assessment of combined mixtures of n-3 LCPUFAs in e.g. fish oil/cod liver oil, see the EFSA opinion from 2012 or the VKM assessment from 2011 (EFSA, 2012; VKM, 2011). In the reviewed literature of this risk assessment, no studies investigating ratios between EPA, DPA, DHA or other fatty acids in mixtures have been identified.

EPA, DPA and DHA are long chain n-3 polyunsaturated fatty acids (n-3 LCPUFA) and in food these fatty acids are incorporated in triacylglycerols (TAGs) and phospholipids (PLs). Dietary sources are fatty fish, cod liver-, seal-, whale-, fish- and krill oils and human milk, containing various ratios of these fatty acids in combination. EPA can be metabolised to eicosanoids such as prostaglandins, prostacyclins and leukotrienes, all groups are biologically active substances. The eicosanoids participate in the regulation of blood pressure, renal function, blood coagulation, inflammatory and immunological reactions. DHA is an essential structural component of the brain, skin, sperm, testicles and retina. DPA can be retro-converted to EPA or converted to DHA. Still little is known of the biological effects of DPA.

Humans have a limited capacity to synthesise EPA, DPA and subsequently DHA from the precursor alpha-linolenic acid (ALA), and this endogenous production is negligible in comparison to the doses used in supplementation studies.

According to information from the NFSA, EPA, DPA and DHA are food supplement ingredients in Norway, and NFSA has requested a risk assessment of these fatty acids in the following doses in food supplements:
EPA: 1500, 1750 and 1825 mg/day
DPA: 100, 125 and 150 mg/day
DHA: 1050 and 1290 mg/day

Children below 10 years were not included in the terms of reference.

Information about intake of EPA, DPA and DHA from the diet is scarce, but calculations performed in the Norwegian Mother and Child Cohort Study indicate a mean total intake (SD) from food and supplements of EPA around 330 (340) mg/day, DPA 43 (30) mg/day and DHA 430 (380) mg/day among pregnant women (2002 to 2008). Mean combined intake of EPA, DPA and DHA from fish oil/ cod liver oil in adults participating in a nationally representative dietary survey was 735 mg/day (VKM, 2014).

The major concerns with high intake of EPA and DHA have been increased bleeding time, adverse effects related to immune function, lipid peroxidation and glucose homeostasis. EFSA concluded in 2012 that long-term supplemental intakes of 5 g/day of the n-3 LCPUFA do not raise safety concerns for adults with regard to an increased risk of spontaneous bleeding episodes or bleeding complications, or affect glucose homeostasis, immune function or lipid peroxidation. In 2011, VKM concluded that an intake n-3 LCPUFA up to 6.9 g/day was not associated with increased risk of any serious adverse events.

Some adverse health effects related to gastrointestinal function, including abdominal cramps, flatulence, eructation, vomiting and diarrhea have been reported, but seem to be associated with intake of an oily substance and not related specifically to EPA, DPA and/or DHA.

EPA

In the report from 2012, EFSA concluded that 1.8 g/day of supplemental EPA does not raise safety concerns in adults. None of the included studies from our literature searches limited to 2012 and onwards have investigated bleeding complications. The dosages of EPA in the three included studies in this report range from 1.8 to 3.8 g/day for 12 weeks. The main endpoints in the studies included lipid peroxidation, inflammation biomarkers of cardiovascular diseases and no serious adverse events were found related to the main endpoints. In general, adverse events were described as gastrointestinal discomforts and not related to dosage.

Studies of longer duration are necessary before an intake above 1.8 g of EPA can be considered safe.

The Norwegian Scientific Committee for Food Safety (VKM) concludes that the specified doses of 1500, 1750, 1825 mg/day of EPA in food supplements are unlikely to cause adverse health effects in adults (≥18 years).

In 2012, EFSA did not make conclusions for children or adolescents for EPA. No new studies with EPA supplementation have been identified in children or adolescents after 2012, and therefore no risk assessment can be made for children (≥10 years) or adolescents.

DPA

No dosage of DPA in food supplements can be evaluated due to lack of data.

DHA

EFSA concluded that 1 g/day of supplemental DHA does not raise safety concerns for the general population (including children and adolescents). The dosages of DHA in the included trials in this report range from 1.0 to 3.6 g/day and the duration from five weeks to four years. Six out of seven studies have used dosages from 1 to 2 g DHA/day. The last study included up to 3.6 g DHA/day for four years and the age spanned from 7 to 31 years. The main endpoints in all studies included lipid peroxidation, inflammation, cognitive performance, blood pressure and biomarkers of cardiovascular diseases and no serious adverse events were found related to the main endpoints. In general, adverse events were described as gastrointestinal discomforts and not related to dosage. VKM therefore considers that the specified daily doses of DHA that moderately exceed 1 g per day (1050 and 1290 mg/day) are unlikely to cause adverse health effects in the general population including children ≥10 years and adolescents.

VKM concludes that the specified doses of 1050 and 1290 mg/day of DHA in food supplements are unlikely to cause adverse health effects in the general population including children (≥10 years), adolescents and adults (≥18 years).

Short summary:

The Norwegian Scientific Committee for Food Safety (VKM) has, at the request of the Norwegian Food Safety Authority, assessed the risk of eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) in food supplements. VKM concludes that:

- The specified doses of 1500, 1750 or 1825 mg/day of EPA in food supplements are unlikely to cause adverse health effects in adults (≥18 years). Due to few studies in children and adolescents, no conclusion can be drawn for these groups for EPA.

- The specified doses of 1050 and 1290 mg/day of DHA in food supplements are unlikely to cause adverse health effects in the general population including children (≥10 years), adolescents and adults (≥18 years).
- No dosage of DPA in food supplements can be evaluated due to lack of data.

It is emphasised that this risk assessment concerns the single fatty acids EPA, DPA or DHA separately and not mixtures of these as found in e.g. fish oil and cod liver oil.

Key words: Adverse health effect, DHA, docosahexaenoic acid, docosapentaenoic acid, DPA, eicosapentaenoic acid, EPA, food supplement, n-3 LCPUFA, negative health effect, Norwegian Scientific Committee for Food Safety, omega-3, other substances, risk assessment, VKM.

Sammendrag på norsk

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

"Andre stoffer" er stoffer som har en ernæringsmessig eller fysiologisk effekt, og som ikke er vitaminer og mineraler. De tilsettes i hovedsak til kosttilskudd, men også til energidrikker og andre næringsmidler. I disse risikovurderingene har VKM ikke sett på potensielle gunstige helseeffekter, men kun vurdert mulige negative helseeffekter.

I denne rapporten har VKM vurdert risiko ved eikosapentaensyre (EPA), dokosapentaensyre (DPA) og dokosaheksaensyre (DHA) som enkeltfettsyrer i kosttilskudd. Risikovurderingen er basert på tidligere risikovurderinger av disse fettsyrene og artikler fra litteratursøk.

Det understrekes at denne risikovurderingen omhandler de enkelte fettsyrene EPA, DPA og DHA hver for seg og ikke blandinger av disse slik som man finner i for eksempel fiskeoljer og tran. For risikovurdering av blandinger av n-3 LCPUFAs i feks. fiskeolje/tran, se EFSAs rapport fra 2012 eller VKMs vurdering fra 2011 (EFSA, 2012; VKM, 2011). Det er ikke avdekket noen studier som har undersøkt ratioer mellom EPA, DPA, DHA eller andre fettsyrer i den litteraturen som er gjennomgått i denne risikovurderingen.

EPA, DPA and DHA er langkjedede flerumettede n-3 fettsyrer (n-3 LCPUFA), og i maten er disse inkorporert i triacylglyserol (TAG) eller fosfolipider (PL). Kilder i kosten er fet fisk, torskelever-, sel -, hval-, fisk- og krilloljer og morsmelk som inneholder ulike kombinasjoner og ratioer av disse fettsyrene. EPA kan omdannes til eikosanoider som prostaglandiner, prostasykliner og leukotriener, som alle er grupper av biologisk aktive substanser. Eikosanoidene tar del i reguleringen av blodtrykk, nyrefunksjonen og blodkoagulering samt inflammatoriske og immunologiske reaksjoner. DHA er en essensiell strukturell komponent i hjernen, hud, sæd, testikler og retina. DPA kan re-konverteres til EPA eller omdannes til DHA. Fortsatt vet vi lite om de biologiske effektene av DPA. Mennesker har en begrenset kapasitet til å syntetisere EPA, DPA og følgelig også DHA fra forløperen alfa-linolensyre (ALA), og den endogene produksjonen er ubetydelig sammenlignet med dosene som brukes i studier.

Ifølge informasjon fra Mattilsynet inngår EPA, DPA og DHA som ingredienser i kosttilskudd som selges i Norge. Oppdraget fra Mattilsynet var å risikovurdere følgende doser i kosttilskudd:
EPA: 1500, 1750 and 1825 mg/dag
DPA: 100, 125 and 150 mg/dag
DHA: 1050 and 1290 mg/dag

Barn under 10 år inngår ikke i oppdraget.

Vi har få data om inntak av EPA, DPA og DHA fra kosten, men beregninger utført i Den norske mor og barn-undersøkelsen indikerer at gjennomsnittlig (SD) totalt inntak av EPA fra mat og tilskudd er omtrent 330 (340) mg/dag, DPA 43 (30) mg/dag and DHA 430 (380) mg/dag blant gravide kvinner 2002-2008. Gjennomsnittlig inntak av EPA, DPA og DHA fra fiskeolje/tran hos voksne i en landsomfattende kostholdsundersøkelse var 735 mg/dag (VKM, 2014).

Bekymringer over høyt inntak av EPA og DHA har hovedsakelig vært knyttet til økt blødningstid eller relatert til immunforsvar, lipidperoksidering og glukose-homeostasen. I 2012 konkluderte EFSA med at et langvarig inntak av EPA i kombinasjon med DHA opp til totalt 5 g/dag fra tilskudd ikke medfører økt risiko for spontane blødninger eller blødningskomplikasjoner hos voksne, og heller ikke påvirker glukose-homeostasen, immunfunksjon eller lipidperoksidering. I 2011 konkluderte VKM at et inntak opptil totalt 6,9 g/dag av EPA kombinert med DHA ikke var forbundet med noen alvorlige negative helseeffekter.

Det er rapportert om enkelte negative helseeffekter knyttet til gastrointestinale funksjoner, inkludert magekramper, oppblåsthet, oppstøt, oppkast og diaré, men disse ser ut til å være forbundet med inntak av olje som sådan, og ikke å være spesifikt knyttet til EPA, DPA og/eller DHA.

EPA

I rapporten fra 2012 konkluderte EFSA med at 1,8 g/dag av EPA fra tilskudd ikke medfører noen helserisiko hos voksne. Ingen av de inkluderte studiene fra våre litteratursøk (begrenset tilbake i tid fra og med 2012) har undersøkt blødningskomplikasjoner. Doser av EPA i de tre inkluderte studiene i denne rapporten spenner fra 1,8 til 3,8 g EPA/dag og intervensjonsperiodene var 12 uker. De inkluderte studiene har undersøkt lipidperoksidering, immunfunksjon og glukose- og lipid- homeostase, og ingen av dem har rapportert om negative effekter i doser opp til 3,8 g EPA/dag. Generelt er de bivirkningene som er rapportert beskrevet som gastrointestinale plager og ikke knyttet til dose.

Det er behov for studier av lenger varighet før det kan vurderes om inntak av EPA over 1,8 kan vurderes som trygt.

VKM konkluderer med at det er usannsynlig at de spesifiserte dosene på 1500, 1750 eller 1825 mg/dag EPA i kosttilskudd vil forårsake negative helseeffekter hos voksne (≥18 år).

I 2012 konkluderte ikke EFSA for barn eller ungdom vedrørende EPA. VKM har ikke identifisert noen nye kosttilskuddsstudier med EPA til barn eller ungdom, og dermed kan heller ikke VKM foreta en risikokarakterisering av EPA for barn (≥10 år) eller unge.

DPA

Ingen av de spesifiserte dosene for DPA i kosttilskudd kan vurderes på grunn av mangel på data.

DHA

EFSA konkluderte med at 1 g/dag av DHA fra tilskudd ikke medfører noen helserisiko i befolkningen generelt inkludert barn og unge. Doser av DHA i de inkluderte studiene i denne rapporten spenner fra 1,0 til 3,6 g DHA/dag, og intervensjonsperioden spenner fra fem uker til fire år. Seks av syv studier har brukt doser på 1-2 g/dag. En av studiene ga opp til 3,6 g DHA/dag i fire år til personer i alderen 7-31 år. De viktigste endepunktene i alle de inkluderte studiene omfatter lipidperoksidering, inflammasjon, kognitiv prestasjon, blodtrykk og biomarkører for hjerte- og karsykdommer, og det er ikke rapportert om alvorlige negative helseeffekter i disse studiene. Generelt er de bivirkningene som er rapportert beskrevet som gastrointestinale plager og ikke knyttet til dose. VKM anser at det er usannsynlig at en moderat overskridelse over 1 g DHA per dag (1050 og 1029 mg/dag) som enkeltfettsyre i tilskudd vil forårsake negative helseeffekter i den generelle befolkningen inkludert barn fra (≥10 år) og unge.

VKM konkluderer med at det er usannsynlig at de spesifiserte dosene på 1050 eller 1290 mg/dag DHA i kosttilskudd vil forårsake negative helseeffekter i den generelle befolkningen inkludert barn (≥10 år), unge og voksne (≥18 år).

Kort sammendrag til nettmelding: Vitenskapskomiteen for mattrygghet (VKM) har på oppdrag for Mattilsynet har vurdert risiko ved inntak av eikosapentaensyre (EPA), dokosapentaensyre (DPA) og dokosaheksaensyre (DHA) som enkeltfettsyrer i kosttilskudd. VKM konkluderer med at:

- Det er usannsynlig at de spesifiserte dosene på 1500, 1750 eller 1825 mg/dag av EPA i kosttilskudd vil forårsake negative helseeffekter hos voksne (≥18 år). På grunn av få studier med barn og unge gir VKM ingen konklusjoner for barn og unge for EPA.
- Det er usannsynlig at de spesifiserte dosene på 1050 eller 1290 mg/dag DHA i kosttilskudd vil forårsake negative helseeffekter i den generelle befolkningen inkludert barn (≥10 år), unge og voksne (≥18 år).
- Ingen doser DPA i kosttilskudd kan evalueres på grunn av mangelfulle data.

Det understrekes at denne risikovurderingen omhandler de enkelte fettsyrene EPA, DPA og DHA hver for seg og ikke blandinger av disse slik som man finner i for eksempel fiskeoljer og tran.

Abbreviations and/or glossary

Abbreviations

AA	- arachidonic acid
ASEAN	- Spanish Agency for Food Safety and Nutrition
AV	- anisidine value
bw	- body weight
COX	- cyclooxygenase
CRP	- C-reactive protein
DHA	- docosahexaenoic acid
DPA	- docosapentaenoic acid (in this report referring to n-3 DPA if not specified as n-6 DPA)
EFSA	- European Food Safety Authority
EPA	- eicosapentaenoic acid
HbA1c	- hemoglobin A1c
HDL	- high density lipoprotein
HOMA-IR	- homeostatis model assessment-insulin resistance
ICAM	- intercellular adhesion molecule
IL	- interleukin
LA	- linoleic acid
LCPUFA	- long-chain polyunsaturated fatty acid
LDL	- low density lipoprotein
MDD	- major depressive disorder
NFSA	- Norwegian Food Safety Authority [*Norw.*: Mattilsynet]
OPUS	- Optimal well-being, development and health for Danish children
PGI	- prostaglandin I also called prostacyclin I
PPAR	- peroxisome proliferator-activated receptor
PL	- phospholipid
PLA_2	- phospholipase A_2
PUFA	- polyunsaturated fatty acid
PV	- peroxide value
TAG	- triacylglycerol
TG	- triglyceride
TNF-alpha	- tumor necrosis factor-alpha
TXA	- thromboxane A
TXB	- thromboxane B
UL	- tolerable upper intake level
VCAM	- vascular cell adhesion molecule

VKM - Norwegian Scientific Committee for Food Safety [*Norw.*: Vitenskapskomiteen for Mattrygghet]
VLDL - very low density lipoprotein

Glossary

"Other substances": a substance other than a vitamin or mineral that has a nutritional or physiological effect (European Regulation (EC) No. 1925/2006, Article 2; http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32006R1925&from=en).

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

An adverse event is considered serious if it:

- results in death
- is life-threatening
- requires or prolongs hospitalisation
- is a congenital anomaly or birth defect
- is a persistent or significant disability/incapacity
- is another serious or important medical event

Background as provided by the Norwegian Food Safety Authority

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

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

While at the EU level, these substances fall under the scope of the European Regulation (EC) No. 1925/2006 on the addition of vitamins, minerals and certain other substances to foods and the European Regulation (EC) No 258/97 concerning novel foods and novel food ingredients, "other substances" remain largely unregulated. In order to ensure safe use of "other substances" many countries have regulated their use at a national level. For example, Denmark regulates these substances in a positive list i.e. a list of substances with maximal daily doses, permitted for use in food supplements and other foods (https://www.retsinformation.dk/Forms/R0710.aspx?id=163394).

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

Terms of reference as provided by the Norwegian Food Safety Authority

The Norwegian Food Safety Authority (NFSA) requested the Norwegian Scientific Committee for Food Safety (VKM) to assess the safety of eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) at the following doses:

EPA: 1500, 1750 and 1825 mg/day
DPA: 100, 125 and 150 mg/day
DHA: 1050 and 1290 mg/day

NFSA requested VKM to assess the safety of "other substances" (in accordance to the guidance document developed in Phase 2) at the doses specified (Phase 3).

Safety assessments for "other substances" present in food supplements shall be carried out for a general population, ages 10 years and above.

Assessment

1 Introduction

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

This risk assessment regards the single substances eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) per se, and no specific products.

VKM has in this series of risk assessments of "other substances" not evaluated any potential beneficial effects from these substances, but merely possible adverse effects at specified doses used in Norway.

According to information from the Norwegian Food Safety Authority (NFSA), EPA, DPA and DHA are food supplement ingredients in Norway, and NFSA has requested a risk assessment of EPA in the following doses in food supplements: 1500, 1750 and 1825 mg/day; of DPA in the doses of 100, 125 and 150 mg/day and of DHA in the doses of 1050 and 1290 mg/day.

EPA (20:5n-3), DPA (22:5n-3) and DHA (22:6n-3) are referred to as omega-3 or n-3 long chain polyunsaturated fatty acids (n-3 LCPUFAs), and are naturally found in fish and seafood. Mean intake of EPA, DPA and DHA from fish oil/ cod liver oil in adults participating in a nationally representative dietary survey was 735 mg/day, n=1787 (1982 mg/day in consumers only, n=663) (VKM, 2014). Concentrations of the n-3 LCPUFAS in cod liver oil may vary, and a recommended dose of 5 ml may contain 400 mg EPA, 60 mg DPA and 600 mg DHA.

The n-3 LCPUFAs are naturally bound to major lipid classes such as triacylglycerols (TAGs) or phospholipids (PLs). In food supplements these fatty acids can be bound to TAGs and PLs, but given as single fatty acids most formulations are as ethyl esters or as free fatty acids. EPA can be transformed to eicosanoids such as prostaglandins, prostacyclins and leukotrienes, all groups are biologically active substances. Eicosanoids participate in the regulation of blood pressure, renal function, blood coagulation, inflammatory and immunological reactions. DHA is an essential structural component of the brain, skin, sperm, testicles and retina. DPA can be retro-converted to EPA or converted to DHA. Still little is known of DPA's specific biologic effects.

2 Hazard identification and characterisation

2.1 Literature

This risk assessment is based on previous risk assessments of EPA, DPA or DHA and articles retrieved from literature searches.

2.1.1 **Previous risk assessments**

Risks related to EPA, DPA and/or DHA have previously been evaluated by the Institute of Medicine, USA (IOM, 2005), VKM (2011)and the European Food Safety Authority (EFSA) with regard to food supplements (EFSA, 2012).

Since these fatty acids are absorbed almost completely regardless of the source, i.e. free fatty acids, ethyl esters, phospholipids or triacylglycerols, the reports concluded that there was no need to undertake separate safety assessments for different sources of n-3 LCPUFA.

Dietary reference intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids, USA 2005

In the IOM report the potential hazards of EPA and DHA intake were identified. Concerns were given to immune function, bleeding and increased risk of haemorrhagic strokes and oxidative damage. In the IOM report twenty publications from 1985 to 2000 are referred to which show suppression of various immune function in vitro or ex vivo studies in different cell cultures from individuals provided EPA and DHA supplements or as experimental diets. IOM concluded that due to differences in study design it was not possible to draw any conclusions on the impact on immune functions of EPA and DHA supplementation. It was also mentioned that a few animal studies had shown effect of n-3 fatty acid supplementation on infection response (IOM, 2005), but in 2005 there was no data for support in humans.

Twelve short-term studies (4 to 11 weeks) were reported to have found increased bleeding time, whereas three studies using similar intake found no impact of n-3 LCPUFAs supplementation. It was reported that there was a dose dependent increase in bleeding time of EPA and DHA supplementation, but that none of the studies reported excessive bleeding time, bleeding episodes or bruising. Ecological studies suggested an increased risk of haemorrhagic stroke among Greenland Eskimos with an average intake of 6.5 g/day of EPA and DHA. However, due to uncontrolled conditions these studies were not taken into consideration.

LCPUFAs are vulnerable to lipid peroxidation, but in studies with vitamin E supplementation in addition the oxidative damage was reduced or prevented.

In addition, IOM described two subpopulations that should take supplements containing EPA and DHA with caution: those individuals who already exhibit glucose intolerance or diabetic conditions that require increased doses of hypoglycemic agents and individuals with familial hypercholesterolemia using anticoagulants. However, IOM considered the scientific data insufficient to establish an UL.

VKM Evaluation of negative and positive health effects on n-3 fatty acids as constituents of food supplements and fortified foods, Norway 2011.

The VKM report is an evaluation of negative and positive health effects of supplemental n-3 fatty acid use (VKM, 2011). The evaluation of negative health effects was based on human studies retrieved in a literature search limited to the years 2000-2009. VKM concluded that no clear adverse effects from combined EPA and DHA up to the dosage 6.9 g/day were identified. An increased bleeding time had been found after intake of 6.9 g/day EPA and DHA in coronary heart disease patients on anti-thrombotic medication. However, no negative health effects regarding bleeding complications in connection with EPA and DHA supplementation had been reported. Possible negative health effects of combined EPA and DHA had been reported in various patient groups at doses above 3.5 g/day, including increases in biomarkers indicative of lipid peroxidation and endothelial cell activation. No tolerable upper intake level was established. No evaluation was done for EPA, DPA and DHA given as single fatty acids in food supplements.

Scientific Opinion on the Tolerable Upper Intake Level of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA), EFSA 2012

The mandate in the EFSA opinion was to establish a tolerable upper intake level (UL) for EPA, DPA or DHA individually or combined in the general population and vulnerable groups. If ULs could not be established EFSA was asked to provide advice on a daily intake of these fatty acids which does not give rise to concerns about adverse health effects either individually or combined. The EFSA opinion is based on data from human studies and the safety assessment of EPA, DHA and DPA referred to long-term consumption (EFSA, 2012). Since these fatty acids are absorbed almost completely regardless of the chemical forms, the EFSA Panel considered that there was no need to undertake separate safety assessments for this factor. EFSA concluded that 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 to affect glucose homeostasis, immune function or lipid peroxidation, provided the oxidative stability of the n-3 LCPUFAs is guaranteed. EFSA

concluded further that available data are not sufficient to establish a tolerable upper intake level for the n-3 LCPUFAs individually or in combination for any population group.

A study by Clarke et al. (1990) reporting epistaxis episodes among 11 adolescents (11-21 years old) who received up to 5 g/day of EPA and DHA was cited in the EFSA opinion. However, this was a non-randomised study with few participants and a later open labelled study in nine children (mean age 11.4 years) found no effects on bleeding after 8 weeks with an intake up to 16.2 g EPA/DHA per day (Sorgi et al., 2007). In 2012 the EFSA Panel omitted the results from the Clark study in their conclusions.

It was concluded that supplemental intakes of 1.8 g/day of EPA did not raise safety concerns for adults based on bleeding complications, bleeding time and platelet function. No conclusions were made for infants, children and adolescents for EPA, and no conclusion was made for safe intake of DPA due to few studies and insufficient knowledge about biological mechanisms of this fatty acid.

Supplemental intakes of 1 g/day of DHA alone do not raise safety concerns for the general population (including children and adolescent). No information was provided regarding how they reached their conclusion of up to 1 g DHA per day.

2.1.2 **Literature search**

A main literature search was performed in MEDLINE and EMBASE in order to retrieve publications on adverse effects caused by EPA, DPA or DHA after 2011. The latest previous report (EFSA) is from 2012, but we could not identify if EFSA had been able to include all studies from 2011, and we therefore included studies from 2011 in our search. This is probably an overlap of EFSA's search. The literature search was performed 30 April 2015. The strategy for the main search is included in Appendix 1. When writing the risk characterisation and conclusions for children and adolescents, it was considered that extra literature searches for children and adolescents were warranted as was a literature search for animal studies with DPA. These additional searches were performed 4 June 2015 (specified for children and adolescents) and 16 June 2015 (including both human and animal studies with DPA).

The strategies for these additional searches are also included in Appendix 1.

The main search and the additional searches specified for children and adolescents were all limited back in time to include papers from the beginning of 2011, except for the additional search for animal studies with DPA which was unlimited back in time.

2.1.2.1 Publication selection

The study types for inclusion in this opinion have been human studies. Animal studies were not included in the main literature search due to numerous human studies with EPA and DHA. The criteria for inclusion were:

- EPA, DPA or DHA in relation to adverse effect must be addressed in the abstracts of the paper
- Outcome not affected by other substances than EPA, DPA or DHA
- Oral route of exposure to EPA, DPA or DHA in human studies
- Human studies were performed in apparently healthy individuals or patient groups who are assumed to have normal EPA, DPA or DHA absorption and metabolism.

In vitro studies were not included. Also papers in languages other than English, Norwegian, Danish or Swedish were excluded. The main literature search 30 April 2015 identified 252 articles.

The additional literature searches 4 June 2015 specified for children and adolescents identified 29 articles for EPA, 10 articles for DPA and 91 articles for DHA (several papers included in more than one search).

The additional literature search for animal studies with DPA 16 June 2015 identified 82 articles. Most studies reported on DPA n-6 and only one study concerning DPA n-3 has been included in this evaluation.

Study titles and abstracts were first reviewed by the authors of this report, resulting in selection of 28 full text articles. After review of the available full text articles using the same inclusion criteria as above, 14 articles were included.

A final total of 14 publications were identified and included in the results in this report (see Figure 2.1.2.1-1).

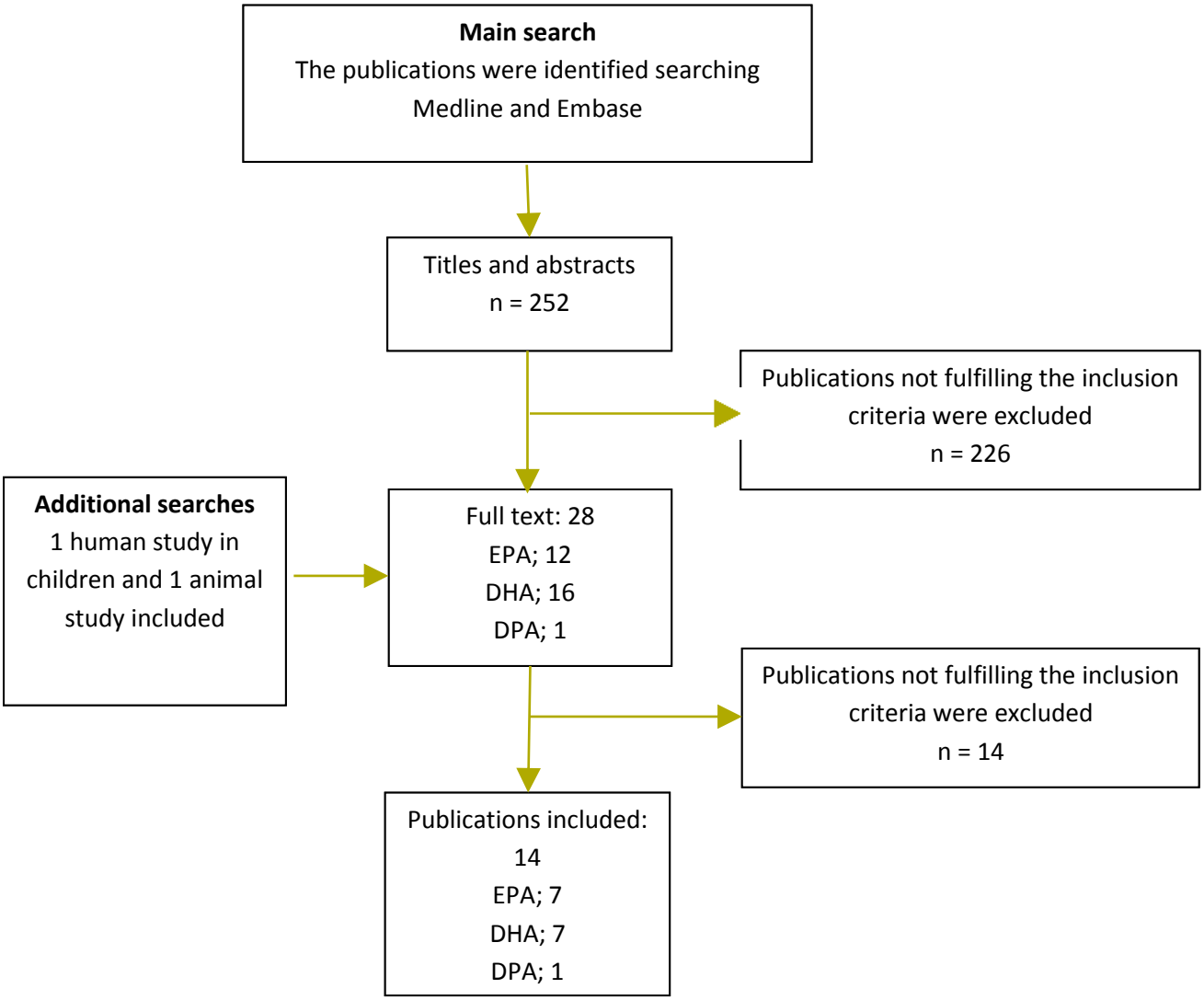

Figure 2.1.2.1-1: Flow chart for publication selection for EPA, DPA and DHA literature search.

2.2 General information

2.2.1 Chemistry

The n-3 LCPUFA have their first double bond between carbon atoms number 3 and 4 from the methyl end, hence they are named n-3 or omega-3 fatty acids.

EPA (20:5Δ4c,7c,10c,13c,16c) is an n-3 LCPUFA. The CAS number for EPA is 10417-94-4.

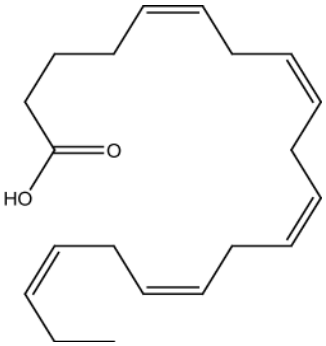

Figure 2.2.1-1: Structural formula for EPA.

DPA (22:5Δ4c,7c,10c,13c,16c) The CAS number for DPA is 25182-74-5.

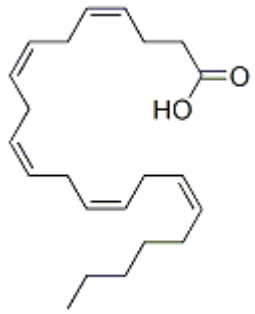

Figure 2.2.1-2: Structural formula for DPA.

DHA (22:6Δ4c,7c,10c,13c,16c,19c) The CAS number for DHA is 6217-54-5.

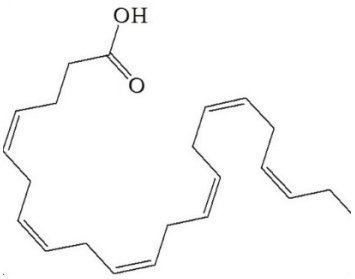

Figure 2.2.1-3: Structural formula for DHA.

2.2.2 **Occurrence**

Regular foods contain combinations of EPA, DPA and DHA, and food supplements or fortified foods are the only sources for isolated single EPA, DPA and DHA. The endogenous production of EPA, DPA and DHA from ALA is limited and insignificant compared to doses used in supplementation studies.

Main dietary sources of n-3 LCPUFAs are fatty fish, human milk, cod liver-, seal-, whale-, fish- and krill-oils. N-3 LCPUFA supplements are also produced from marine algae and fungi.

2.3 Absorption, distribution, metabolism and excretion

Alpha-linolenic acid (ALA, 18:3 n-3) is the essential n-3 fatty acid which is found in seeds (chia, flaxseed), nuts (notably walnuts), and in many common vegetable oils. ALA is the precursor for EPA, DPA and DHA. However, humans have a limited capacity for endogenous production of the n-3 LCPUFAs because ALA competes with linoleic acid (LA, 18:2 n-6) for the same enzyme system of elongases and desaturases for the endogenous production of arachidonic acid (AA, 20:4 n-6). In a western diet, the LA:ALA ratio varies between 5-9, which favors the production of AA. Analyses with radioisotope labelled ALA suggest that less than 10% of ALA is converted to EPA and further 0.1 to 9% to DHA (Williams and Burdge, 2006). The conversion of ALA to EPA is further dependent on gender and is more limited in men compared with women, possibly due to the regulatory effects of estrogen (Burdge, 2004).

The intakes of EPA, DPA and DHA from regular foods are in the form of triacylglycerol (TAG) and phospholipids (PLs). In food supplements these fatty acids can be bound to TAGs and PLs, but given as single fatty acids most formulations are as ethyl esters or as free fatty acids. According to EFSA (2012), EPA, DPA and DHA are absorbed almost completely regardless of the source. New studies show that the bioavailability of the n-3 LCPUFAs as free fatty acids is higher in comparison with ethyl esters (Maki et al., 2013; Offman et al., 2013).

In the intestine TAG, PLs and ethyl esters undergo lipolysis by different lipases and the n-3 LCPUFAs are absorbed into the enterocytes. This step does not apply to the free fatty acids. The enterocytes incorporate the n-3 LCPUFA into TAGs or PLs which together with cholesterol, cholesterol esters and apo-lipoproteins are assembled into chylomicrons that enter the circulation via the lymphatic system. Circulating chylomicrons release the n-3 LCPUFAs to adipose tissue and other tissues and organs.

In the liver, EPA, DPA and DHA are together with other fatty acids (both exogenous and endogenous) incorporated into very low-density lipoprotein (VLDL) particles which enter directly to the venous blood circulation. Circulating VLDL releases these fatty acids to various tissues and organs before the remaining low-density lipoprotein (LDL) is taken up by the liver through LDL receptors. However, in the event that the LDL particle becomes oxidised (oxLDL) it is taken up by macrophages, which ultimately leads to formation of foam cells and the formation of fatty streaks that is the initial step in atherosclerosis (Lusis, 2000).

EPA is incorporated into cell membranes and may thus impact cellular metabolism, signal transduction and regulation of gene expression. EPA can be transformed to eicosanoids, a

group of biologically active substances including prostaglandins, prostacyclin 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 the 5 series of leukotrienes. In addition, both EPA and DHA are metabolised to specialised pro-resolving lipid mediators like resolvins, poxytrins, neuroprotectins and maresins, which are thought to be involved in resolution of inflammatory responses (Chen et al., 2011; Dobson et al., 2013; Serhan et al., 2008; Spite et al., 2014).

DHA is a structural component of cell membranes and contributes to various membrane functions such as fluidity, permeability, activity of membrane-bound enzymes and receptors, and signal transduction in nervous tissue and retina. DHA is not a substrate for mitochondrial beta-oxidation, i.e. energy production (energy as adenosine triphosphate (ATP)), but DHA can be beta-oxidised by peroxisomes (yielding energy as heat). In addition, DHA is metabolised to F_4-neuroprostanes and endocannabinoids.

DPA can be retro-converted to EPA and only minimally to DHA (EFSA, 2012). More recently, it was shown that interconvertion of DPA to EPA and DHA was very limited and there was no increase in EPA levels after a 5 hour postprandial period following a breakfast supplemented with 2 g DPA (Linderborg et al., 2013).

Few studies have investigated the biologic effects of DPA alone, but it is suggested that DPA may affect membrane structure differently from EPA and DHA and thereby have independent effects on enzyme activity and gene expression. DPA might inhibit platelet aggregation and stimulate endothelial cell migration more efficiently than both EPA and DHA (Kaur et al., 2011; VKM, 2011; Yazdi, 2013). Supplementation with 8 g of either pure DPA or EPA resulted in different and specific incorporation patterns into plasma lipid classes and red blood cell PLs (Miller et al., 2013).

2.4 Adverse effects noted in previous reports

Adverse effects of high intakes of n-3 LCPUFA are described as bleeding episodes, impaired immune function, increased lipid peroxidation, and impaired lipid and glucose metabolism (EFSA, 2012; VKM, 2011). Most studies have been performed with food supplements containing EPA and DHA in combination and as TAG. Few studies refer to single fatty acid supplementation.

2.4.1 **Bleeding complications and bleeding time**

Increased tendency to bleed from the nose and urinary tract, and increased mortality from haemorrahagic stroke have been reported in observational studies of Greenlandic Eskimos

(mean intakes of n-3 LCPUFAs about 6.5 g/day) as well as prolonged bleeding time and reduced platelet aggregation (Dyerberg and Bang, 1979). These studies were uncontrolled for factors other than the intake of n-3 LCPUFA.

Bleeding complications were studied in an open label human intervention study (Yokoyama et al., 2007) cited in VKM (2011) and EFSA (2012), which investigated the effects of 1.8 g/day of EPA as ethyl esters consumed for five years in combination with statins (n=9326) *vs*. statins alone (n=9319) in hypercholesterolemic, high fish consumers on primary and secondary prevention of coronary heart disease. In this study adverse outcomes were assessed and published in a separate paper (Tanaka et al., 2008) cited in EFSA 2012. Bleeding (cerebral and fundal bleedings, epistaxis, and subcutaneous bleeding combined) was more frequently reported in the EPA group than in controls. EFSA (2012) noted that nose- or subcutaneous bleeding was self-reported, and that self-reported side effects are subject to high reporting bias in open label studies. EFSA further noted that no statistically significant differences in the total incidence of stroke, or in the incidence of cerebral or subarachnoid haemorrhage, were observed between groups. EFSA considered that an intake of EPA alone at doses up to 1.8 g/day for five years does not increase the risk of bleeding complications.

The aim of a review article from 2014 was to look at the effects of n-3 fatty acids on bleeding complications in a variety of clinical settings and in combinations with different anti-platelet drugs or anticoagulant therapies (Wachira et al., 2014). It was stated that although platelets are affected by n-3 fatty acids supplementation, impact on bleeding time and bleeding complications is minor. New insights indicate that fish oil can somewhat lower platelet arachidonic acid levels and possibly slightly diminish the cyclooxygenase (COX)-derived signalling cascade. Besides COX, n-3 fatty acids are substrates for several enzymes that produce active metabolites which can affect platelets function.

Neither in the present nor in the EFSA opinion, were any studies with single DPA or DHA supplementation investigating bleeding complications or bleeding time identified.

2.4.2 **Immune function**

According to the EFSA opinion from 2012, there are no human intervention studies available that have investigated the effects of n-3 LCPUFA supplementation on the risk of infections (EFSA, 2012).

There are some indications, from *ex vivo* and *in vitro* studies performed in peripheral blood white 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 as reviewed in IoM, 2005. However, the clinical relevance of these changes is unknown.

Furthermore, there is no available information on the effect of a high intake of n-3 LCPUFA on the risk of chronic diseases with an inflammatory component. Some markers of the so-called low-grade systemic inflammation (e.g. high-sensitivity C-reactive protein, and some cytokines) and vascular (e.g., sICAM-1, VCAM-1, and E-selectin) 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 that report on the effects of EPA and DHA on markers of systemic and vascular inflammation are small and generally not designed for the 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 nor a significant decrease in sICAM-1 (Yang et al., 2012) included in EFSA 2012). The majority of the studies report either no effect or a decrease in systemic markers of inflammation, including hs-CRP (high-sensitivity C-reactive protein) and TNF-alpha (Bloomer et al., 2009; VKM, 2011).

EFSA (2012) noted 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.

2.4.3 **Peroxidation**

PUFAs are generally more prone to peroxidation compared with saturated fatty acids. Enhanced oxidative stress and increased lipid peroxidation either locally in the vessel wall or systemically have been associated with the pathogenesis of atherosclerosis in humans and the relation to adverse effects (IOM, 2005; Steinberg et al., 1989; VKM, 2011).

The majority of the human intervention studies used fish oils stabilised with antioxidants, but some studies did not report whether sources of EPA, DHA, or both, contained antioxidants. Only a few studies reported on the concentration of primary and secondary oxidation products in the supplements administered. The addition of antioxidants to food supplements containing n-3 LCPUFA to ensure product stability appears to be optional (EFSA, 2012).

F2-isoprostanes measured in urine or plasma are reliable markers of *in vivo* lipid peroxidation. In 2011, VKM identified nine controlled human intervention studies that used n-3 LCPUFA-rich oils stabilised with antioxidants, mostly with vegetable oils as control (olive, maize, sunflower, safflower or soy oil), and reported on plasma or urinary F2-isoprostanes (VKM, 2011). Studies were conducted in newborns (following maternal supplementation with 4 g/day EPA and DHA from fish oil from 20 weeks of gestation until delivery) (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/adolescents 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 (0.8-4 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 with the controls (Barden et al., 2004; Higdon et al., 2000; Mas et al., 2010; Mori et al., 2000; Mori et al., 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).

Susceptibility of LDL to oxidation has in a number of studies been reported to be increased, decreased or unchanged during consumption of EPA and DHA either from fish oil or as ethyl esters. 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) showed no effect (of EPA and DHA) in comparison with control (mostly vegetable oils) at doses up to about 5 g/day (VKM, 2011). In two studies in which the diet was supplemented with salmon providing EPA + DHA, 1.5 g/day or 2.9 g/day (Seierstad et al., 2005) or herring providing EPA + DHA, 1.2 g/day (Lindqvist et al., 2009) the intervention had no effect of on plasma oxidised LDL concentrations in comparison with controls (EFSA, 2012).

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

2.4.4 **Impaired lipid and glucose homeostasis**

From intervention studies, mostly uncontrolled, adverse effects on lipid- and glucose metabolism from supplemental intake of n-3 LCPUFA (≥10g/day) have been described. EFSA concluded that an intake of EPA and DHA combined up to 5 g/day consumed for 12 weeks does not significantly affect glucose homeostasis in healthy or diabetic subjects, but that scientific data is not available to conclude whether the same doses administered as EPA or DHA alone would have a different effect.

Several human studies have addressed the effects of supplementation with n-3 LCPUFA on blood LDL-cholesterol concentrations. EFSA (2012) concluded that 2-6 g/day of supplemental EPA and DHA combined or 2-4 g/day of mostly DHA, increases the blood concentration of LDL-cholesterol by about 3% and that such an increase is accompanied by a decrease in TAG with no changes in HDL-cholesterol concentrations. Supplementation of mostly EPA had no such effect on cholesterol concentrations. The small increase in LDL-cholesterol by EPA+DHA supplementation or DHA supplementation alone was not considered as an adverse effect by the EFSA Panel.

2.5 Human studies published after 2010

Fourteen human studies included from our literature searches are listed in the table below.

Table 2.5-1: An overview of human studies published after 2011 investigating EPA and adverse health effects.

Reference	Participant characteristics, age groups	Country (study start)	Number in treatment group		Doses, g/day	Main endpoints	Length of follow-up	Adverse effects
			EPA	Control				
RCTs								
Tatsuno et al. (2013)	Patients with a history of hypertri-glyceridemia	Japan	195	205 and 210	0.6 g x 3 EPA ethyl ester /day, n=195 EPA+DHA 2 g/day, n=205 and EPA+ DHA 4 g/day, n=210	Effects on blood fatty acid profile	12 wk	Nasopharyngitis, pharyngitis, increased blood creatine phosphokinase and gastrointestinal problems but no serious adverse events in any treatment group
Linderborg et al. (2013)	Healthy women aged 20-30 years. Randomised cross over study	Finland	10	10	2 g of EPA/day, 2 g DPA/day or 2 g olive oil /day	Postprandial metabolism	5 hours	Different postprandial effect of EPA and DPA
Ballantyne et al. (2012)	Patients with hypertri-glyceridemia, statin treated	USA, The Netherlands	469	233	1.9 g EPA/day, n=236, 3.8 g EPA/day, n=233, placebo, n=233	Efficacy and safety of treatment	12 wk	No difference in the three groups with regard to adverse events
Bays et al. (2011)	Patients with a stable high TAG with or without statins	USA, The Netherlands	153	76	1.9 g EPA/day, n=76, 3.8 g EPA/day, n=77, Placebo =76	Changes in TAG concentration	12 wk	Similar in treatment and placebo groups
Other human studies								
Takada et al. (2014) Open labelled study	20 male, age 50-75 years, patients who had undergone coronary stent implantation at least 8 months earlier	Japan			0.9 g x 2 EPA daily as ethyl esters	Platelet functions	12 wk	EPA treatment had suppressed collagen-induced platelet aggregation in patients with a high plasma EPA/AA ratio

Reference	Participant characteristics, age groups	Country (study start)	Number in treatment group		Doses, g/day	Main endpoints	Length of follow-up	Adverse effects
			EPA	Control				
Damsgaard et al. (2014)	713 children, aged 8-11 years, participating in the OPUS-project. Cross sectional study	Denmark			No supplemen-tation study	EPA and DHA concentration and cardio-metabolic risk makers, adjusted for confounders		Total blood EPA conc. was positively associated with total cholesterol, both LDL and HDL. EPA was positively associated with increased diastolic blood pressure among boys
Sato et al. (2013)	1 pregnant woman age 29-years with hypertri-glyceridemia	Japan			1.8 g EPA from gestational week 31 to 34 and 2.7 g EPA from week 34 to 37. EPA was the only treatment	Hypertri-glyceridemia and child outcomes		The child was born with respiratory distress syndrome but recovered after 7 days without need for treatment

Table 2.5-2: An overview of human studies published after 2011 investigating DHA and adverse health effects.

Reference	Participant characteristics	Country (study start)	Number in treatment group		Dose	Main endpoint	Length of follow-up	Adverse effect
			DHA	Control				
RCTs								
Mischoulon et al. (2015)	Adults with major depressive disorder (MDD)	USA	66 and 65	65	1 g DHA /day or 1 g EPA/day. Placebo soy oil	Treatment of MDD	8 wk	Of the 21 physical symptoms assessed, only 2 were significantly different by treatment group (constipation: 13.3% for EPA-enriched, 14.3% for DHA enriched, and 0% for placebo; P=0.010; and tremors: 1.7% for EPA-enriched, 8.9% for DHA-enriched, and 0% for placebo; P=0.020
Hughbanks-Wheaton et al. (2014)	Males with X-linked retinitis pigmentosa, age 7-31 years	USA	33	27	30 mg DHA/kg bw/day (1.5 to 3.6 g/day). Placebo corn/soy oil	Free radical induced oxidation	4 yr	No significant difference in antioxidant activity or oxidised LDL. Mild gastrointestinal discomfort

Reference	Participant characteristics	Country (study start)	Number in treatment group		Dose	Main endpoint	Length of follow-up	Adverse effect
			DHA	Control				
Stonehouse et al. (2013)	Healthy adults age 18-45	New Zealand	85	91	1.16 g DHA/day. Placebo was sunflower oil	Cognitive performance	6 mo	Self-rated burping and unpleasant breath. Adverse effects were not investigated
Azizi-Soleiman et al. (2013)	Middle aged patients with type 2 diabetes,	Iran	14	17	1 g DHA/day	Inflammation, oxidative stress and fat mass	12 wk	Intestinal side effects
Sagara et al. (2011)	Middle aged men with hypertension and/or hypercholesterolemia	Scotland	15	23	2 g DHA/day	Blood pressure, heart rate, triglycerides and VLDL	5 wk	Registered, none reported
Singhal et al. (2013)	Healthy volunteers (n=328) age 18-37 yrs	UK	162	162	1.6 g DHA/day	Branchila artery flow-mediated endothelial dependent vasodilation (primary outcome), surrogate vascular markers for atherosclerosis and conventional biochemical risk factors for CVD	16 wk	No difference
Neff et al. (2011)	Healthy overweight and obese adults, age 18-65	USA	19	17	2 g DHA/day	Plasma lipids and lipoprotein concentrations, and size	4.5 mo	Without significant side effects

2.5.1 **Randomised controlled trials (RCTs)**

A double-blind, randomized controlled clinical trial comparing eicosapentaenoic acid versus docosahexaenoic acid for depression, Mischoulon et al. 2015

196 adults (53% female; mean age 44.7 years) with major depressive disorder (MDD) were randomised to 8 weeks intervention with oral EPA or DHA enriched n-3 preparations as monotherapy 1 g/day or placebo (Mischoulon et al., 2015). Adverse effects were measured using the Patient-Rated Inventory of Side Effects (PRISE). Because many subjects endorsed PRISE symptoms at baseline, the analysis focused on adverse effects that emerged or worsened during treatment. Self-reported safety data were available for 173 subjects. No significant differences across treatment groups were observed. Of the 21 physical symptoms assessed, only two were significantly different between treatment groups (constipation: 13.3% for EPA-enriched, 14.3% for DHA-enriched, and 0.0% for placebo; P=0.010; and tremors: 1.7% for EPA-enriched, 8.9% for DHA-enriched, and 0% for placebo; P=0.020. One EPA-enriched group subject discontinued due to worsening depression, and one placebo patient discontinued due to an unspecified "negative reaction to pills". The authors concluded that both n-3 preparations were well tolerated.

Safety assessment of docosahexaenoic acid in X-linked retinitis pigmentosa: The 4-year DHAX trial, Hughbanks-Wheaton et al. 2014.

Males (n=78; 7-31 years with retinitis pigmentosa were randomised to receive 30 mg DHA/kg bw (n=33) or to placebo = corn/soy oil (n=27) for at least 1 year, but was continued for 4 years and the principle of intention to treat was applied (Hughbanks-Wheaton et al., 2014). All related or unrelated possible adverse events, both self-reported and through blood analyses, were recorded but only treatment related adverse events were evaluated. Thirteen compared to 6 possible treatment related adverse events were seen in the DHA treated group compared to placebo group and mostly gastrointestinal discomfort, but also dehydration (2 cases), increased sensitivity to allergens (1 case), hypothyroidism (1 case), and hypercholesterolemia (1 case) was reported. Of the blood parameters only 7 adverse events were seen in the DHA treated group compared to 13 in the placebo group. The authors concluded that the adverse events were minor and sporadic in occurrence and duration and no significant difference was seen in antioxidant activity or oxidised LDL. Mean fasting glucose levels were marginally reduced after the 4-year DHA intervention, but was not significantly different compared to placebo (P= 0.08). No significantly altered lipoprotein cholesterol or TAG levels were observed in this trial.

Effects of pure eicosapentaenoic and docosahexaenoic acids on oxidative stress, inflammation and body fat mass in patients with type 2 diabetes, Azizi-Soleiman et al. 2013

Sixty patients with type 2 diabetes were randomly allocated to receive daily either ~1 g EPA or ~1 g DHA, or canola oil as placebo for 12 weeks in a randomised triple-blind, placebo-controlled trial (Azizi-Soleiman et al., 2013). With regard to side effects withdrawals from the study were due to swallowing four capsules per day and intestinal side effects in EPA and DHA groups, and personal reasons in the placebo group. However, no figures were given for withdrawal. The main findings of this trial were that both EPA and DHA did not significantly affect oxidative stress, inflammation, blood lipids, body weight or fat mass in patients with type 2 diabetes as compared to placebo.

Postprandial metabolism of docosapentaenoic acid (DPA, 22:5n-3) and eicosapentaenoic acid (EPA, 20:5n-3) in humans, Linderborg et al. 2013

Ten healthy, normal weight women between the age of 20 and 30 years participated in this randomised cross over study with three different supplements included in a standard breakfast meal (Linderborg et al., 2013). The standard breakfast contained 18 g of olive oil. Each period lasted three days with the standard breakfast and on the third day either 2 g of olive oil, or 2 g of DPA or 2 g of EPA was included in the breakfast. Blood was drawn each hour for 5 hours after intake of the DPA, EPA or olive oil rich breakfast. Each test period was followed by a 2-week wash-out period. During the study the participants were asked to

refrain from intake of fatty fish or other supplements containing fatty acids. This study, designed to test if dietary DPA was significantly less absorbed than EPA, showed that plasma TAGs remained at fasting level after the DPA breakfast and a significantly lower concentration of chylomicrones was seen 1 to 3 hours after the DPA rich meal. A reduced incorporation of DPA compared with EPA into chylomicrons was seen 5 hours after the meals. The authors suggested that one possible mechanism explaining the DPA induced chylomicronemia could be that DPA acted as a pancreatic lipase inhibitor. This hypothesis was supported by the recorded observation that three out of the ten subjects reported diarrhea or upset stomach 3 hours after the DPA breakfast. Another explanation suggested by the authors was that the TAGs could be stored in the enterocytes and released over a longer time span than the 5 hours. Furthermore, there was negligible conversion of DPA and EPA to DHA. Further studies are required on the effect of pure DPA since DPA seems to be differently metabolised and causes an effect different from that of EPA and DHA (Linderborg et al., 2013). No safety evaluations were performed in this study.

Docosahexaenoic acid supplementation, vascular function and risk factors for cardiovascular disease: A randomised controlled trial in young adults, Singhal et al. 2013

Healthy volunteers (n=328), aged 18 to 37 years, were randomly assigned to 1.6 g DHA/day (from a microalgae source) together with 2.4 g/day carrier oil (index group) or to 4.0 g/day olive oil (control) (both given in eight 500-mg capsules/day for 16 weeks) (Singhal et al., 2013). Flow-mediated endothelium-dependent vasodilation of the brachial artery (primary outcome) was measured before and after the intervention (n=268) using high-resolution vascular ultrasound. Data on adverse effects and tolerance were recorded for the preceding 7 days during monthly phone calls. There were no serious adverse events in either dietary group and both diets were well tolerated. No participant dropped out of the study due to adverse effects associated with either dietary supplement (the most common reason for dropping out was social). Tolerance data were obtained in 133 and 129 participants from control and DHA-supplemented groups, respectively. Most study participants did not experience any adverse events (86/133 and 84/129 in control and DHA-supplemented groups, respectively). The most common problems were gastrointestinal, but the incidence of these was similar in both randomised groups (23% [n=31/133] and 25% [n=32/129] in control and DHA-supplemented groups, respectively) (for specific symptoms in control and DHA-supplemented groups, respectively: abdominal pain, n=3 and 5; nausea, n=2 and 5; bloating, n=6 and 7; flatulence, n=12 and 9; diarrhea, n=4 and 4; and constipation, n=4 and 2). There was no effect on fasting concentrations of insulin, glucose, or CRP. Fasting concentrations of VLDL and TAG were lower in the DHA-supplemented compared with the control group.

DHA supplementation improved both memory and reaction time in healthy young adults: a randomized controlled trial, Stonehouse et al. 2013

Healthy adults (n=176); age range 18-45 years; non-smoking and with a low intake of DHA completed a 6 month randomised, placebo-controlled, double-blinded intervention with 1.16 g DHA/day or a placebo (Stonehouse et al., 2013). At the end of the study, each participant completed a computer-based tolerance questionnaire that included side effects such as dizziness, diarrhea, nausea, burping, heartburn/reflux, unpleasant breath/bad taste and feeling tired. With regard to side effects, a significantly greater proportion of participants in the DHA-treatment group reported burping and unpleasant breath [burping: 49% compared with 22%, respectively ($P<0.001$); unpleasant breath: 39% compared with 18%, respectively ($P<0.001$)], but the adverse effects were rated as minor (1 and 2 on a scale from 1 to 10). No data on blood lipids, glucose or insulin levels were given and no serious adverse effects were reported.

Efficacy and safety of TAK-085 compared with eicosapentaenoic acid in Japanese subjects with hypertriglyceridemia undergoing lifestyle modification: The omega-3 fatty acid randomized double-blind (ORD) study, Tatsuno et al. 2013

In this randomised, double-blind study subjects with hypertriglyceridemia was investigated (Tatsuno et al., 2013). One group was given 2 g of TAK-085 once daily (n=205), one group got 2 g of TAK-085 twice daily (n=210) and one group was given EPA as ethyl ester 0.6 g three times daily (n=195) for 12 weeks. One gram of TAK-085 contains a combination of concentrated EPA (465 mg) and DHA (375 mg), mostly as ethyl esters. Safety was monitored via the occurrence of adverse advents and changes in clinical laboratory tests (haematology, serum chemistry and urine analysis) at each visit (weeks 0, 4, 8, 10 and 12). There were no significant difference in adverse events between the three groups and there were no apparent relation with the dose of TAK-085. There were no clinically relevant changes in haematology, serum chemistry, urine analysis or vital signs. All three treatments were well tolerated, but no control group was included.

Efficacy and safety of eicosapentaenoic acid ethyl ester (AMR101) therapy in statin-treated patients with persistent high triglycerides (from the Anchor study) Ballantyne et al. 2012

This is multicenter, placebo-controlled, randomised, double-blinded 12-week clinical study in high-risk, statin treated patients with hypertriglyceridemia (TAG levels ≥ 200 and <500 mg/dl).One group was given 4 g AMR101/day (n=233), second group got 2 g AMR101/day (n=236) and the third group got placebo (paraffin) (n=233) (Ballantyne et al., 2012). AMR101 contains 96% EPA as ethyl ester, equaling 3.8 g and 1.9 g of pure EPA as ethyl ester. Adverse events were self-reported and CRP was determined. Adverse events were mild or moderate in severity and considered unrelated to study drug. Diarrhea, nausea, nasopharyngitis and arthralgia occurred in 3% of the patients. Only arthralgia occurred in a larger percentage of patients treated with AMR101 versus placebo, but was highest in the group treated with 2 g of AMR101/day of EPA (no dose effect). The most common treatment

related adverse events were gastrointestinal disorders, which occurred in a larger percentage of patients in the placebo group. Eructations were reported by 2, 1, and 4 patients receiving 4 g, 2 g AMR101 daily or placebo, respectively.

Eicosapentaenoic acid ethyl ester (AMR101) therapy in patients with very high triglyceride levels (from the multi-centre, placebo-controlled, randomised, double-blind, 12-week study with an open-label extension [MARINA] trial), Bays et al. 2011

This is another multicenter, placebo-controlled, randomised, double-blinded 12-week clinical study in patients with hypertriglyceridemia (TAG levels≥500 and <2000 mg/dl) (Bays et al., 2011). One group was given 4 g AMR101/day (n=77), second group got 2 g AMR101/day (n=76) and the third group got placebo (paraffin) (n=76). AMR101 contains 96% EPA as ethyl ester. In this study not all patients were on statin treatment. The safety evaluation was done using reported adverse events, clinical laboratory assessments, 12-lead electrocardiographic findings, physical examination findings, weight and body mass index and vital signs like heart rate and blood pressure. Adverse events were recorded by the investigator (who was unaware of the treatment regimen) as related or not related to the study drug. Treatment-related adverse events were defined as those adverse events that had newly occurred or had worsened in severity during the double-blind treatment period. Most treatment related adverse events were mild to moderate in severity. About 35% of the patients reported treatment-related adverse events in all three groups. Most common were diarrhea, nausea and eructation, but these were more common in the placebo treated group. Changes in fasting plasma glucose and hemoglobin A1c (HbA1c) concentrations did not differ significantly that of the placebo group. Neither did the treatment with AMR101 at any dose change vital signs, electrocardiographic parameters, alanine aminotransferase, aspartate aminotransferase or creatine kinase values (Bays et al., 2011).

Algal Docosahexaenoic acid affects plasma lipoprotein particle size distribution in overweight and obese adults, Neff et al. 2011

Enrolled participants were between the ages of 18 and 65 years, healthy and obese, with a BMI of 30.0–39.9 kg/m^2, or overweight, with a BMI of 25.0–29.9 kg/m^2 and a waist circumference ≥ 102 cm in men or ≥ 88 cm in women (Neff et al., 2011). Thirteen individuals did not complete the study due to withdrawal of consent, unrelated adverse events, or noncompliance with the study protocol, and their data were excluded from the data analyses. The authors stated that DHA supplementation was well tolerated without significant side effects. Total cholesterol increased more with DHA than with placebo (P = 0.031). There were no significant between-group differences in changes in TG, VLDL cholesterol, LDL cholesterol, or HDL cholesterol in response to treatment. However, in paired-sample analyses, TG levels decreased over time in the DHA group (P = 0.001) but not the placebo group (P = 0.15). In the DHA group, mean TG level decreased by 21% with

treatment. Nuclear magnetic resonance (NMR)-based calculations suggested that the DHA group had greater decreases in both total TG (P = 0.006) and VLDL TG (P = 0.009) than the placebo group. Treatment-associated changes in calculated HDL cholesterol level did not differ between the DHA and placebo groups (P = 0.53). No significant differences were detected between the DHA and placebo groups in changes in fasting glucose or insulin levels, HOMA-IR score, HbA1c, or the area under the curve for glucose or insulin during the oral glucose tolerance test. Changes in 24-hours monitored systolic and diastolic blood pressures over the course of the study did not differ significantly between the DHA and placebo groups. Compared with placebo, DHA had no significant effect on clotting parameters, including prothrombin time, activated partial thromboplastin time, and fibrinogen (data not shown). Markers of inflammation that were assessed in all participants included IL-1b, IL-6, IL-10, TNFα, lipopolysaccharidebinding protein, and CRP. Of these, the only significant between-group difference in change was a greater increase in plasma concentration of IL-10 (P = 0.021) after treatment in the DHA group than in the placebo group (Neff et al., 2011).

Effects of Docosahexaenoic acid supplementation on blood pressure, heart rate, and serum lipids in Scottish men with hypertension and hypercholesterolemia, Sagara et al. 2011

The study was a randomised double-blind placebo-controlled trial involving 156 males aged 45–59 years. The study was conducted in two separate stages (Sagara et al., 2011). Stage 1 was a screening survey for high-risk subjects for developing cardiovascular disease, included a structured questionnaire to obtain information about demographic characteristics, medical history and medication, and a clinical evaluation of height and weight, blood pressure, heart rate, and fasting blood lipid profile. Stage 2 was a five-week dietary supplementation period. Among the 56 high risk subjects 25 and 31 participants were assigned to the DHA group and to the active placebo group, respectively, randomly in a double-blind fashion. In the DHA group, DHA powder containing calcium encapsulated DHA (2 g/day) mixed in bread rolls were consumed in addition to the usual diet. The active placebo group received bread rolls containing calcium encapsulated olive oil powder (1 g/day). During each visit, dietary counselling, adverse events, compliance assessments, and any medication changes were documented. Participants completed a one-page questionnaire intended to identify any adverse effects of the supplements and they were asked to return the food record forms. The authors reported no adverse events. There was a significant reduction in mean systolic blood pressure (P < 0.001), diastolic blood pressure (P < 0.01), and heart rate (P < 0.05) in DHA supplemented group but not in the placebo group. The analysis of serum lipid profiles showed an increase in HDL-cholesterol and decrease in total cholesterol and non-HDL-cholesterol/HDL-cholesterol ratios both in the placebo and DHA groups. There was no significant change in the total cholesterol and non-HDL-cholesterol during the 5 weeks of intervention in either group (Sagara et al., 2011).

2.5.2 **Other human studies**

Eicosapentaenoic acid and docosahexaenoic acid in whole blood are differentially and sex-specifically associated with cardiometabolic risk makers in 8-11 year old Danish children, Damsgaard et al. 2014

This is a cross sectional study performed in 713 children aged 8-11 years. One of the aims was to investigate associations between n-3 LCPUFA status measured as EPA and DHA in whole blood, and early cardiometabolic risk factors (Damsgaard et al., 2014). The study is a part of the OPUS project (Optimal well-being, development and health for Danish children through a healthy New Nordic Diet). In this study a negative association was found between whole blood EPA and TAG, and a positive association between EPA and cholesterol as well as a sex specific association with increased blood pressure in boys. Both EPA and DHA were negatively associated with heart rate and DHA with insulin resistance. Blood pressure was measured only at one occasion and just before blood sampling which was an important limitation of the study. However, the authors referred to a pilot study where DHA concentration in whole blood was associated with increased blood pressure among 8-11 year olds and in 7 year-old offsprings of mothers who received fish oil versus olive oil supplementation in pregnancy.

Effects of Eicosapentaenoic Acid on Platelet Function in Patients Taking Long-Term Aspirine Following Coronary Stent Implantation, Takada et al. 2014

In this open labelled trial, 18 men (age 50-75 years) on stable medication (100 mg acetylsalicylic acid/day and stable statin treatment) who had undergone coronary stent implantation at least 8 months earlier, were given 1.8 g/day of EPA ethyl ester for 12 weeks. EPA was given in capsules (2 x 0.9 g) daily with meals (Takada et al., 2014). The main outcome of this study was the effect of EPA on platelet aggregation and platelet activation in patients under aspirin treatment after coronary stent implantation. In the group with the highest EPA/arachidonic acid (AA) ratio after treatment (n=10), there was a significant suppressed collagen-induced maximum platelet aggregation, but there were no change in normal platelet aggregation. In the patients with low EPA/AA ratio after treatment there were no changes in platelet aggregation.

Effect of eicosapentaenoic acid agent on aggravated hypertriglyceridemia during pregnancy, Sato et al. 2013

This study is a case report of a 29 year old multiparous pregnant women who was transferred to a hospital in week 29 + 0 of gestation due to hypertriglyceridemia (TAG = 898 mg/dl) (Sato et al., 2013). She was treated with 1.8 g EPA/day and TAG concentration was reduced to 550 mg/dl. At week 37 + 2 of gestation she gave birth to a 2667 g baby who was borne with respiratory distress syndrome. After one week treatment, all symptoms of

respiratory problems were resolved. The authors concluded that treatment with EPA in patients with hypertriglyceridemia in pregnancy is a possible therapeutic approach although it might increase the risk of foetal respiratory distress syndrome.

2.5.2.1 Interactions

There was no information concerning interactions between any of the single fatty acids and other nutrients, drugs or any substance in the literature reviewed in the present risk assessment.

2.5.2.2 Allergic sensitisation and adjuvant effects

In one study increased sensitivity to allergens was reported after 4 years with 30 mg/kg bw DHA supplementation (Hughbanks-Wheaton et al., 2014). No other study reported information concerning allergic sensitisations and/or adjuvant effects of EPA, DPA or DHA in humans.

2.5.3 **Animal studies**

One animal study investigating DPA as single fatty acid was identified in the additional literature search (see section 2.1.2). Fard et al. (2014) conducted an animal study to examine the results for DPA from a human study, and to test whether DPA was metabolised differently than EPA. The rats were randomly divided into four groups and the first group was sacrificed at day 0. The rats were then fed a special diet with no EPA, DHA or DPA for 9 days and on days 5, 6 and 8 the rats were feed either 250 mg/day of EPA or DPA as free fatty acids or olive oil. There was no statistically significant difference in the total amount of faecal fat between the three groups over the 9 day study, but faecal DPA excretion was 4.6 –fold higher compared with EPA excretion. There was no significant difference in the content of DPA and DHA in plasma fatty acids at day 9, but the concentration of EPA was significantly higher in the groups fed DPA and EPA. It was also found that liver DPA, EPA and total amount of n-3 LCPUFA levels were significantly increased by DPA and EPA feeding compared with olive oil fed controls. In the heart, DPA feeding increased the DPA content and both DPA and EPA feeding increased the total n-3 LCPUFA level. The authors concluded that the DPA and EPA are metabolised differently, confirming the results from their human study (Linderborg et al., 2013).

2.5.4 **Mode of action for adverse effects**

Possible adverse effects of EPA, DPA and DHA include prolonged bleeding time, suppression of immune reactions, oxidation of LDL particles or lipoproteins and impact on lipid and glucose homeostasis in patients with type 2 diabetes (EFSA, 2012; IOM, 2005; VKM, 2011).

EPA and DHA are incorporated into phospholipids of all cell membranes. EPA and DHA are incorporated in the sn-2 position of phospholipids and thereby replacing other unsaturated fatty acids of the n-6 family such as arachidonic acid (AA). This may alter the fluid characteristics of the membranes, effecting changes in protein/enzyme function and ion channel conductance. The enzyme phospholipase A_2 (PLA_2) liberates EPA and DHA from the membrane into the cytoplasm, where they are subject to one of two oxidation pathways, the enzymatic or the non-enzymatic pathway.

In the enzymatic pathway, n-6 and n-3 LCPUFAs are metabolised to eicosanoids, which can enter the nucleus where they down regulate expression of genes that promote inflammation. Eicosanoids act locally and they can also travel through the bloodstream where the n-3 derived eicosanoids reduce platelet aggregation and the accumulation of fatty acids in the arteries. N-3 LCPUFAs are also subject to spontaneous oxidation by reactive oxygen species, which can attack EPA and DHA, both when free and when incorporated in membranes. These reactions result in the formation of lipid peroxides, such as hydroperoxy-DHA and -EPA, which can activate transcription factors such as peroxisome proliferator-activated receptors (PPARs) and nuclear factor erythroid-derived 2 and up-regulate gene expression in the nucleus (Serhan et al., 2008; Spite et al., 2014).

EPA

EPA is known to influence platelet function. Platelet aggregation is mediated through the production of eicosanoids, especially thromboxane A_2 (TXA_2) and prostaglandin A_2 (PGI_2) from arachidonic acid. Upon platelet activation cell membrane fatty acid availability is of importance. If intake of n-3 fatty acids is high and the ratio of n-3 to n-6 fatty acids is increased the production will shift from TXA_2 and PGI_2production from arachidonic acid to production of TXA_3 and PGI_3 from EPA. TXA_3 and PGI_3 have local vasodilitatory and antiplatelet activating effects and reduce the production of platelet activating factor thereby increasing bleeding time.

The production of the less proinflammatory eicosanoids participating in the regulation of inflammatory and immunological reactions might be positive for reduction of inflammation in obesity, diabetes and autoimmune diseases. However, immunosuppression may also increase the risk of infections.

DPA

Little is known about the effects of DPA because few studies have been conducted, but lately interest in this fatty acid has increased because human milk contains high concentration of DPA (Yu et al., 1998). From studies performed so far, it has been suggested that DPA could act as a lipase inhibitor since faecal excretion of DPA was 4.6 fold higher than equivalent

amounts of EPA. However, the incorporation of DPA and EPA in plasma TAGs were similar which was explained by EPA being a substrate for β-oxidation.

DHA

DHA can be transformed into a variety of metabolites such as resolvins, epoxides, poxytrins, neuroprotectins and maresins, some of which are able to inhibit platelet aggregation at low micromolar concentrations responses (Chen et al., 2011; Dobson et al., 2013; Serhan et al., 2008; Spite et al., 2014). The extent to which their relatively subtle effects on platelet function may be of clinical significance is a matter of debate. However, the fact that they alter important platelet signalling pathways and thereby may mute haemostatic processes is clear.

One of the described studies in the EFSA (2012) opinion specifically assessed whether DHA and EPA could have differential effects on platelet aggregation. In a double-blind placebo-controlled trial of parallel design, 59 treated hypertensive type 2 diabetic men and postmenopausal women were randomised 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, the EFSA (2012) opinion describes another study comparing 4 g/day EPA compared to 4 g/day of fish oil in which EPA was found more effective in decreasing platelet aggregation. The latter may be a dose effect.

A reduction in platelet-mediated thrombin generation by which DHA (and EPA) may retard haemeostasis is a newly discovered mechanism (Larson et al. 2013). The delayed, but not eliminated, thrombin activation can still allow for relatively normal haemostasis while also reducing the risk of thrombosis (Wachira et al. 2014).

N-3 LCPUFA

Because n-3 LCPUFAs have 5-6 double-bonds these fatty acids could be more prone to peroxidation than fatty acids with less double-bonds.

The main side effect of n-3 LCPUFA supplementation is gastrointestinal discomfort, which has been reported by some subjects in all supplementation studies.

2.5.5 **Vulnerable groups**

Concern was raised in an intervention study in children that reported nose bleeding episodes associated with the consumption of fish oil (Clarke et al., 1990). In our literature search, no new studies reporting on bleeding complication related to EPA or DHA supplementation in

children were identified. In a new Danish cross sectional study by (Damsgaard et al., 2014), whole blood EPA was positively associated with higher diastolic blood pressure in boys (8 to 11 years). No information about dietary intake was available in the article. According to the authors, similar results were found in a pilot study where increased blood pressure was correlated with whole blood DHA concentration (Damsgaard et al., 2014). Respiratory distress syndrome was reported in a child born to a mother using 1.8 g EPA from week 31 to 34 and 2.7 g EPA from week 34 to 37 during pregnancy (Sato et al., 2013). The child recovered after 7 days without further need for treatment. This was a case-report and no further studies were recognised reporting adverse outcome following supplementation during pregnancy.

2.6 Summary of hazard identification and characterisation

There are several RCTs studying the effect of n-3 LCPUFAs in combination, but still a relatively modest number addressing supplements with the single fatty acids EPA, DPA or DHA. Only few studies with EPA, DPA or DHA supplements performed after 2011 have addressed possible adverse effects of supplementation (included safety concerns). Most of the included studies have investigated dosages that are below or at the dosage considered as safe by EFSA. None of the included randomised supplementation studies were undertaken in children, adolescents or pregnant women.

EFSA concluded in 2012 that up to 1.8 g/day of supplemental EPA does not raise safety concerns for adults, and up to 1 g/day of DHA does not raise safety concern for the general population. EFSA did not conclude for DPA because data was not sufficient for evaluation. The safety concerns related to n-3 LCPUFAS combined or as single substances in previous reports are related to bleeding complications, immune function, peroxidation and impaired glucose or lipid homeostasis.

For risk assessment of combined mixtures of n-3 LCPUFAs in e.g. fish oil/cod liver oil, see the EFSA opinion from 2012 or the VKM assessment from 2011 (EFSA, 2012; VKM, 2011). In the reviewed literature of this risk assessment, no studies investigating ratios between EPA, DPA, DHA or other fatty acids in mixtures have been identified.

None of the included studies from our literature searches published from 2011 onwards have investigated bleeding complications. The included studies have investigated lipid peroxidation, immune function and glucose and lipid homeostasis. None of the studies included reported adverse effects related to immune function, lipid peroxidation or lipid or glucose homeostasis.

EPA

Four randomised controlled trials and three other human studies were included. Three of the RCTs are conducted in patients with hypertriglyceridemia. Dosage used i range from 1.8 to 3.8 g/day of EPA for 12 weeks. The endpoints include immune function, blood pressure and heart rate. Diarrhea, nausea, nasopharyngitis, and arthralgia were the most common adverse events and no serious adverse events were reported in any of the four randomised controlled studies. Furthermore, adverse events reported were not related to dosage.

Two of the included randomised studies investigated EPA at doses above 1.8 g/day (1.9-3.8 g/day) as a single fatty acid. Supplemental intakes of EPA at doses up to about 3.8 g/day for 12 weeks did not change glucose homeostasis and similar numbers of nasopharyngitis as a measure of immune function were seen in treatment group and placebo (Ballantyne 2010, Bays 2011).

In 2012, EFSA did not draw conclusions concerning the safety of EPA for children or adolescents. We have identified only one recent cross-sectional study in children (Damsgaard et al., 2014) in which the concentration of EPA in blood in 8 to 11 years old children correlated positively with blood pressure in boys. However, since no new studies with EPA supplementation have been identified in children or adolescents, no provisional safe level of use for children or adolescents can be set.

Animal studies on EPA have not been included in this report as it was considered that EPA is thoroughly investigated in humans.

In summary; it is well documented that 1.8 g supplemental EPA per day is unlikely to cause adverse health effects in adults. In two studies doses up to 3.8 g/day were given for 12 weeks without reported adverse effects. However, these two studies were of short duration, i.e. 12 weeks and studies of longer duration are necessary for an assessment of higher intakes of EPA.

As value for comparison in the risk characterisation of EPA, VKM will use 1.8 g/day. This is based on the opinion from EFSA from 2012, and also taking into account newer literature.

DPA

Information about effects of DPA is scarce, but one study in 10 healthy normal weight women given 2 g of supplemental DPA served for breakfast and followed for 5 hours post-prandially indicated a different incorporation of DPA compared with EPA into various cell membranes. Furthermore, 2 g/day of DPA inhibited incorporation of other fatty acids into chylomicrons (Linderborg et al., 2013). In a study in rats, the different incorporation of DPA into the various body compartments was confirmed (Fard et al., 2014). However, the importance and relevance of these findings still have to be elucidated.

In summary, no value for comparison can be established for DPA due to lack of data.

DHA

Although there are several human intervention trials with supplementation of DHA alone, studies addressing possible adverse effects of DHA supplements for healthy adults and the general population are missing. In 2012, EFSA assessed the impact of DHA supplementation on bleeding time, platelet function, glucose homeostasis, LDL-cholesterol, and lipid peroxidation. For DHA it was concluded that supplemental intakes of DHA up to about 4 g per day are not considered to cause adverse effects; it was not associated with an increased risk of clinical complications (e.g. spontaneous bleeding). Regarding possible increase in LDL-cholesterol it was concluded that supplemental intakes of 2 to 4 g DHA per day is not adverse in relation to CVD risk. A supplemental intake of up to about 4 g DHA per day for six weeks did not induce lipid peroxidation as assessed by F_2-isoprostanes. Moreover, doses up to about 5 g DHA per day for up to 16 weeks did not induce changes in lipid peroxidation. Their final conclusion was that supplemental intakes of up to 1 g/day of DHA do not raise safety concerns for the general population. No information was provided regarding how they reached their conclusion of up to 1 g DHA per day.

In this risk assessment, we have included seven studies with both patients and healthy adults (Table 2.5-2). The dosages of DHA ranged from 1.0 to 3.6 g DHA/day and the duration from five weeks to four years. Six out of seven studies used dosages from 1 to 2 g DHA/day. The last study included up to 3.6 g DHA/day for four years and the age spanned from 7 to 31 years, but there were few participants, n=33 in the treatment groups. The main endpoints in all studies included lipid peroxidation, inflammation, cognitive performance, blood pressure, and/or biomarkers of cardiovascular diseases. No serious adverse events were found related to the main endpoints. In general, adverse events were described as gastrointestinal discomfort and were not related to dose.

In this report, one safety study of supplemental DHA on vulnerable groups such as pregnant women, children and adolescents was identified. Animal studies on DHA have not been included in this report as previous risk assessments have found no serious adverse events with doses of DHA up to 5 g per day and combined doses of EPA and DHA up to 6.9 g/day (VKM 2011, EFSA 2012).

In summary; due to a limited number of studies with supplemental doses above 1 g DHA per day, the risk associated with supplemental DHA above 1 g DHA per day could not be assessed. However, a daily dose of DHA that moderately exceed 1.0 g from food supplements is not considered to lead to adverse health effects in the general population (including children ≥10 years and adolescents).

As value for comparison in the risk characterisation of DHA, VKM will use 1 g/day. This is based on the opinion from EFSA from 2012, and also taking into account newer literature.

3 Exposure / Intake

Exposures of EPA, DPA and DHA were estimated from the intake of food supplements. For food supplements, the intakes of EPA, DPA and DHA were estimated for the age groups 10-14 years, 14-18 years and adults (≥ 18 years).

3.1 Food supplements

NFSA requested VKM to perform a risk assessment of 1500, 1750 and 1825 mg/day of EPA, 100, 125 and 150 mg/day of DPA and 1050 and 1290 g/day of DHA in food supplement for children 10 years and above, adolescents and adults. The default body weights (bw) for age groups determined by EFSA were used: 10 to<14 years = 43.4 kg, 14 to <18 years = 61.3 kg and adults = 70.0 kg. The estimated exposures from supplemental EPA, DPA and DHA in children, adolescents and adults are given in Table 3.1-1.

Table 3.1-1 Estimated exposure of EPA, DPA and DHA in children, adolescents and adults from food supplements.

Groups	Daily doses (mg)	Body weight (kg)	Exposure (mg/kg bw per day)
Children (10 to <14years)	EPA 1500, 1750 and 1825 DPA 100, 125, 150 DHA 1050, 1290	43.4	EPA: 35, 40 and 42 DPA: 2.3, 2.9 and 3.5 DHA: 24 and 30
Adolescent (14 to <18 years)	EPA 1500, 1750 and 1825 DPA 100, 125, 150 DHA 1050, 1290	61.3	EPA: 25, 29 and 30 DPA: 1.6, 2.0 and 2.4 DHA: 17 and 21
Adults (≥18 years)	EPA 1500, 1750 and 1825 DPA 100, 125, 150 DHA 1050, 1290	70.0	EPA: 21, 25 and 26 DPA: 1.4, 1.8 and 2.1 DHA: 15 and 18

Information about intakes of EPA, DPA and DHA from the diet is scarce, but calculations performed in the Norwegian Mother and Child Cohort Study indicate a mean total intake (SD) from food and supplements of EPA around 330 (340) mg/day, DPA 43 (30) mg/day and DHA 430 (380) mg/day among pregnant women (2002 to 2008), see Table 3.1-2.

Mean intake of EPA, DPA and DHA from fish oil/ cod liver oil in adults participating in a nationally representative dietary survey was 735 mg/day (VKM, 2014).

Concentrations of the n-3 LCPUFAS in cod liver oil may vary, and a recommended dose of 5 ml may contain 400 mg EPA, 60 mg DPA and 600 mg DHA.

3.2 Other sources

Use of EPA, DPA or DHA as single fatty acids in a supplement is relatively new and the actual intake and usage is not known in the general Norwegian population.

4 Risk characterisation

EPA

The doses requested from NFSA are 1500, 1750 and 1825 mg EPA per day in food supplements, and the exposures for children (10 years and above), adolescents and adults are given in chapter 3.

The value for comparison used in this risk characterisation of EPA is 1.8 g/day.

Two supplementation studies with 3.8 g EPA per day for 12 weeks did not report adverse effects, but these studies were of too short durations to conclude on chronic effect. VKM however, considers that the highest specified daily dose of EPA 1825 mg/day is unlikely to cause adverse health effects.

One study included children aged 8 to 11 years, in which a positive association was found between EPA blood concentration and blood pressure in boys, indicating that children might be more vulnerable than adults for high body concentrations of EPA. However, this was not a supplementation study and the authors also reported on uncontrolled situations when blood pressure was measured. No safe level for EPA supplementation in children or adolescents was set in the EFSA report from 2012. Taken together, the EFSA conclusion from 2012 and newer studies in children no risk characterisation can be made for children and adolescents with regard to EPA supplementation.

VKM considers that:

In adults, (18 years and above) the specified doses of 1500, 1750 and 1825 mg EPA in food supplements are unlikely to cause adverse health effects.

No safe dose can be stated for children and adolescents because of insufficient data.

DPA

The doses requested from NFSA are 100, 125 and 150 mg DPA per day in food supplements, and the exposures for children, adolescents and adults are given in chapter 3. For DPA there is not sufficient data to assess the risk of adverse effects for any population group.

DHA

The doses received from NFSA are 1050 and 1290 mg DHA per day in food supplements, and the exposures for adults, children (10 years and above) and adolescents are given in chapter 3.

The value for comparison used in this risk characterisation of DHA is 1 g/day. This value is valid for the general population (including children 10 years and above and adolescents).

A previous risk assessment report (EFSA, 2012) has concluded that doses up to 1 g DHA/day do not raise safety concern for the general population. However, in the same report no serious adverse events were described with supplemental intakes up to 5 g DHA per day. In the present risk assessment, the included studies encompass adults and children, both patient groups and healthy subjects, and the dosages of DHA range from 1.0 to 3.6 g/day with duration of interventions ranging from five weeks to four years. VKM therefore considers that the specified daily doses of DHA, 1050 and 1290 mg/day, are unlikely to cause adverse health effects in adults, children (10 years and above) and adolescents.

5 Uncertainties

No studies on children and adolescents were identified in the new literature search and it is therefore possible only to make assumptions about the safety for these groups.

The risk assessment is based on previous risk assessments of EPA, DPA and DHA containing no information on vulnerable groups, interactions or allergy.

The majority of the studies considered are studies specifically designed to investigate positive effects such as reduction of hypertriglyceridemia and depression or cognitive impairment and do not focus on adverse health effects. In the human studies, the adverse effects reported are mainly based on self-reporting questionnaires, and to lesser extent biomarkers for negative health effects e.g. bleeding time, peroxidation or immune function.

6 Data gaps

The few studies on negative health effects related to supplemental intake of EPA or DHA are only performed in different patient groups and have high heterogeneity in both design and results, and are limited to adults with the exception that one study includes patients aged 7-31 years. The majority of studies are of short duration, mostly 12 weeks, and studies of one to two years duration are lacking.

There is a general lack of studies addressing supplemental use of individual n-3 LCPUFAs in vulnerable groups such as pregnant women and children.

There are generally lacking safety studies related to supplemental intakes of either EPA, DPA or DHA, particularly in children, adolescents and pregnant women.

7 Conclusions with answers to the terms of reference

The Norwegian Food Safety Authority (NFSA) requested the Norwegian Scientific Committee for Food Safety (VKM) to assess the safety of EPA, DPA and DHA in food supplements at the following doses for the general population, ages 10 years and above:

EPA: 1500, 1050 and 1825 mg/day
DPA: 100, 125 and 150 mg/day
DHA: 1050 and 1290 mg/day

It is emphasised that this risk assessment concerns the single fatty acids EPA, DPA or DHA separately and not mixtures of these as found in e.g. fish oil/cod liver oil. For risk assessment of combined mixtures of n-3 LCPUFAs in e.g. fish oil/cod liver oil, see the EFSA opinion from 2012 or the VKM assessment from 2011 (EFSA, 2012; VKM, 2011). In the reviewed literature of this risk assessment, no studies investigating ratios between EPA, DPA, DHA or other fatty acids in mixtures have been identified.

In the EFSA report 2012 it was concluded that doses up to 1.8 g per day of EPA in adults and 1 g per day of DHA in adults, adolescents and children for one year is unlikely to cause adverse health effects.

Some adverse health effects related to gastrointestinal function, including abdominal cramps, flatulence, eructation, vomiting and diarrhea have been reported in the reviewed literature, but seem to be associated with intake of an oily substance and not related specifically to EPA, DPA and/or DHA.

The major concerns with high intake of EPA or DHA have been increased bleeding time or related to immune function, lipid peroxidation and glucose homeostasis.

Due to lack of clinical studies and/ or randomised controlled trials, vulnerable groups like pregnant /lactating women have not been evaluated in this report.

VKM concludes that:

- The specified daily doses of 1500, 1750 or 1825 mg/day of EPA in food supplements are unlikely to cause adverse health effects in adults (≥18 years).
- No conclusion can be made for children or adolescents for EPA.
- No dosage of DPA in food supplements can be evaluated due to lack of data.

- The specified daily doses of 1050 and 1290 mg/day of DHA in food supplements are unlikely to cause adverse health effects in the general population including children (≥10 years), adolescents and adults (≥18 years).

An overview of the conclusions is presented in Table 7.1.

Table 7.1: An overview of the conclusions for EPA and DHA in food supplements.
Green: Estimated exposures to EPA or DHA are unlikely to cause adverse health effects.
Grey: No conclusions have been made.

	Eicosapentaenoic acid (EPA)			**Docosahexaenoic acid (DHA)**	
Doses / Age groups	1500 mg/day	1750 mg/day	1825 mg/day	1050 mg/day	1290 mg/day
Children (10 to <14 years)					
Adolescents (14 to <18 years)					
Adults (≥18 years)					

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9 Appendix

Strategy for main literature search

Database: Ovid MEDLINE(R) <1946 to April Week 4 2015>, Embase <1974 to 2015 April 30>

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1. eicosapentaen*.ti. (3583)
2. EPA.ti. (2549)
3. docosahexaen*.ti. (5133)
4. DHA.ti. (1959)
5. docosapentaen*.ti. (166)
6. DPA.ti. (383)
7. 1 or 2 or 3 or 4 or 5 or 6 (11830)
8. (risk* or safety or adverse or side-effect*1 or hazard* or harm* or bleeding* or negative or contraindicat* or contra-indicat* or interact* or toxicity or toxic).tw. (8874887)
9. 7 and 8 (2467)
10. (conference abstract* or letter* or editorial*).pt. (4382040)
11. not 10 (2158)
12. limit 11 to (danish or english or norwegian or swedish) (2092)
13. limit 12 to human (1298)
14. remove duplicates from 13 (747)
15. limit 14 to yr="2011 -Current" (252)

Strategies additional literature searches for EPA, DPA and DHA, respectively, and children and adolescents

Database: Ovid MEDLINE(R) <1946 to June Week 1 2015>, Embase <1974 to 2015 June 04>

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1. (eicosapentaenoic acid* or EPA).ti. (5448)
2. (child* or adolescent* or teenage* or college* or high school*).mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, an, tn, dm, mf, dv, kw] (5855060)
3. 1 and 2 (214)
4. (conference abstract* or letter* or editorial*).pt. (4464924)
5. 3 not 4 (175)
6. limit 5 to (danish or english or norwegian or swedish) (172)
7. limit 6 to human (164)
8. remove duplicates from 7 (118)
9. limit 8 to yr="2011 -Current" (29)

Database: Ovid MEDLINE(R) <1946 to June Week 1 2015>, Embase <1974 to 2015 June 04>

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1. (docosahexaenoic acid* or DHA).ti. (6358)
2. (child* or adolescent* or teenage* or college* or high school*).mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, an, tn, dm, mf, dv, kw] (5855060)
3. 1 and 2 (552)
4. (conference abstract* or letter* or editorial*).pt. (4464924)
5. 3 not 4 (454)
6. limit 5 to (danish or english or norwegian or swedish) (433)
7. limit 6 to human (397)
8. remove duplicates from 7 (252)
9. limit 8 to yr="2011 -Current" (91)

Database: Ovid MEDLINE(R) <1946 to June Week 1 2015>, Embase <1974 to 2015 June 04>

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1. (docosapentaenoic acid* or DPA).ti. (524)
2. (child* or adolescent* or teenage* or college* or high school*).mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, an, tn, dm, mf, dv, kw] (5855060)
3. 1 and 2 (43)

4. (conference abstract* or letter* or editorial*).pt. (4464924)
5. 3 not 4 (41)
6. limit 5 to (danish or english or norwegian or swedish) (21)
7. limit 6 to human (18)
8. remove duplicates from 7 (10)

Strategy for additional literature search for DPA including animal studies

Database: Ovid MEDLINE(R) <1946 to June Week 1 2015>, Embase <1974 to 2015 June 16>

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1. docosapentaenoic acid*.ti. (147)
2. dokosapentaensyre*.ti. (0)
3. 1 or 2 (147)
4. remove duplicates from 3 (82)