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SCIENTIFIC OPINION

Scientific Opinion on health benefits of seafood (fish and shellfish) consumption in relation to health risks associated with exposure to methylmercury[1]

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

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ABSTRACT

Following a request from the European Commission to address the risks and benefits as regards fish/seafood consumption related to relevant beneficial substances (e.g. nutrients such as n-3 long-chain polyunsaturated fatty acids) and the contaminant methylmercury, the Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver a Scientific Opinion on health benefits of seafood consumption in relation to health risks associated with exposure to methylmercury. In the present Opinion, the NDA Panel has reviewed the role of seafood in European diets and evaluated the beneficial effects of seafood consumption in relation to health outcomes and population subgroups that have been identified by the FAO/WHO Joint Expert Consultation on the Risks and Benefits of Fish Consumption and/or the EFSA Panel on Contaminants in the context of a risk assessment related to the presence of mercury and methylmercury in food as relevant for the assessment. These included the effects of seafood consumption during pregnancy on functional outcomes of children's neurodevelopment and the effects of seafood consumption on cardiovascular disease risk in adults. The Panel concluded that consumption of about 1-2 servings of seafood per week and up to 3-4 servings per week during pregnancy has been associated with better functional outcomes of neurodevelopment in children compared to no consumption of seafood. Such amounts have also been associated with a lower risk of coronary heart disease mortality in adults and are compatible with current intakes and recommendations in most of the European countries considered. These associations refer to seafood *per se* and include beneficial and adverse effects of nutrients and non-nutrients (i.e. including contaminants such as methylmercury) contained in seafood. No additional benefits on neurodevelopmental outcomes and no benefit on coronary heart disease mortality risk might be expected at higher intakes.

KEY WORDS

fish, shellfish, benefit, neurodevelopment, coronary heart disease

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SUMMARY

Following a request from the European Commission to address the risks and benefits as regards fish/seafood consumption related to relevant beneficial substances (e.g. nutrients such as n-3 LCPUFA) and the contaminant methylmercury, the Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver a Scientific Opinion on health benefits of seafood consumption in relation to health risks associated with exposure to methylmercury.

In this Opinion, the term **seafood** denotes vertebrate and invertebrate aquatic animals whether of marine or freshwater origin, whether farmed or wild, except aquatic mammals (e.g. whales and dolphins), aquatic reptiles (e.g. turtles and crocodiles), echinoderms (e.g. sea urchins and starfish), and jellyfish, and does not include aquatic plants.

In the present Opinion the NDA Panel has: a) reviewed the role of seafood in European diets; b) evaluated the beneficial effects of seafood consumption in relation to health outcomes and population subgroups previously identified by the FAO/WHO Joint Expert Consultation on the Risks and Benefits of Fish Consumption and/or the CONTAM Panel as relevant for the assessment. These include the effects of seafood consumption during pregnancy on functional outcomes of children's neurodevelopment, and the effects of seafood consumption on cardiovascular disease risk in adults; c) addressed which nutrients in seafood may contribute to the beneficial effects of seafood consumption in relation to the above-mentioned outcomes; and d) considered whether the beneficial effects of seafood consumption in relation to the above-mentioned outcomes could be quantified.

On the basis of the data available, the Panel concludes that:

a) Seafood is a source of energy and protein with high biological value, and contributes to the intake of essential nutrients, such as iodine, selenium, calcium, and vitamins A and D, with well established health benefits. Seafood also provides n-3 long-chain polyunsaturated fatty acids (LCPUFA), and is a component of dietary patterns associated with good health. Most European Food-Based Dietary Guidelines recommend (a minimum of) two servings of fish per week for older children, adolescents, and adults to ensure the provision of key nutrients, especially n-3 LCPUFA, but also vitamin D, iodine and selenium. Recommendations for children and pregnant women refer to the type of fish and are also based on safety considerations, i.e. presence of contaminants. Available data suggest a large variation in the amount of fish and other seafood consumed across European countries and age groups, as well as in the type of seafood and species eaten, although data from European surveys are difficult to compare, the type of seafood consumed is largely unknown in some countries, and data are particularly scarce for infants. Seafood provides the recommended amounts of n-3 LCPUFA in most of the European countries considered and contributes to the needs of other essential nutrients, such as vitamin D, iodine or selenium, in some countries.

b) Consumption of about 1-2 servings of seafood per week and up to 3-4 servings per week during pregnancy has been associated with better functional outcomes of neurodevelopment in children compared to no seafood. Such amounts have also been associated with a lower risk of coronary heart disease (CHD) mortality in adults and are compatible with current intakes and recommendations in most of the European countries considered. These associations refer to seafood *per se* and include beneficial and adverse effects of nutrients and non-nutrients (i.e. including contaminants such as methylmercury) contained in seafood. No additional benefits on neurodevelopmental outcomes and no benefit on CHD mortality risk might be expected at higher intakes.

c) The observed health benefits of seafood consumption during pregnancy may depend on the maternal status with respect to nutrients with an established role in the development of the central nervous system of the foetus (e.g. docosahexaenoic acid (DHA) and iodine) and on the contribution of seafood (relative to other food sources) to meet the requirements of such nutrients during pregnancy. No effect of these nutrients on functional outcomes of children's neurodevelopment is expected when maternal

requirements are met. The health benefits of seafood consumption in reducing the risk of CHD mortality are probably owing to the content of n-3 LCPUFA in seafood.

d) Quantitative benefit analyses of seafood consumption during pregnancy and children’s neurodevelopmental outcomes, and of seafood consumption in adulthood and risk of CHD mortality, have been conducted, but are generally hampered by the heterogeneity of the studies which have investigated such relationships. Such studies differ in the tools used to estimate seafood consumption, in the tools used to measure (or ascertain) the outcomes of interest, and in the adjustment for confounding variables.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The Commission has asked the Scientific Panel on Contaminants in the Food Chain (CONTAM Panel) to issue a scientific opinion on the risk for public health related to the presence of mercury and methylmercury in food. The scientific opinion is currently under preparation[4].

Fish consumption is known to have beneficial effects on human health due to its nutrients, e.g. long chain n-3 polyunsaturated fatty acids that have beneficial effects on the neurodevelopment of children. On the other hand, fish contains methylmercury, the most toxic form of mercury which is known to have adverse effects on children's neurodevelopment. It is therefore important that fish consumption is such that benefits are maximised while risks are minimised.

As a follow up and second step to the question on mercury and methylmercury in food, the Commission therefore asks EFSA to carry out a risk benefit analysis as regards the risks and benefits analysis to human health of fish/seafood consumption related to methylmercury. The risk benefit analysis should fully take into account the information and conclusions drawn in the scientific opinion on mercury and methylmercury in food as well as the conclusions of the Joint FAO/WHO Expert consultation on the risks and benefits from fish consumption on 25-29 January 2010[5]. This will enable the Commission and the Member States to take appropriate risk management action, e.g. to give dietary advice to consumers of fish.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002 the European Commission asks the European Food Safety Authority for a scientific opinion on the risks and benefits of fish/seafood consumption to human health related to methylmercury.

In particular, the opinion should

- address risks and benefits as regards fish/seafood consumption related to relevant beneficial substances (e.g. nutrients such as long-chain n-3 polyunsaturated fatty acids) and the contaminant methylmercury

- address risks and benefits for relevant sub groups of the population (e.g. maternal fish consumption during pregnancy and breastfeeding, infants, children, general adult population, etc.)

The opinion should fully take into account all the findings and conclusions of the EFSA opinion on the risks for public health related to the presence of mercury and methylmercury in food as well as the dietetic benefits of eating fish. The conclusion of the Joint FAO/WHO Expert consultation on the risks and benefits from fish consumption should also be taken into account.

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[4] A Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food was adopted by the CONTAM Panel on 22 November 2012.

[5] WHO TRS 959, Seventy-second report of the Joint FAO/WHO Expert Committee on Food Additives, 16-25 February 2010.

ASSESSMENT

1. Introduction and definition of terms

On the basis of the International Standard Statistical Classification of Aquatic Animals and Plants (ISSCAAP) used by FAO for the purposes of collecting and compiling fishery statistics, the term **seafood** denotes in this Opinion vertebrate and invertebrate aquatic animals whether of marine or freshwater origin, whether farmed or wild (FAO/WHO, 2010), except aquatic mammals (e.g. whales and dolphins), aquatic reptiles (e.g. turtles and crocodiles), echinoderms (e.g. sea urchins and starfish), and jellyfish, and does not include aquatic plants. The Panel is aware that the term seafood has been used to denote only marine animals (and occasionally plants) in other contexts. The term **fish** will be used as a synonym of **finfish** for vertebrates, and the term **shellfish** for invertebrates. **Shellfish** includes crustaceans (e.g. shrimps, crabs and lobsters) and molluscs (bivalves, gastropods and cephalopods).

The terms “seafood”, “fish”, “white fish”, “lean fish”, “oily fish”, “fatty fish” and “shellfish” have been widely used in the scientific literature and by regulatory bodies referring mostly to aquatic animals consumed by humans. However, the use of such terms has been inconsistent and their meaning ill defined.

The terms “fatty fish”, “oily fish”, “lean fish” and “white fish” will be avoided in this Opinion, unless used by others in scientific articles, reports or national recommendations without a clear definition of these terms. In such cases, the terms will be quoted in inverted commas.

Omega-3 polyunsaturated fatty acids (n-3 PUFAs) contain one of the double bonds located at three carbon atoms from the methyl end. The main n-3 PUFAs in the diet are α-linolenic acid (ALA; 18:3Δ9c,12c,15c), eicosapentaenoic acid (EPA; 20:5Δ5c,8c,11c,14c,17c), docosahexaenoic acid (DHA; 22:6Δ4c,7c,10c,13c,16c,19c) and docosapentaenoic acid (DPA; 22:5Δ7c,10c,13c,16c,19c). EPA, DHA and DPA are usually referred to as n-3 LCPUFAs, i.e. n-3 PUFA with 20 or more carbon atoms (EFSA NDA Panel, 2010a, 2012). Therefore, in this opinion, the term n-3 LCPUFA refers to EPA, DHA and DPA, and does not include ALA.

2. Background

The Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) convened a Joint Expert Consultation on the Risks and Benefits of Fish Consumption from 25 to 29 January 2010. The tasks of the Expert Consultation were to review data on levels of nutrients (long-chain omega-3 polyunsaturated fatty acids (n-3 LCPUFAs)) and specific chemical contaminants (including methylmercury) in a range of seafood species and to compare the health benefits of seafood consumption and nutrient intake with the health risks associated with contaminants present in seafood. The Expert Consultation concluded the following in relation to the health benefits of fish consumption and the risks derived from the presence of methylmercury in seafood:

- Consumption of fish provides energy, protein and a range of other important nutrients, including n-3 LCPUFAs.

- Eating fish is part of the cultural traditions of many people. In some populations, seafood is a major source of food and essential nutrients.

- Among the general adult population, consumption of seafood, particularly fatty fish, lowers the risk of mortality from coronary heart disease (CHD). There is an absence of probable or convincing evidence of risk of CHD associated with methylmercury.

- When comparing the benefits of n-3 LCPUFAs consumption with the risks of exposure to methylmercury among women of childbearing age, in most of the circumstances evaluated the risk of suboptimal neurodevelopment in offspring of women consuming seafood is lower than in the offspring of women not eating seafood.

- Among infants, young children and adolescents, the available data are currently insufficient to derive a quantitative framework of the health risks and health benefits of eating seafood.

In 2011, EFSA was asked by the European Commission to consider new developments regarding methylmercury toxicity and to evaluate whether the Joint FAO/WHO Expert Committee on Food Additives (JECFA) Provisional Tolerable Weekly Intake (PTWI) for methylmercury of 1.6 µg/kg body weight (b.w.) was still appropriate[6]. This PTWI was based on neurodevelopmental endpoints from epidemiological studies. The point of departure behind this PTWI was based on the mean of the highest No Observed Effect Levels (NOEL) for prenatal exposure in the Seychelles main cohort (15.3 mg/kg in maternal hair) and the 95 % lower confidence limit of the Benchmark Dose ($BMDL_{05}$) for neurodevelopmental effects at age seven years in the Faroese Cohort 1 (12 mg/kg in maternal hair), giving a point of departure of 14 mg/kg in maternal hair. The EFSA CONTAM Panel (2012) issued a Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food. The EFSA CONTAM Panel established a Tolerable Weekly Intake (TWI) for methylmercury of 1.3 µg/kg b.w., expressed as mercury, which was also based on neurodevelopmental endpoints. The point of departure was calculated from a methylmercury concentration of 11 mg/kg in maternal hair as the apparent NOEL in the Seychelles Child Development Nutrition Study for neurodevelopmental effects at 9 and 30 months of age, which was adjusted for maternal blood concentrations of DHA, and the $BMDL_{05}$ for neurodevelopmental effects at age seven years in the Faroese Cohort 1 (12 mg/kg in maternal hair), giving a point of departure of 11.5 mg/kg in maternal hair. The EFSA CONTAM Panel also noted that evidence for adverse effects of methylmercury on cardiovascular outcomes in adults was inconclusive, although conflicting results from observational studies on the association between exposure to methylmercury and risk of myocardial infarction could possibly be explained by differences in the method used in the studies to adjust for the beneficial effects of n-3 LCPUFA on that outcome (i.e. biomarkers vs. dietary intakes).

Taking into consideration the above, the NDA Panel will:

a) Review the role of seafood in European diets;

b) Evaluate the beneficial effects of seafood consumption in relation to health outcomes and population subgroups previously identified by the FAO/WHO Joint Expert Consultation on the Risks and Benefits of Fish Consumption and/or the CONTAM Panel as relevant for the assessment. These include the effects of seafood consumption during pregnancy on functional outcomes of children's neurodevelopment and the effects of seafood consumption on cardiovascular disease risk in adults;

c) Address which nutrients in seafood may contribute to the beneficial effects of seafood consumption in relation to the above-mentioned outcomes;

d) Consider whether the beneficial effects of seafood consumption in relation to the above-mentioned outcomes can be quantified.

3. Existing dietary guidelines for seafood consumption in Europe

A total of 35 European countries were asked to supply information on their current national recommendations for fish and shellfish consumption through the EFSA focal points[7] using a questionnaire developed for that purpose (**Appendix A**). Answers were received from 21 countries (Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Malta, the Netherlands, Norway, Poland, Romania, Slovakia, Slovenia, Switzerland and the United Kingdom). All countries reported having Food-Based Dietary Guidelines (FBDG) which

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[6] WHO TRS 959, Seventy-second report of the Joint FAO/WHO Expert Committee on Food Additives, 16-25 February 2010.

[7] http://www.efsa.europa.eu/en/fp/fpmembers.htm

have been updated between 2004 and 2012. In the majority of countries, governmental bodies (n = 20) and/or scientific societies (n = 14) were involved in the development of FBDG, while in some countries industrial bodies (n = 3), non-profit organisations (n = 5) or other bodies (n = 4) were also involved. FBDG were specially developed for the specific country in nearly all countries (n = 20), while the WHO Countrywide Integrated Non-communicable Disease Intervention (CINDI) dietary guide was taken as a basis and adapted in one country only. All FBDG but two include recommendations on fish consumption (**Appendix B**).

Recommendations for fish consumption are generally given for the general population, and less often for specific population subgroups, such as infants and children, elderly or pregnant and lactating women. Specific recommendations refer to the amount of fish rather than the type of seafood (fish vs. shellfish) or the species of fish to be consumed.

For adults or the general population, national FBDGs advise a consumption of fish ranging from 100 g per week to 200 g per day, but most recommend two servings of about 150 g each per week. In some countries these recommendations refer to the minimum amounts which should be consumed, whereas in other countries it is unclear whether recommendations refer to minimum or target amounts. When the type of fish to be consumed is specified, most recommendations refer to the consumption of half of the fish as “fatty fish”, while only one FBDG recommends exclusive consumption of “lean cooked fish”.

Only one FBDG contains specific information for infants, and suggests a fish consumption of 10 g per week from seven to nine months of age, and of 20 g per week thereafter. The few FBDG which are addressed to children recommend intakes of 40 g, 50 g, and two servings of 100 g of fish per week for children aged one year, two to six years, and older than six years, respectively. For children and adolescents, recommendations for fish consumption range from 100 to 300 g per week, of which no more than 100 g per week of large carnivorous fish.

Some countries give special recommendations for pregnant and lactating women, taking into account concentrations of contaminants in certain types of seafood. The recommendations are to prefer seafood low in pollutants (e.g. trout, ocean perch, cisco, sardine, white halibut, salmon, mackerel, herring, sprats, anchovies, carp and prawns); not to eat swordfish, dogfish, marlin, shark, and ray; and to consume at most one serving of fresh tuna or pike per week, and a maximum of four servings of canned tuna per week. One FBDG also recommends avoiding fish from the Baltic Sea.

Diet-related health problems considered in the development of the recommendations for fish consumption are primarily cardiovascular diseases and related disorders, such as dyslipidaemia, hypertension and obesity, followed by pregnancy outcomes and cancer. Other diet-related health problems are mentioned in FBDG only by a few countries. The main purpose of the recommendations for fish consumption is to ensure the provision of key nutrients, especially n-3 LCPUFA, as specified in nearly all of the FBDG, and also vitamin D, iodine and selenium, among others. In some FBDG, fish consumption is also recommended in order to replace foods or nutrients with putative adverse health effects if consumed in excess, such as saturated fat and cholesterol.

About half the FBDG on fish consumption address safety aspects, for example hazards related to contamination with bacteria (*Listeria*, *Clostridium*) and related toxins and/or with chemicals, namely heavy metals (mercury, methylmercury), pesticides, dioxins, furans, polychlorinated biphenyls and brominated flame retardants.

The Panel notes that FBDG from 19 out of 21 European countries which answered to the questionnaire include dietary recommendations for fish consumption. The recommended intakes for adults range from 100 g per week to 200 g per day, but most countries recommend (a minimum of) two servings of about 150 g per week. FBDG relative to the type and amount of fish to be consumed were not only based on health benefits, but also on risks to health. The main purpose of the recommendations was to ensure the provision of key nutrients, especially n-3 LCPUFA, but also vitamin D, iodine and selenium.

4. Dietary intakes

4.1. Methodological considerations

4.1.1. Comprehensive European Food Consumption Database

The EFSA Comprehensive European Food Consumption Database (EFSA, 2011), which was built from existing national information on food consumption at a detailed level in order to estimate dietary exposure for risk assessment, has been used to calculate dietary intakes of fish, crustaceans and molluscs in this Opinion[8].

Briefly, summary statistics from the Comprehensive Database for seafood consumption have been calculated using data from 28 dietary surveys carried out in 17 EU Member States concerning different age groups, as shown in **Appendices C and D**. Surveys with only one observation day per subject or which used food frequency questionnaires (FFQ) for data collection have been excluded. In the majority of surveys either three-day dietary records (11 surveys) or two 24-hour dietary recalls (nine surveys) were used for data collection. However, in some countries (Denmark, France, UK, Ireland and Sweden), 7-day dietary records were used. Only two surveys (in Bulgaria and Italy) collected data on infants.

The food classification system used in the Comprehensive Database (FoodEx 1) is a hierarchical system in which the food category “fish and other seafood (including amphibians, reptiles, snails and insects)” at Level 1 is further divided into the subgroups “fish and other seafood (including amphibians, reptiles, snails and insects) (unspecified)”, “fish meat”, “fish products”, “fish offal”, “crustaceans”, “water molluscs”, and “amphibians, reptiles, snails, insects” at Level 2. “fish oil” at Level 2 is one out of six subgroups under “animal and vegetable fats and oils” at Level 1, and “Fish and seafood based meals” at Level 2 is one out of the 12 subgroups under “composite food (including frozen products)” at Level 1. Since most data providers were able to codify the large majority of foods at least at Level 2, the amounts consumed under “fish and other seafood (including amphibians, reptiles, snails and insects) (unspecified)” are negligible. Level 3 identifies 25 species of fish under “fish meat”, seven species under “crustaceans” and 10 species under “water molluscs”. Whenever fish species were not reported by the data providers or whenever the species reported could not be found, the upper category at Level 2 (e.g. “Fish meat (unspecified)”) was chosen. “fish products” include “fish balls”, “fish fingers”, “fish paste”, “fish pâté”, and “fishcakes”.

4.1.2. European Nutrient Composition Database

EFSA’s Nutrient Composition Database has been used to calculate nutrient intakes from seafood in selected European countries. The nutrient database was constructed with data collected through a procurement project CFT/EFSA/DCM/2011/03 (Roe et al., 2013) and contains nutrient composition information for about 2 600 food items based on the FoodEx 2 food classification system (EFSA, 2011). In this project, national food composition database compilers mapped food codes in their published food composition datasets to EFSA FoodEx 2 codes using guidelines agreed by the project and EFSA. Where possible, codes were matched exactly and close matches were used when no exact match was available. To ensure that a complete nutrient dataset was provided, values were borrowed from another country when data were not available in a national database. Nutrient information was provided for over 100 nutrients.

For the calculation of energy intakes and intakes of protein, fat, n-3 LCPUFA (EPA and DHA), vitamin D, calcium, iodine, selenium, zinc from seafood, the average nutrient content for fish, crustaceans and molluscs codes appearing in the EFSA Comprehensive Food Consumption Database was compiled. Nutrient values were retrieved for 45 food terms, including 20 fish species, six “Fish products”, five crustacean species, nine water mollusc species and five averaged upper level food categories (i.e. seafood, fish meat (unspecified), fish products, crustaceans and water molluscs) appearing in the Comprehensive Food Consumption Database (FoodEx 1 Level 2). Some missing

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[8] http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb.htm

values mainly for n-3 LCPUFA and selenium were filled in by borrowing nutrient values from similar seafood species or the average value of a group. Nutrient composition information was used as unit/100 g of raw food, as information on food processing in the Comprehensive Food Consumption Database is limited.

Nutrient intakes from fish and other seafood were calculated by multiplying food consumption of a particular species as g/day by the nutrient composition of the corresponding species or group as unit/100 g. Nutrient intakes were calculated both as means for the whole population group and as medians for consumers only.

4.1.3. Limitations of the data used to calculate nutrient intakes from seafood

A high degree of intra-species (analytical and physiological) variability for all nutrients (except protein) has been reported for seafood. Sources of physiological variability in the nutrient content of the same species, particularly in relation to the amount and type of fat, include geographical region, season, life stage, origin (sea vs. freshwater; farmed vs. wild), eating habits or feeding practices, and sampling protocols (EFSA, 2005). For example, the total fat content is up to 50 % higher in farmed than in wild specimens of some fish species (e.g. salmon), whereas no differences are observed in other species (e.g. trout). As for wild fish, total fat content is related to feed availability, which in turn depends on the season (more in summer, less in winter). Similarly, the state of maturation strongly determines fat distribution of wild and farmed fish: fat accumulates in tissues and organs at early stages and is then transferred to the eggs prior to spawning. Even the distribution of adipose tissue in fish fillets is not uniform: in general it decreases from head to tail and from dorsal to ventral, being higher below the skin and in red muscle (EFSA, 2005).

The fatty acid profile is sensitive to water temperature (unsaturated fatty acids increase as water temperature decreases to maintain membrane fluidity) and dietary lipids. In farmed fish, the fatty acid composition of phospholipids and triglycerides closely mimics the fatty acid composition of the feed as long as minimum requirements of the LCPUFA EPA, DHA and arachidonic acid (ARA) are met. Food processing and cooking habits also influence the nutritional value of fish as eaten, as well as whether bones of small fishes are eaten or not. For example, whereas much of the fat (and n-3 LCPUFA) of fresh tuna is lost during the canning process, frying may significantly increase the total amount of fat that is consumed with fish, and may eventually modify the fatty acid profile.

There are a number of uncertainties in relation to the nutrient intake data from fish and other seafood calculated using the available EFSA food consumption and nutrient composition databases. Firstly, different levels of detail were provided by different surveys about seafood consumption, and the list of fish species under the “fish meat” category in the Comprehensive database was unable to accommodate all species consumed, which were then recorded as “unspecified”. In addition, FoodEx 1 does not differentiate between processed and unprocessed fish (e.g. canned vs. fresh). Regarding the nutrient composition database, the Panel notes that no information is available about the region where the fish was harvested or caught, the sampling season, the stage of maturation of the fish, the anatomical area or the number of samples analysed, or the analytical methods used.

4.2. Dietary intakes of fish and other seafood

Table 1 summarises dietary intakes of fish and other seafood by age group in European populations. Intakes of “amphibians, reptiles, snails, insects” (considered irrelevant for this Opinion), intakes of “fish offal”, “fish oil”, and “fish and seafood based meals”, and intakes of any fish category by infants, which were negligible, are not reported. **Appendix E** provides details on mean intakes of fish and other seafood by country and age group, as well as the number of subjects included in each survey by population subgroup.

Table 1: Range of mean intakes of "fish and other seafood" by age group in European countries.

	Lowest mean intakes – highest mean intakes (g/day)					
Fish category	**Toddlers (12-35 mo)**	**Other children (36 mo-9 y)**	**Adolescents (10-17 y)**	**Adults (18-64 y)**	**Elderly (65-74 y)**	**Very elderly (≥ 75 y)**
Fish meat	1.2-29	2.2-30.8	4.4-36.4	4.8-47.7	19.7-35.5	18.3-26.3
Fish products	1.9-2.6	1.0-7.4	2.0-7.4	0.6-5.3	0.5-2.5	0.8-1.6
Crustaceans	-	0.2-2.4	0.7-5.7	0.6-5.2	0.2-2.5	1.9-2.0
Molluscs	-	0.6-8.8	1.1-13.8	0.1-12.0	2.4-8	1.6-4

Intakes of "fish meat" showed greater variability among countries and were generally higher than intakes of any other category of seafood. The highest mean consumption of fish and other seafood was reported in Italy and Spain for almost all age groups and included all seafood categories, except for (manufactured) fish products, for which mean intakes were highest in Sweden (adolescents and adults), France (elderly) and Germany (very elderly).

Appendix F lists median intakes of fish and other seafood, the 5th (P5) and 95th (P95) percentiles by country and age group in consumers only. Consumers are defined as subjects reporting consumption within a certain category on at least one survey day. The Panel notes that this approach may underestimate the proportion of occasional consumers in the general population (i.e. consumers not having seafood on survey days) in countries using two or three sampling days for data collection, compared to countries (Denmark, France, UK, Ireland and Sweden) where 7-day dietary records were obtained. Dietary intakes of fish and other seafood by age group in consumers only are summarised in Table 2.

Table 2: Range of percentages of seafood consumers and range of median dietary intakes of "fish and other seafood" in consumers by age group.

	Toddlers (12-35 mo)	**Other children (36 mo-9 y)**	**Adolescents (10-17 y)**	**Adults (18-64 y)**	**Elderly (65-74 y)**	**Very elderly (≥ 75 y)**
Consumers (%)	13.7-63.9	15.5-88.0	11.9-84.8	12.8-90.2	9.7-91.9	8.8-95.0
	Lowest median intakes – highest median intakes (g/day)					
Fish category	**Toddlers**	**Other children**	**Adolescents**	**Adults**	**Elderly**	**Very elderly**
Fish meat	9.1-50.0	9.7-50.0	9.2-66.3	13.4-75.0	20.0-72.5	19.0-59.4
Fish products	17.3-26.1	18.8-27.8	14.3-56.3	13.6-72.5	11.5-67.5	16.5-54.0
Crustaceans	-	3.2-7.8	0.6-35.7	1.7-30.0	2.3-20.0	1.3-31.2
Molluscs	-	15.0-25.0	8.6-45.9	8.3-44.6	15.4-80.0	10.0-41.9

The percentage of infants consuming any seafood was very low in Bulgaria (1.7 %). The Italian survey was too small to provide reliable data for this population subgroup (**Appendix F**). The percentage of toddlers consuming crustaceans, molluscs and "fish products" was negligible in all countries, whereas toddler consumers of "fish meat" ranged from 7.1 % in the Netherlands to 55.6 % in Italy, where median "fish meat" consumption in toddler consumers (50 g/d) was about five times higher than in any other country except Bulgaria (twice as high).

For children and adolescents, Denmark (7-day food records), Finland, France, Spain and Italy reported the highest percentages of "fish meat" consumers (around 50-80 %). However, median intakes of "fish meat" in consumers from these countries varied considerably (from 10-15 g/day in Denmark, Finland and France to around 50-60 g/day in Spain and Italy). In the remaining countries, "fish meat" was consumed by 7-30 % of children and adolescents, with median intakes around 15-60 g/day and a tendency to higher intakes with increasing age. A substantial amount of crustacean consumers among children and adolescents (around 19-34 %) was reported for Denmark, France and Spain, but generally in low amounts (median < 13 g/day). Molluscs were a relevant food among children and adolescents in

Italy, Sweden and Belgium (children only), with around 20-30 % of consumers and median intakes of 25-40 g/day.

Median consumption of “fish meat” in adult consumers was more consistent throughout surveys, with around half of the surveys reporting intakes of 50-60 g/day. The highest median “fish meat” consumption was reported for the Czech Republic at 75 g/day and the lowest for Denmark at 13.4 g/day. The percentage of “fish meat” consumers in this population ranged from 12 % in the Netherlands to 85 % in Denmark. Crustaceans were consumed by a relevant percentage of the population (18-50 %) in Denmark, France, Spain, Sweden and the UK, with median intakes in consumers of between 1.7 g/day in Denmark and 17 g/day in Sweden. Molluscs were of some relevance in adults (around 17-42 % consumers) in France, Italy and Spain, with a median consumption ranging from 11 g/day (France) to 45 g/day (Italy).

In the population of elderly and very elderly, the countries with the highest percentage of consumers of “fish meat” and crustaceans were France and Denmark (80-95 % for “fish meat” and 20-55 % for crustaceans), with a median intake in consumers of 20-30 g/day for “fish meat” and 1-4 g/day for crustaceans. Countries with lower percentages of consumers usually reported higher median intakes (50-70 g/day for “fish meat” and around 20 g/day for crustaceans) for consumers. Molluscs were consumed by 20 % of the population of elderly and very elderly in France and Italy, with median intakes of 10-40 g/day. Median intakes in Belgium (3 % of consumers) reached 40-80 g/day. However, the number of surveys available for this age group was limited and the data given may not be able to depict the variability of seafood intakes across European countries in this population group.

Available data for adults (15 surveys from 14 countries) with respect to the species of seafood consumed in different European countries is summarised in **Appendices G and H**. The information available for seafood consumption was variable between surveys: from 1 % (Denmark) to 86 % (Sweden) of the fish consumed and 1 % (Belgium) to 100 % (Czech Republic) of crustaceans consumed could not be attributed to a particular species. Conversely, only up to 2 % of mollusc consumption could not be attributed to a particular species in the 11 countries reporting mollusc intakes.

Some species of fish were consumed in almost all 14 countries (cod and whiting, herring, salmon and trout, tuna) although their relative contribution to total fish consumption was variable. These species combined accounted for one third to three fourths of total fish consumption in all countries. Three countries (Czech Republic, Latvia, and Sweden) with about 50 % or more of fish consumption unspecified were not considered. Anchovies and mackerel were widely consumed (in 10 and 13 countries, respectively), but their contribution to total fish intake was low (< 8 %). Bass, halibut, lophiiformes, rays, sprat and whitefish were consumed in four countries or less and never represented more than 8 % of total fish intake in any country (generally < 5 %). Carp, hake and plaice were consumed in more than five countries and, in at least one of them, each accounted for ≥ 17 % of total fish intake (up to 29 %). The two surveys conducted in Spain were in fair agreement regarding the fish species consumed and their contribution to total fish intake.

Consumption of crustaceans and molluscs was even more variable among countries. In five countries (Italy, France, Denmark, Latvia, and Finland), shrimps contributed > 80 % of total intake of crustaceans. Prawns accounted for 84 % of the total in Ireland and 100 % of crustaceans consumed in Hungary were crayfish. Lobsters (*Homarus vulgaris* or *Nephrophs norvegicus*) and crabs were hardly consumed in eight countries each, except lobsters in Spain (20-30 % of all crustaceans). Only 11 (out of 14) countries reported consumption of molluscs. Mussels were the molluscs more widely consumed (10 countries), followed by squid (9 countries) and scallops (7 countries). Mussels corresponded to more than three quarters of mollusc consumption in three countries (the Netherlands, Finland, Belgium). In Italy, France and the UK, no single species of molluscs accounted for more than 35 % of total consumption, whereas in Denmark, Finland and the Netherlands, single species accounted for about 80 % of the total or more.

The data presented suggest a large variation in the amount of fish and other seafood consumed across European countries and age groups, as well as in the type of seafood and species eaten. Methodological differences among the surveys used as sources of data (e.g. sampling, size, methods used for data collection) may account for part of the variation. The Panel also notes that the type of seafood consumed in some European countries is largely unknown, and that many reasons could account for this fact (e.g. underreporting by consumers, question not included in dietary surveys, some fish species identified by consumers not present in the EFSA database). Fish intake data are particularly scarce for infants.

4.3. Nutrient intakes from seafood

4.3.1. Nutrient composition

The nutrient composition of seafood varies widely from one type to another (fish vs. crustaceans or molluscs), and among fish species, with the exception of protein. The nutrient content of the species of fish, crustaceans and molluscs mostly consumed in Europe, as extracted from the nutrient composition database of EFSA, is shown in **Appendices I and J**.

Fish contains significant amounts of all amino acids, is a good source of B vitamins, and the species that are high in fat are usually rich in n-3 LCPUFA and the fat soluble vitamins A and D. As for minerals and trace elements, fish is a valuable source of iodine, selenium, zinc, calcium, phosphorus, iron and copper.

The nutrient content of fish varies greatly among species. In the fish species most commonly consumed in Europe, mean n-3 LCPUFA content varies from 200 mg/100 g (cod and whiting) to 2 500 mg/100 g (herring and tuna). Also Atlantic salmon provides n-3 LCPUFA in high amounts (1 800 mg/100 g). The most consumed freshwater fish, i.e. carp and trout, have an n-3 LCPUFA content of around 300 and 600 mg/100 g, respectively. Vitamin D concentrations in different fish species also vary widely, from around 0.5-2 µg/100 g in carp, hake, mackerel and plaice to around 10-18 µg/100 g in trout, anchovies and herring. Calcium concentrations have been reported to be between 15-20 mg/100 g in Atlantic salmon, cod and tuna and 100-135 mg/100 g in anchovy and herring. Iodine content in the commonly consumed freshwater fish species (carp, trout) is lower (around 2-12 µg/100 g) than in sea fish (30-160 µg/100 g), with the highest concentrations observed in cod (160 µg/100 g) and hake and mackerel (110 µg/100 g each). Concentrations of selenium and zinc are less variable among fish species. Mean selenium concentrations have been reported to be between 21 µg/100 g (trout) and 75 µg/100 g (tuna), with most values falling between 25 and 30 µg/100 g. Mean zinc concentrations of different species are mostly in the range of 0.3-0.7 mg/100 g with the highest concentrations (1.1 mg and 2.2 mg/100 g) reported for herring and anchovy, respectively. The Panel notes that these figures are average values of nutrients for one fish species, and that the variability between the different samples analysed for the same species is high (up to four-fold, in rare cases greater than four-fold).

The nutrient content among the different species of crustaceans and molluscs consumed mostly in Europe varies less than among different fish species. Concentrations of n-3 LCPUFA in crustaceans are in the range of 370-520 mg/100 g, with the exception of crayfish (60 mg/100 g), and are lower in molluscs (160-350 mg/100 g). Vitamin D concentrations in crustaceans and molluscs are low (0-0.5 µg/100 g) except for clams and mussels (around 5 µg/100 g). Calcium concentrations in both crustaceans and molluscs are about 30-100 mg/100 g. Iodine varies greatly both among crustaceans and among molluscs. In crustaceans, the highest iodine concentrations have been reported for lobster (around 360 µg/100 g) and the lowest for crayfish (around 65 µg/100 g). In molluscs, the iodine content of squid, octopus and cuttlefish has been reported at 20 µg/100 g, and that of clams and mussels at 120-140 µg/100 g. As for fish, selenium and zinc concentrations are less variable among species, with a range of 20-75 µg/100 g for crustaceans and of 50-65 µg/100 g for molluscs for selenium and of 1.4-2.5 mg/100 g for zinc (for both crustaceans and molluscs) except for crab, which has higher zinc content (6.5 mg/100 g). Variability was high for the species and nutrients for which this information was available.

4.3.2. Nutrient intakes

Harmonised nutrient intake data from seafood for adults in the five European countries (Denmark, Hungary, Italy, Spain, UK) with the highest percentage of seafood consumed specified at species level in dietary surveys are shown in **Appendix K**. Geographical balance and a wide range of seafood intakes were also considered for the selection of these countries in order to illustrate the variability of nutrient intakes from seafood across Europe. Dietary surveys were conducted between 2001 and 2006 and intake data were estimated using three- or seven-day dietary or food records (**Appendix C**).

Harmonised nutrient intake data from the whole diet for these countries are not available at present, and therefore the contribution of seafood to nutrient intakes within these countries cannot be calculated. Alternatively, Dietary Reference Values (DRVs) for the European population have been used to depict the importance of seafood in the diet as a source of nutrients (**Appendix K**).

As expected, mean intakes of energy and of some essential nutrients (i.e. protein, fat, n-3 LCPUFA, vitamin D, calcium, iodine, selenium and zinc) from seafood were generally higher in countries with the highest intakes of seafood, like Spain or Italy. Seafood covered 100 % of the DRV for n-3 LCPUFA in all countries but Hungary (about 50 %), where mean intakes of seafood were very low (8.8 g per day). Mean intakes of calcium and zinc from seafood relative to the DRV for these nutrients were low in all countries, whereas mean intakes of vitamin D, iodine and selenium varied widely depending on the amounts of seafood consumed and accounted for > 50 % of the DRV only in Spain, where mean intakes of seafood were highest. This picture did not change significantly by considering nutrient intakes from seafood among consumers only, except for Hungary, where median intakes of n-3 LCPUFA were beyond the DRV. The Panel notes that seafood is an important dietary source of n-3 LCPUFA and provides the recommended amounts of n-3 LCPUFA in most of the European countries considered. The Panel also notes that seafood significantly contributes to the needs of other essential nutrients, such as vitamin D, iodine or selenium, in some countries.

5. Seafood as source of essential nutrients

Seafood is a source of energy and protein with high biological value and contributes to the intake of essential nutrients, such as iodine, selenium, calcium, and vitamins A and D. Although such nutrients may be obtained from other dietary sources, seafood may become the main contributor to vitamin D intake and therefore status when endogenous synthesis is low (i.e. limited exposure to UV-B radiation), and also to iodine intake and status. Seafood also provides n-3 LCPUFA, which, in contrast to other nutrients, are obtained mainly from seafood.

6. Seafood consumption and dietary patterns

The identification of dietary patterns has been proposed as a necessary step in the development of FBDG (EFSA NDA Panel, 2010b) taking into account that the health benefits associated with some diets in epidemiological studies cannot be attributed to a single food or nutrient.

6.1. Methodological considerations

Briefly, two types of methods have been used to study dietary patterns (EFSA NDA Panel, 2010b).

The *a priori* approach is based on prevailing knowledge concerning favourable or adverse effects of various dietary constituents. Diets are assessed for the presence or absence of certain foods or nutrients, and the results are converted into a score.

The *a posteriori* approach is data driven, and exposure is summarised using factor or cluster analysis. Factor analysis may be considered as a pattern detection method that reduces the number of dietary variables by transforming an original large set of correlated variables into a smaller set of uncorrelated variables, which are called principal components or factors. The proportion of the total variance in food intakes explained by the principal components may vary within a large range, for example, from < 20 % to > 50 %, depending on the choices made. Cluster analysis classifies persons into mutually exclusive groups on the basis of a similarity in reported food intakes. The number of clusters (generally from two to six) that are retained for the description of the dietary patterns and the proportion of the total variance explained by these patterns depends on the choices made (EFSA NDA Panel, 2010b).

6.2. Associations among dietary patterns, health outcomes and other factors

"Healthy dietary patterns" usually include seafood. Irrespective of the method used to derive them, dietary patterns characterised by the consumption of fruit, vegetables, whole grain, seafood, and poultry have been associated with selected biomarkers of health and disease risk in the expected direction. Examples of health/disease outcomes include total mortality, cardiovascular disease (CHD, stroke), metabolic syndrome, diabetes, cognitive function, and Alzheimer disease (Kant, 2004; Mente et al., 2009; Kastorini et al., 2011; Smithers et al., 2011; Sherzai et al., 2012; Wirfalt et al., 2013). In studies investigating associations between dietary patterns (mostly the "Mediterranean diet") and disease risk, the association between individual components of the pattern and health outcomes was much weaker than that of the overall dietary pattern (Jacques and Tucker, 2001; Sanchez-Villegas et al., 2009; Trichopoulou et al., 2009).

"Healthy dietary patterns" have also been associated with other dietary (e.g. higher intakes of essential nutrients, lower intakes of nutrients that if consumed in excess can be detrimental for health) and non-dietary (e.g. higher socioeconomic status, higher education, being married, higher level of physical activity, less smoking) factors which could have a positive impact on health.

6.3. Conclusion

Seafood is a component of dietary patterns associated with good health. However, studies investigating the relationship between dietary patterns and health do not allow conclusions to be made on the beneficial effects of an individual component of that pattern, for example seafood *per se,* or to quantify the contribution of a component in a given pattern, such as seafood, to health outcomes.

7. Health benefits of seafood consumption on functional outcomes of children's neurodevelopment

7.1. Role of nutrients in seafood

Among the essential nutrients contained in seafood in substantial amounts, DHA and iodine have a well-established role in the development of the central nervous system (CNS) of the foetus during pregnancy (EFSA NDA Panel, 2010a, 2014).

DHA accumulates rapidly in the brain during the third trimester of pregnancy and after birth (Clandinin et al., 1980a, 1980b). In the normal term infant, total DHA content of the body is about 3.8 g (Cunnane et al., 2000) and n-3 PUFA accretion during the last trimester has been estimated to be 34.1 mg/kg b.w. per day, of which most is DHA (Lapillonne and Jensen, 2009). The growth spurt of the brain starts in the 28th week of gestation and continues to one year, whilst the demand for DHA continues to two years of age (Martinez, 1992, 1994). Whole body and brain DHA accumulation may be limited by DHA availability due to a low maternal DHA status. In addition, recent data indicate that there is inter-individual variation in the ability to convert the precursor ALA to n-3 LCPUFA and particularly to DHA, which is related to common polymorphisms in the human Δ-5 and Δ-6 desaturase genes $FADS_1$ and $FADS_2$ (Schaeffer et al., 2006). DHA is preferentially transferred across the human placenta to the foetus mediated by specific transfer proteins (Larque et al., 2003; Larque et al., 2006). Adequate intakes of DHA between 100 and 200 mg per day have been estimated for pregnant and lactating women in order to accommodate the needs of their infants for deposition of DHA in the brain and retina during the last trimester of pregnancy and during breastfeeding (EFSA NDA Panel, 2010a). DRVs for DHA during pregnancy have been set on the basis of structural requirements (rate of DHA accumulation in the brain) and not based on requirements in relation to children's neurodevelopmental outcomes.

Maternal iodine deficiency during pregnancy results in foetal iodine deficiency, which impairs early brain development with consequent physical and mental retardation and lower cognitive and motor performance in later life (Zimmermann, 2012). Adequate intakes of iodine (200 µg/day) have been estimated for pregnant and lactating women in order to accommodate the increased requirements for iodine (50 µg/day) during pregnancy and lactation (EFSA NDA Panel, 2014). In geographical areas where iodine intakes from other foods are insufficient, seafood consumption during pregnancy may have a major role in the development of the CNS of the foetus. DRVs for iodine during pregnancy have been set on the basis of increased maternal requirements for that nutrient, and not on the basis of requirements in relation to children's neurodevelopmental outcomes.

7.2. Methodological considerations

7.2.1. Type of studies considered

The Panel considers that the available evidence on the health benefits of seafood consumption during pregnancy in relation to children's neurodevelopment consists of observational studies in which such relationship has been investigated by adjusting for relevant confounding variables, i.e. factors with an impact on neurodevelopmental outcomes independently of seafood intakes or factors associated with seafood intakes with no impact on neurodevelopment. The Panel also considers that such studies provide information about the relationship between seafood consumption *per se*, including nutrients and non-nutrients (e.g. contaminants such as methylmercury) contained in seafood, and neurodevelopment. Biomarkers of n-3 LCPUFA (mostly DHA) in maternal samples obtained during pregnancy or at delivery, and biomarkers of maternal iodine status, have also been investigated in relation to children's neurodevelopmental outcomes in some of these observational studies.

In addition, and taking into account that n-3 LCPUFAs are obtained mainly from seafood, the Panel will review studies which have explored the relationship between n-3 LCPUFA and neurodevelopmental outcomes but have not reported on seafood intakes during pregnancy. These include: (a) observational studies which report n-3 LCPUFA intakes during pregnancy calculated from seafood intakes, but do not report on seafood intakes as such; (b) observational studies which report on biomarkers of maternal or umbilical cord blood n-3 LCPUFA (mostly DHA), but not on seafood intakes; (c) intervention studies which investigate the effect of n-3 LCPUFA (mostly DHA) supplementation from all sources (e.g. DHA in fish oil, algal oils, egg phospholipids) during pregnancy on neurodevelopmental outcomes in infants and children. The Panel notes that the impact of seafood consumption during pregnancy on neurodevelopment may not be limited to its content of n-3 LCPUFA, and that any health effects of n-3 LCPUFA supplementation cannot be extrapolated to all types of seafood.

Summary publications (systematic reviews and meta-analyses) will be used to describe the data available whenever possible.

7.2.2. Estimates of seafood consumption

With few exceptions, observational studies on the association between seafood consumption during pregnancy and neurodevelopmental outcomes have used validated semi-quantitative FFQs to retrieve data on fish (and often shellfish) consumption during pregnancy. In some cases, the questions on seafood consumption have been reported in the publication. For data analysis, total seafood intakes and/or fish intakes, and occasionally intakes of "white fish", "oily fish" and "shellfish", have been expressed as number of eating occasions, number of servings and/or number of grams per day/week/month. Thereafter, the study population has been divided into categories of fish/seafood consumption according to: (a) quartiles or quintiles of intake; (b) predefined categories according to US recommendations of 2 servings of seafood per week during pregnancy (i.e. no seafood; > 0 to ≤ 2 servings or 1-340 grams per week; > 2 servings or > 340 grams per week); or (c) fixed categories also taking into account the distribution of intakes in the study population. In a few cases, seafood consumption has been considered as a continuous variable in data analysis. The Panel notes that using pre-defined categories of seafood intake to explore the association between seafood consumption and health outcomes may lead to largely uneven groups in the extreme categories of intake.

7.2.3. Biomarkers of n-3 LCPUFA

As an alternative to self-reported dietary intakes of seafood or n-3 LCPUFAs, which are prone to measurement errors and recall or reporting bias, neurodevelopmental outcomes have been explored in relation to various biomarkers of maternal n-3 LCPUFAs during pregnancy or at delivery. These include n-3 LCPUFA measurements in total plasma, plasma phospholipids, red blood cell (RBC) membranes, and in colostrum, and are expressed as a percentage of the entire fatty acid profile. Biomarkers of n-3 LCPUFAs have also been measured in umbilical cord blood at delivery in some studies. These markers have the advantage of being objective measurements of potential foetal accessibility to n-3 LCPUFA, and unlike dietary estimates of n-3 LCPUFA intake they are not subject to recall or reporting bias. However, they do not only reflect intakes of n-3 LCPUFAs during pregnancy, but also their absorption, metabolism and incorporation into plasma fractions, cells or tissues (which is determined by genetic background and health/disease status), as well as the intakes of other fatty acids in the diet. In addition, at the end of pregnancy, a general decline in n-3 LCPUFAs takes place in the maternal compartment, which is largely independent of differences in dietary habits and ethnic origin (Otto et al., 1997), together with a progressive transfer of maternal n-3 LCPUFAs to the umbilical cord and then the infant’s blood (Agostoni et al., 2011). Thus, markers of maternal or cord blood n-3 LCPUFA may reflect only in part intakes of seafood during pregnancy (Silva et al., 2014).

7.2.4. Children’s neurodevelopmental outcomes

Assessment of neurodevelopment in children from birth to adolescence is complex because of the broad range of developmental domains which include neurological and brain function, cognition (memory, attention, learning, intelligence, language, problem solving), visual function, motor skills, temperament, and mental health. Different assessments exist within each domain, and there is wide variation in performance measures and psychometric characteristics. For example, assessments of visual function include both behavioural and electrophysiological measures of acuity determined by discrimination of visual angle or stereoacuity, as well as recordings of electrical responses in the retina and visual cortex.

Test characteristics vary considerably with children’s age. Standardised age-normed tests for assessing infant development (e.g. the Bayley Scales of Infant Development (BSID)) measure the timely achievement of developmental milestones, but provide only a crude and global assessment of development which is not comparable to childhood measures of intelligence (e.g. intelligence quotient (IQ)). Other infant tests measure specific abilities such as speed of processing, attention, problem solving and working memory, but most are not standardised and age-normed, and for most there is no agreed procedure for administering the test. These factors make it difficult to interpret and compare the results from different studies, especially when assessments have been conducted at different ages.

A greater proportion of tests for older children are standardised and age-normed, and many provide information about both global and specific abilities. For example, the McCarthy Scales of Children’s Abilities provide a global measure of intelligence (General Cognitive Index), as well as specific measures of verbal, perceptual-performance, quantitative memory, and motor abilities. The use of standardised and age-normed instruments permits meaningful comparisons between studies using different assessments, especially when the tests are administered at different ages. Some tests administered by trained staff provide relatively objective measures, whereas other tests are questionnaires completed by parents, and therefore subject to bias and error. Many tests are available in shortened versions which provide measures of neurodevelopment derived from a reduced number of test items, and are therefore less sensitive compared to the full version. Caution must be taken when comparing the results obtained by these different types of test.

The Panel notes that there is currently no consensus as to what are the most appropriate and sensitive tests for assessing neurodevelopment in infants and children. Studies have therefore included assessments from a range of possible neurodevelopmental outcomes, and across a span of ages which vary from newborns to adolescents.

7.2.5. Other methodological considerations

In observational studies on the association between maternal seafood consumption (or biomarkers of maternal or cord blood n-3 LCPUFA) and children's neurodevelopmental outcomes, statistical analyses have been adjusted for a number of potentially confounding variables (range: 2 to 28). The majority include a measure of maternal education (e.g. number of years in education, highest educational qualification) as an estimate of maternal intelligence and socioeconomic status. Some studies also include paternal education, but few studies include a direct measure of maternal IQ. The majority of studies also include a measure of socio-economic status, such as parental income, social class based on employment, or type of housing. Other variables frequently included are maternal smoking and alcohol use in pregnancy, duration of breast-feeding, duration of gestation, infant sex, birth weight, and child age at test administration. Very few studies have included post-natal maternal and child seafood consumption, or incidence of *post-partum* depression or maternal mental health problems.

Statistical models to explore the relationship between biomarkers of n-3 LCPUFA (and more rarely seafood consumption) and children's neurodevelopmental outcomes have also been occasionally adjusted for variables that could have influenced children's neurodevelopmental outcomes but which are not independent of seafood intakes. Such variables include concentrations of contaminants (including methylmercury) in maternal or umbilical cord blood. Whenever available, only outcomes from statistical models which did not consider such variables will be reported below.

7.3. Observational studies on seafood consumption during pregnancy

A number of prospective cohort studies have examined the relationship between seafood consumption in pregnancy and measures of neurodevelopment in childhood. The majority of the studies have addressed multiple outcomes, either in the main cohort or in subsets of the main cohort, and these have been reported separately in different publications.

7.3.1. Prospective cohort studies conducted in Europe

The Avon Longitudinal Study of Parents and Children study

The Avon Longitudinal Study of Parents and Children (ALSPAC) study recruited expectant mothers residing in Bristol (UK) and surrounding areas with an expected delivery date between April 1991 and December 1992. Of 14 541 pregnancies, 13 988 children survived for at least 12 months. About 85 % of eligible expectant mothers participated. A total of 11 875 pregnant women completed a semi-quantitative FFQ with questions on 43 different foods at 32 weeks of gestation, which included three questions to assess seafood consumption, namely the number of times they currently consumed: (a) "white fish" (cod, haddock, plaice, fish fingers, etc.), (b) "dark or oily fish" (tuna, sardines, pilchards, mackerel, herring, kippers, trout, salmon, etc.), and (c) shellfish (prawns, crabs, cockles, mussels, etc.). During the study period, no formula milks supplemented with DHA were commercially available in the UK. Developmental outcomes (including behaviour, cognitive and motor development) were assessed at ages 6, 15, 18, 30, 42, 81 months and 8 years (Daniels et al., 2004; Hibbeln et al., 2007; Steer et al., 2013), whereas visual outcomes were assessed at 3.5 years (Williams et al., 2001).

Out of the 10 092 singleton, term (≥ 37 completed weeks of gestation) children whose mothers answered questionnaires throughout pregnancy and developmental assessments for their children at 15 and 18 months, complete datasets were available for 7 421 children (Daniels et al., 2004). Maternal fish intake combining "oily" and "white" fish (shellfish were not considered) was classified as rarely or never, once in 2 weeks, 1-3 times per week, and ≥ 4 times per week. A serving size of 127.6 g was used to calculate the median amount of fish consumed per week, and resulted in an ordinal variable with the values 0, 63.8, 255.1 and 510.3 g per week. Eighty-eight percent of the women in this cohort ate fish during pregnancy, 80 % of which ate fish at least once per week and 65 % ate both "white" and "oily" fish varieties. The MacArthur Communicative Development Inventory (MCDI) and an ALSPAC adaptation of the Denver Developmental Screening Test (DDST) were completed by the mother when the child was 15 and 18 months of age, respectively. The MCDI child's vocabulary comprehension and social activity scores and the individual DDST components that assessed the child's social and language skills, as well as the DDST total score (which aggregates scores for the child's language, social, fine, and gross motor skills), were considered for analysis. Generalised linear models were used to estimate children's mean developmental scores for each level of maternal seafood consumption and to evaluate trends. Models were adjusted by infant's fish intake, child's age at testing, sex, birth order, breast-feeding status, maternal age, education, dental treatment, smoking and alcohol use during pregnancy, and by the Home Observation for Measurement of the Environment (HOME) score. Odds ratios (ORs) and 95 % confidence intervals (CIs) were estimated by logistic regression to assess the relation of exposure to low and high developmental scores.

MCDI vocabulary comprehension and social activity scores and DDST total and language scores significantly increased across increasing categories of fish intake during pregnancy (p for trend = 0.03, 0.002, 0.03, and 0.004, respectively), whereas differences across categories of maternal fish intake were not significant for the DDST social score. The largest differences were observed for the MCDI comprehension score among children whose mothers ate fish at least once per week during pregnancy compared with those whose mothers did not eat fish (nearly five point difference, 7 %). For the MCDI social activity score, the greatest differences occurred between no fish and the first category of fish consumption (once in two weeks), with additional fish intakes only slightly strengthening the association. The DDST total score was 2 % higher among children whose mothers ate fish 1-3 times per week compared with those whose mothers ate no fish. Most developmental scores increased only fractions of a point with each increase in fish intake during pregnancy. The authors reported that the results were similar when maternal intakes of different types of fish ("white fish" or "oily fish") were considered separately, although the data and results were not reported. Fish intake during pregnancy was associated with a significantly lower chance of low MCDI social activity scores (but not of MCDI vocabulary comprehension scores) and an increased chance of high MCDI vocabulary comprehension and social scores. Fish intake during pregnancy was associated with a significantly lower chance of low DDST total and language scores (but not DDST social scores) and an increased chance of high DDST language scores (but not DDST total or social scores).

Hibbeln et al. (2007) reported data from singleton and first-twin births (n = 8 946) for whom data were available for 28 key social, demographic, and other confounding variables. Mothers answered questions about development or behaviour of their children at ages 6, 18, 30, and 42 months and 7 years (number completing at least one valid response and with complete information on confounders = 8 801), and their children had their IQ measured by the Wechsler Intelligence Scale for Children III^{UK} (WISC-III^{UK}) at eight years of age (n = 5 449). The consumption of seafood ("white fish", "oily fish" and shellfish) rather than fish consumption was considered in relation to developmental outcomes. Seafood consumption was categorised as rarely or never, once in two weeks, 1-3 times per week, 4-7 times per week, and more than once a day, which corresponded to an estimation of 0, 0.5, 2, 5.5 and 10 servings per week, respectively. Serving sizes for each type of seafood were based on typical UK eating patterns, and total seafood consumption was calculated as the total estimated number of servings multiplied by the estimated serving size for each type of seafood. Analyses were based on three categories of estimated seafood consumption: none; 1-340 g per week (i.e. up to three servings); and > 340 g per week. The ALSPAC adapted DDST questionnaire was completed when children were 6, 18, 30 and 42 months old. The DDST total score (which aggregates scores for the child's language, social, fine, and gross motor skills) and the DDST individual scores were considered for analyses. Suboptimum development was defined as a score at the lower end of the distribution and closest to 25 %. The Strengths and Difficulties Questionnaire (SDQ) was completed by mothers when children were aged seven years, and measured children's problem behaviour symptoms and positive behaviour on five subscales (hyperactivity, emotional problems, conduct problems, peer problems, and pro-social), as well as a total difficulties score. The low tails of the distribution of gender-specific scores (closest to 10 %) were chosen to create a binary outcome, indicating sub-optimum behavioural outcomes for each subscale. IQ at eight years of age was obtained with a shortened form of the WISC-III which was administered to children by a trained tester at a research clinic, and provided measures of full-scale, verbal, and performance IQ. Suboptimal cognitive outcomes were defined as the lowest 25 % of scores for full scale, verbal, and performance subscales. Data were analysed by logistic regression analyses using those whose mothers ate > 340 g of seafood per week as the reference category to assess trends. Models were adjusted by 28 potentially confounding variables (including 12 food categories, as well as maternal education and measures of socio-economic status).

Maternal seafood consumption ranged from 0 to 3 268 g per week (mean: 235 g per week, standard deviation (SD): 202). In total, 12 % of women ate no seafood during pregnancy, 65 % 1–340 g per week, and 23 % more than 340 g per week. Only 205 (1.7 %) of women in the study consumed fish oil supplements during pregnancy. The likelihood of suboptimal developmental scores in infants and children significantly increased across decreasing categories of seafood intake for verbal and full-scale IQ (but not for performance IQ) at eight years (about 5 000 children), for one out of six behavioural outcomes at seven years (about 6 000 children), for communication skills at 6 and 18 months, for social development at 30 and 42 months (but not at 6 or 18 months), and for fine motor skills at 18 and 42 months (but not at 6 or 30 months or for gross motor skills at any age). Analyses at early ages (6-42 months) included from about 7 700 to about 8 700 children. Benefits in relation to these variables were mostly observed when the highest category of seafood consumption during pregnancy was compared to the lowest, whereas no significant differences were observed between the medium and highest categories of seafood consumption. The Panel notes that the results for the lowest (no fish) and medium (1-340 g per week) categories of seafood consumption were not directly compared in the statistical analyses.

A subset of 5 222 women had blood samples taken in "late pregnancy" for the measurement of fatty acids in RBC phospholipids. Maternal fatty acid data and results from the WISC-III^{UK} at age eight years were available for 2 839 children (Steer et al., 2013). Linear regression analyses, adjusted for 18 confounders including maternal education, were conducted separately for maternal DHA in the lowest and highest quartiles. Lower maternal DHA concentrations were associated with lower verbal IQ and full-scale IQ, but not with performance IQ, within the lowest quartile of maternal DHA. There were no significant associations between maternal DHA within the highest quartile of maternal DHA and any IQ measure.

Urine samples from the first trimester of pregnancy (defined as ≤ 13 weeks of gestation) and a measure of IQ in the offspring at eight years of age were available for 1 040 mothers (Bath et al., 2013). Trained psychologists assessed children's reading speed, accuracy, and comprehension using the Neale Analysis of Reading Ability at nine years of age. Suboptimal outcomes in any of these measures were defined as scores in the lowest quartile. This population of pregnant women was classified as having mild-to-moderate iodine deficiency on the basis of a median urinary iodine concentration of 91.1 μg/L (iodine-to-creatinine ratio 110 μg/g, interquartile range 74–170 μg/g). Women's results for the iodine-to-creatinine ratio in urine samples were dichotomised for data analysis to < 150 μg/g or ≥ 150 μg/g on the basis of WHO criteria for iodine deficiency or sufficiency in pregnancy. After adjustment for 21 socioeconomic, parental, and child factors as confounders, children of women with an iodine-to-creatinine ratio < 150 μg/g were more likely to have scores in the lowest quartile for verbal IQ (OR 1.58; 95 % CI 1.09–2.30; p = 0.02), reading accuracy (OR 1.69; 95 % CI 1.15–2.49; p = 0.007), and reading comprehension (OR 1.54; 95 % CI 1.06–2.23; p = 0.02) than those of mothers with ratios ≥ 150 μg/g. When the < 150 μg/g group was subdivided, children's IQ and reading scores decreased consistently across groups of maternal urinary iodine (≥ 150 μg/g, 50–150 μg/g, and < 50 μg/g).

Maturity of stereoacuity at age 3.5 years was assessed in 641 children, a random sample taken from the last six months of recruitment (Williams et al., 2001). Stereoacuity matures through three stages (peripheral, or poor; macular, or moderate; and foveal, or adult). "White fish", "oily fish", and shellfish consumption were considered as categorical variables (yes/no), yes meaning at least once every two weeks, in logistic regression analyses, which were adjusted for confounding variables including maternal education, socioeconomic factors, infant feeding practices, and measures of maternal lifestyle during pregnancy. Mothers who ate "oily fish" during pregnancy were more likely to have children who achieved foveal stereoacuity at age 3.5 years compared to mothers who did not eat "oily fish". Such association was not observed for "white fish" or shellfish.

The Panel notes that the results from the ALSPAC cohort showed significant positive associations between fish consumption during pregnancy and measures of children's neurodevelopment. Improved scores on parental assessments of development in children aged from 6 to 42 months were reported for mothers who consumed fish/seafood compared to mothers who consumed no fish/seafood (Daniels et al., 2004; Hibbeln et al., 2007). Significant positive associations were also reported between maternal fish/seafood consumption and scores on objective measures of neurodevelopment administered by trained observers. More mature visual steroacuity was observed in children whose mothers consumed "oily fish" compared to mothers who consumed no "oily fish" (Williams et al., 2001). An improved performance on measures of full-scale and verbal IQ (but not of performance IQ) was reported in eight-year old children whose mothers consumed seafood compared to mothers who consumed no seafood (Hibbeln et al., 2007). The same association was observed between maternal DHA in late pregnancy and children's full-scale and verbal IQ at eight years in a subset of 2 839 women within the lowest quartile of maternal DHA but not within the highest quartile of maternal DHA, suggesting a non-linear association between maternal DHA and children's neurodevelopmental outcomes (Steer et al., 2013). In a subset of 1 040 women classified as having mild-to-moderate iodine deficiency, an association was also seen between maternal iodine status in early pregnancy and children's verbal IQ at eight years and reading abilities at nine years (Bath et al., 2013). The Panel also notes that different measures of intakes were used for data analysis in the different publications (fish, seafood, "oily fish" vs. "white fish"), that the lowest amount of fish/seafood which was associated with better neurodevelopmental outcomes varied depending on how data were analysed in each particular publication and on the outcomes measured (from once every two weeks to more than two servings per week), and that such associations were observed for up to about 4 servings per week compared to no fish/seafood consumption.

The Panel considers that the results from the ALSPAC cohort study suggest that the observed health benefits of fish/seafood consumption during pregnancy on functional outcomes of children's neurodevelopment may depend on the maternal status with respect to nutrients with an established role in the development of the CNS of the foetus and on the independent contribution of seafood (relative to other food sources) to meet the requirements of such nutrients during pregnancy.

The Danish National Birth Cohort study

The Danish National Birth Cohort (DNBC) enrolled 101 042 pregnant women, for whom data on 28 958 and 25 446 mother-child pairs were available in a study of fish consumption during pregnancy and children's neurodevelopment at ages 6 and 18 months, respectively (Oken et al., 2008a). Seafood consumption was estimated from responses to a semi-quantitative FFQ completed at 25 weeks of gestation with > 360 questions about the intake of foods and supplements during the previous month, including information on frequency and type of fish consumed. Fish intake in g/day was calculated by using assumptions about standard serving sizes. Quintiles of fish intakes were used for the primary analysis. Fish intakes were also analysed as a continuous variable in weekly servings and also according to US recommendations for weekly fish intake during pregnancy, with the categories of no fish, 1–2 servings/week (1–340 g/week), or > 3 servings/week (> 340 g/week). Child's neurodevelopment was assessed from maternal yes/no answers to a series of 13 questions (at six months) or nine questions (at 18 months) about developmental milestones. Scores were obtained for total development, motor development and social/cognitive development. Mothers interviewed when their child was 18 months also reported the total number of words currently used by the child, and the ages at which the child sat unsupported and walked unassisted. No information was provided about the sources of the questions asked or the validity of the questionnaires that were created for the study. Multivariate, cumulative, ordinal, logistic regression analyses for each of the three outcomes (motor, social/cognitive, and total development) at 6 and 18 months were performed.

Most (86.3 %) of the women reported consuming 1-2 fish servings/week (1-340 g/week), and 11.0 % consumed ≥ 3 fish servings/week (> 340 g/week), whereas only 2.8 % of women never consumed fish. Cod, plaice, salmon, herring and mackerel accounted for about 85 % of the fish consumed. Mean maternal fish intake was 5.4 g/day (range: 0–10.5 g/day) in the lowest quintile of intake, 22.3 g/day (range: 18.2–26.8 g/day) in the middle quintile and 58.6 g/day (range: 39.4-493.9 g/day) in the highest quintile, corresponding to about < 1, about 1.5 and about 3.5 servings of fish per week, respectively. After adjustment for potential confounding variables, which included parental social class and education, higher maternal pre-natal fish intake was significantly associated with higher child developmental scores at 18 months, with an OR of 1.29 (95 % CI 1.20-1.38) for the highest compared with the lowest quintile. Estimates were similar for the lowest (reference category) and second quintile, and then increased across the three highest quintiles of intake. When fish intake was expressed as a continuous variable, the OR for higher development was 1.49 (95 % CI 1.33-1.66) for each additional fish serving/week. When fish intake was analysed according to US guidelines for intake during pregnancy, ORs (95 % CIs) for higher total development at 18 months were 0.98 (0.85-1.12) for 1–340 g fish consumption per week and 1.20 (1.04-1.40) for > 340 g/week, compared with no fish. The Panel notes the low percentage of women falling within the extreme categories of fish intake using this fixed categorisation system. Estimates of the associations of pre-natal fish intake with motor and social or cognitive development were similar. Results were also similar for developmental milestones reported at six months.

The Panel notes that the Danish National Birth Cohort is the largest prospective observational study, and that it found significant positive associations between maternal fish consumption and measures of children’s neurodevelopment. The Panel also notes that although parental reports of achievement of developmental milestones provide only a global assessment of development which may have been influenced by parental bias, the measure has been shown to correlate with IQ and social achievement later in life (Taanila et al., 2005; Murray et al., 2007). Positive associations between fish consumption during pregnancy and better developmental milestones were observed for mean fish intakes of 1.5 servings per week and up to 3.5 servings per week as compared to low intakes (<1 serving per week), with higher benefits associated with the highest intakes.

The Faroe Islands study

Budtz-Jorgensen et al. (2007) re-analysed previously published data on the association between pre-natal methylmercury exposure and functional outcomes of children’s neurodevelopment in the Faroe Islands (Grandjean et al., 1992; Grandjean et al., 1997; Debes et al., 2006). Frequency of “fish” consumption (number of “fish dinners” per week) during pregnancy was obtained by a questionnaire completed shortly after childbirth. It is unclear whether shellfish was taken into consideration. Data were re-examined with structural equation modelling methods to determine the association between “fish” intake during pregnancy and children’s neurodevelopmental outcomes using analyses adjusted for several confounding variables, including maternal cognitive function (score on Raven’s Progressive Matrices), socioeconomic factors, and cord blood concentrations of methylmercury. The log-transformed number of “fish dinners” during pregnancy was included in the model as a continuous variable. Neurodevelopment at ages 7 and 14 years was assessed with a battery of items taken from several standard tests, which included the Neuropsychological Examination System (NES2), the WISC-Revised (WISC-R), the Wechsler Adult Intelligence Scale-Revised (WAIS-R); Wechsler Memory Scale-III (WMS-III) Spatial Span, the Children’s Category Test, the Stanford-Binet copying, the Catsys reaction time, the Bender Gestalt Test, and the California Verbal Learning Test. Neurodevelopmental outcomes were grouped into a small number of latent variables determined from factor structure which included motor, attention, spatial, verbal, and memory outcomes.

Half of the mothers had “fish” for dinner at least three times per week during pregnancy, and only 2 % ate “fish” for dinner less than once per week. Out of the seven outcomes measured, frequency of “fish” consumption was significantly positively associated only with motor function outcomes, both at 7 and 14 years of age, and with spatial functioning at 14 years.

The Panel notes that this study does not show an association between “fish” consumption and most of the children’s neurodevelopmental outcomes measured. However, the Panel also notes that habitual “fish” consumption in this population, which includes sea mammals (e.g. whales), is much higher than current intakes (and current recommendations) in the majority of European countries.

Other prospective cohort studies conducted in Europe

In one study conducted in the UK (Gale et al., 2008), the relationship between seafood consumption in pregnancy and children's cognition and behaviour at nine years of age was investigated in 217 mother-child pairs. Seafood consumption was estimated at early (15 weeks) and late (32 weeks) gestation from answers obtained from a semi-quantitative FFQ about consumption of 100 foods during the preceding three months. Participants indicated how often they ate: (a)"white fish" (grilled, poached, steamed, in crumbs or batter); (b) fish pie, fish fingers, fish in sauces, (c) "oily fish" (e.g. tuna, sardines, trout, salmon, mackerel), and (d) shellfish (e.g. crab, prawns, mussels). Seafood intakes (all types) were classified in four categories for data analysis (never, n = 19; < 1 time/week, n = 55; 1-2 times/week, n = 102; ≥ 3 times/week, n = 41), and "oily fish" in three categories (never; < 1 time/week; ≥ 1 times/week). Cognitive function of both the mother and her child were obtained with the Wechsler Abbreviated Scale of Intelligence (WASI), which provided measures of full-scale, verbal and performance IQ. Measures of maladaptive behaviour were also obtained using the parental version of the SDQ. This questionnaire contains four subscales of maladaptive behaviour (hyperactivity, emotional symptoms, conduct problems and peer problems), a scale measuring prosocial behaviour, and a total difficulties score. Linear regression analyses, adjusted for eight potential confounders which included maternal IQ and socio-economic status, showed no significant associations between total seafood or "oily fish" consumption in either early or late pregnancy and children's full-scale or performance IQ, or any measures of maladaptive behaviour apart from hyperactivity, which was significantly reduced in the children of mothers who ate "oily fish" less than once per week in either early or late pregnancy. There was a significant trend between greater frequency of seafood consumption in late pregnancy and verbal IQ, but not in early pregnancy. Compared to children whose mothers ate no seafood, verbal IQ was increased by 7.66 points (95 % CI 0.1-15.4) in children whose mothers ate seafood less than once a week, 7.32 points (95 % CI 0.26-14.4) in children whose mothers ate seafood once or twice a week, and 8.07 points (95 % CI 0.28-15.9) in children whose mothers ate fish ≥ 3 times per week.

The Panel notes that seafood intakes of 1-2 times/week and of ≥ 3 times per week in late pregnancy compared to no seafood intakes were associated with large differences in children's verbal IQ (approximately 0.5 SD) at nine years in this small-size study. The Panel also notes that higher verbal IQ scores in children whose mothers consumed seafood in late pregnancy compared to mothers who consumed no seafood were also reported in the much larger ALSPAC cohort (Hibbeln et al., 2007).

Mendez et al. (2009) studied the relationship between seafood consumption in pregnancy and neurodevelopment in 392 Spanish mother-child pairs. Seafood consumption was estimated from interviewer-elicited responses to a 42-item semi-quantitative FFQ that included questions about fish, octopus/squid and shellfish. Fish intake frequencies were categorised as: ≤ 1 time/week; > 1-2 times/week; > 2-3 times/week; and > 3 times/week. Squid and shellfish frequencies were categorised as: ≤ 0.5 times/week; > 0.5-1 times/week; and > 1 time/week. Overall seafood intakes were categorised approximately in quartiles as: ≤ 1.5 times/week; > 1.5-2 times/week; > 2-3 times/week; and > 3 times/week. Children's neurodevelopment at age four years was assessed with the Spanish version of the McCarthy Scales of Children's Abilities (MSCA). The MSCA is a standardised test with measures of performance on five subscales (verbal, perceptual-performance, quantitative, memory, and motor), and the General Cognitive Index (GCI), which is a global measure derived from the verbal, perceptual-performance and quantitative subscales. The relationships between maternal fish and other seafood consumption and MSCA scores were examined with multiple linear regression models adjusting for covariates which included maternal education. Separate analyses were undertaken for children who were breast-fed for less than six months, and children breast fed for six months or longer, as interactions between seafood consumption during pregnancy and breast-feeding duration were statistically significant for general cognitive, memory and numeric MSCA scores. For children who were breast-fed for less than six months (n = 234), maternal fish intake of > 2-3 times/week (n = 28) was associated with significantly higher scores on all MSCA scales than maternal fish intakes ≤ 1 times/week (n = 117). MSCA scores for maternal fish intakes > 3 times/week were not different from those in the reference category of fish intake ≤ 1 time/week, which was probably because of the small number of mother-child pairs in that group (n = 14). The majority of associations between maternal fish consumption and MSCA scores for children breast-fed for ≥ 6 months (n = 143) were not significant, apart from memory, which was significantly lower for fish intakes > 3 times/week (n = 6) compared to fish intakes ≤ 1 time/week (n = 76). In contrast to the results for fish, maternal intakes of other types of seafood were significantly associated with lower GCI, perceptual-performance, verbal and quantitative scores. Lower scores occurred with intakes > 1 times/week (n = 155) compared to > 0.5-1 times/week (n = 107), regardless of duration of breast-feeding. Maternal intakes of all types of seafood combined were not associated with developmental test scores. The Panel notes the uneven (and occasionally very small) number of subjects included in the different categories of fish and seafood intake stratified by breast-feeding duration.

7.3.2. Prospective cohort studies conducted outside Europe

Project Viva

A prospective cohort study (Project Viva) conducted in the US examined the relationship between seafood consumption during pregnancy and measures of cognition (Oken et al., 2005) in the children at six months of age. Measures of receptive vocabulary and visual motor abilities were assessed at three years of age (Oken et al., 2008b). Seafood consumption was estimated from responses to a semi-quantitative FFQ with > 140 foods and beverages completed at 26-28 weeks of gestation. There were four questions about intakes of seafood during the previous three months, including canned tuna fish (one serving defined as 85-114 g); shrimp, lobster, scallops, clams (serving size not defined); “dark-meat fish”, for example, mackerel, salmon, sardines, bluefish, swordfish (85-142 g); and “other fish”, for example, cod, haddock, halibut (85-142 g). Six response options ranged from “never/< 1 serving per month” to “≥ 1 servings per day.” Responses to the four questions were combined to estimate average total seafood intake as servings per week.

Infants from 135 women were given at six months (Oken et al., 2005) a test of visual recognition memory (VRM) in which they were habituated to a pair of identical pictures, and then received two novelty preference tests involving presentation of the familiar and a novel picture. The outcome measure was the average novelty preference score (percentage of time spent looking at the novel picture), which is an index of recognition memory. In tests such as the Fagan Test of Infant Intelligence, higher novelty preference indicates better performance and is correlated with higher childhood IQ. The VRM novelty preference score was positively associated with maternal fish consumption. After adjustment for potentially confounding variables, which included a simple measure of maternal education (college graduate or not), novelty preference increased by 2.8 percentage points for every additional weekly serving of seafood. The Panel notes that the reported VRM assessment involved repeated exposure to pictures until infants were habituated, and that it is the measure of total looking time during habituation which correlates with childhood IQ and provides information about differences in infant cognitive abilities. Novelty preference scores obtained after habituation are not correlated with childhood IQ, and provide no meaningful information about differences in infant cognitive abilities. The Panel notes that no conclusions about the relationship between maternal seafood consumption and infant cognition can be drawn from this study.

A total of 341 children of women in whom seafood consumption was assessed as described above participated in the study at three years of age (Oken et al., 2008a). Receptive vocabulary was measured with the Peabody Picture Vocabulary Test (PPVT) administered by trained assistants, and visual-motor skills were assessed using the Wide Range Assessment of Visual Motor Abilities (WRAVMA). Seafood intake was categorised as never, ≤ 2, and > 2 servings per week. Mean ± SD maternal seafood intake was 1.5 ± 1.4 (range: 0–7.5) servings per week. Forty mothers (12 %) consumed > 2 servings of seafood per week, whereas 47 (14 %) never consumed seafood. Multivariate linear regression analyses, adjusted for potentially confounding variables which included both maternal and paternal education, showed no significant association between seafood intake during pregnancy and PPVT scores. Children of mothers who ate > 2 servings of seafood per week had significantly higher WRAVMA scores than the children of mothers who consumed no seafood.

The Panel notes that no conclusions about the relationship between maternal seafood consumption and infant cognition can be drawn from the publication by Oken et al. (2005), and that a significant association between maternal seafood consumption (> 2 servings/week vs. no seafood) and higher visual motor abilities was reported by Oken et al. (2008a), whereas no association was observed between seafood consumption during pregnancy and vocabulary.

The Seychelles Child Development Nutrition Study

As part of the Seychelles Child Development Nutrition Study (Davidson et al., 2008), seafood consumption during pregnancy was assessed using a food use questionnaire (FUQ) and a four-day food diary at 28 weeks of gestation (n = 225). The FUQ was designed to provide information on frequency of consumption of seafood and seafood-containing meals over a retrospective two-week period. The four-day food diary was used to assess intake of seafood and fish products in grams per day. Unlike in the Faroe Islands, sea mammals are not consumed in the Seychelles. Infant cognition was assessed by the BSID-II mental development index (MDI), which was administered at ages 9 and 30 months. Additional cognitive assessments included the Fagan Test of Infant Intelligence and the Visual Expectation Paradigm, which were administered at five and nine months, and the A not-B and Delayed Spatial Alternation tests which were administered at 25 months. All tests were administered by trained personnel and inter-tester agreement was assessed regularly for BSID-II. Mean ± SD maternal fish intake was 76.7 ± 47.0 g per day (range: 0–346.3 g per day), an average of 537 g/week corresponding to an average of nine seafood-containing meals per week. There were no significant associations between maternal seafood consumption and any measures of infant neurodevelopment at any age, after adjusting by socioeconomic status, home environment, maternal intelligence assessed using the Matrices subtest of the Kaufman Brief Intelligence Test (KBIT) and maternal age, among other confounding variables.

Blood samples were also obtained from women at 28 weeks of gestation and at delivery (Strain et al., 2008). Geometric means of plasma DHA concentrations at these two time points were used for analysis (n = 170); when only one blood sample was available, missing values were imputed assuming a drop in maternal DHA from 28 weeks of gestation to delivery similar to that observed in women for whom two blood samples were available. There were no significant associations between maternal DHA and MDI or Psychomotor Developmental Index (PDI) scores at 9 or 30 months, which is consistent with the lack of association observed between seafood intakes during pregnancy and children’s neurodevelopmental outcomes at either age, including MDI and PDI scores. Measures of finger tapping rate, Preschool Language Scale total language (PLS-TL), verbal ability (PLS-VA) and auditory comprehension (PLS-AC) scores, the KBIT, comprising the verbal knowledge (KBIT-VK) and matrices (KBIT-M) scores, the Woodcock-Johnson Scholastic Achievement Test, second edition, measuring letter-recognition and applied problems, and the Child Behaviour Checklist (CBCL) were obtained at age five years (Strain et al., 2012). There was a significant positive association between maternal DHA and only two (PLS-TL and PLS-VA scores) out of the 10 neurodevelopmental outcomes measured in a model including the n-6 LCPUFA AA, but not in a model including AA and the n-6 PUFA linoleic acid.

The Panel notes that this study does not show an association between seafood consumption or maternal DHA and children’s neurodevelopmental outcomes. The Panel also notes that habitual seafood consumption in this population is much higher than current seafood intakes (and current recommendations) in the majority of European countries.

7.3.3. Conclusion

The Panel notes that two large prospective cohort studies conducted in Europe (UK and Denmark) reported significant positive associations between fish/seafood consumption during pregnancy and functional outcomes of children’s neurodevelopment, one of which included objective measures of IQ, and that similar findings were reported in two smaller studies with comparable seafood intakes (UK and US). The Panel also notes that these associations were observed for fish/seafood intakes of about 1-2 servings per week and up to 3-4 servings per week compared to no fish/seafood intakes, and that no additional benefit might be expected at higher intakes, as suggested by the lack of association between seafood intakes during pregnancy and children’s neurodevelopmental outcomes in two studies where habitual seafood consumption was much higher than current intakes (and recommendations) in the majority of European countries.

7.4. Observational studies on intakes of n-3 LCPUFA from seafood during pregnancy

Bernard et al. (2013) reported on the association between dietary n-3 LCPUFA during the third trimester of pregnancy and children’s neurodevelopmental outcomes at two years of age in a sample of 1 335 mother-child pairs from the EDEN Mother-Child cohort. Information on maternal diet was obtained by completion of a FFQ which included items about intake of seafood and n-3 LCPUFAs. Maternal dietary intake of n-6 and n-3 fatty acids was estimated by using a food composition database. Language development at two years of age was assessed by the French short version of the MSCA, and development at three years of age was assessed by the second French edition of the Ages and Stages Questionnaire (ASQ). The ASQ was completed by parents, and assessed five domains of development (communication, gross motor, fine motor, problem solving, and personal-social). Visual-motor skills were also assessed at three years of age by the Peg Moving Task 5 (PMT-5) and the design copying task taken from the NEPSY (development NEeuroPSYchological assessment) battery. Children also received a verbal fluency test at three years of age. Associations between maternal fatty acid intakes and children’s neurodevelopment scores were examined by multivariable linear regression analyses, adjusted for potential confounders which included parental education and breastfeeding practices. There were no significant associations between maternal DHA intakes and MDI, ASQ, PMT-5 or verbal fluency scores.

Parra-Cabrera et al. (2008) assessed intakes of 104 foods with a FFQ which recorded average frequency of consumption over the preceding year with 10 possible responses ranging from never to six times/day. Maternal n-3 LCPUFA intake was estimated using food composition tables. Brainstem auditory evoked potentials (BAEPs) were recorded in 76 infants at median age 30 days. Logistic regression, adjusted for confounders which included socio-economic status but not maternal education, showed no significant associations between estimated maternal DHA intakes and infant BAEPs.

7.4.1. Conclusion

The Panel notes that there was no association between DHA intakes estimated from seafood consumption during pregnancy and children’s neurodevelopmental outcomes in these studies.

7.5. Observational studies on biomarkers of n-3 LCPUFA

7.5.1. Studies on biomarkers of maternal n-3 LCPUFA during pregnancy or at delivery

Several observational studies have investigated the relationship between maternal levels of n-3 LCPUFAs in pregnancy or at delivery and children’s neurodevelopment. These studies are small, differ in the biomarker of DHA used, and are heterogeneous with respect to the time in which levels of DHA and children’s neurodevelopmental outcomes were assessed, as well as to the tests used for the assessment of cognitive, motor and visual functions.

In a Spanish study (Julvez et al., 2014), the n-3 LCPUFA content of colostrum collected from 434 women in the first 48-96 hours after childbirth was measured. Children’s neurodevelopment was assessed by the Spanish version of the MSCA administered when the children were aged four years. In multivariable linear regression models adjusting for potential confounding variables which included maternal IQ, no significant associations were found between the n-3 LCPUFA content of colostrum and MSCA scores.

Maternal plasma phospholipid fatty acid concentrations were measured in a sample of 17 US women immediately after childbirth (Cheruku et al., 2002). Measures of infant sleep patterns were recorded during the first two days of life. Infant sleep patterns are related to the functional integrity of the CNS, with fewer sleep-wake transitions and more wakefulness indicating more mature development. Women were divided into high DHA (> 3.0 % by weight of total fatty acids (% FA); n = 10) and low DHA

(≤ 3.0 % FA; n = 7) concentrations. Repeated-measures analysis of variance (ANOVA) was used to analyse for the main effects of group and day on the sleep measures, and group by-day interactions, using maternal age and maternal education as confounding variables. Comparisons of sleep patterns showed that infants from mothers with higher DHA had significantly less active sleep than infants from mothers with lower DHA on both days, and significantly less sleep-wake transition and more wakefulness on day two. The Panel notes the small size of the study.

The Panel notes that the two studies considered either did not show an association between maternal DHA and children's neurodevelopmental outcomes (Julvez et al., 2014) or were too small (Cheruku et al., 2002), and therefore uncontrolled for important confounding variables, for conclusions to be drawn.

7.5.2. Studies on biomarkers of n-3 LCPUFA in umbilical cord blood at delivery

7.5.2.1. Prospective cohort studies conducted in Europe

None of the studies conducted in Europe considered concentrations of contaminants (including methylmercury) in cord blood when exploring the relationship between n-3 LCPUFA biomarkers and children's neurodevelopmental outcomes.

In one observational study conducted in the Netherlands, LCPUFA levels in umbilical venous plasma phospholipids were measured in 750 children born at term. Of these, 306 (40.8 %) were followed up at age seven years, when cognitive function was assessed using the Kaufman Assessment Battery for Children (K-ABC) (Bakker et al., 2003), motor function was assessed using the Maastricht Motor Test (MMT) (Bakker et al., 2009), which provides both quantitative and qualitative measures of motor function, and behavioural and emotional problems were obtained using the CBCL, which is a questionnaire completed by parents (Krabbendam et al., 2007). The questionnaire provides scores for internalising behaviour (anxiety, depression, somatic complaints, withdrawn behaviour), and externalising behaviour (aggressive, rule-breaking behaviour). No significant association between DHA at birth and cognitive performance (K-ABC scores) at seven years of age was observed using backward stepwise multiple regression analyses, whereas a positive association between umbilical plasma DHA concentrations and the MMT total and quality scores, but not the MMT quantitative scores, was reported. A significant negative association was found between infant cord blood DHA and internalising problem behaviour, but there was no significant association between cord blood DHA and externalising behaviour. There was also a significant interaction between DHA and feeding type on internalising problem behaviour, with the negative association present in formula-fed infants, but not in breast-fed infants.

Ghys et al. (2002) measured fatty acid levels in cord plasma and RBC membranes in 246 infants. Of these, 128 (52 %) were followed up at the age of four years when cognitive function was assessed using the K-ABC and the Groningen Development Scale (GOS). In multiple linear regression analyses adjusted for relevant confounding variables, no significant associations were found between infant cord plasma or RBC DHA and K-ABC or GOS scores.

Fatty acid levels in blood from umbilical veins and arteries were measured in a sample of 317 Dutch infants. Neurodevelopment was assessed on day 10–14 after birth, according to the neonatal neurological examination technique described by Prechtl (Dijck-Brouwer et al., 2005). The results of the examination were rated as normal, mildly abnormal or definitely abnormal, and were also interpreted using a neurologic optimality score (NOS). Neurodevelopment at three months (n = 262) was assessed as the quality of general movements (Bouwstra et al., 2006a), and at 18 months by the BSID and the Hempel scores (Bouwstra et al., 2006b). The technique described by Hempel assesses motor functions (grasping, sitting, crawling, standing, and walking), the quality of motor behaviour, and muscle tone, reflexes and the function of the cranial nerves. A NOS was derived from these measures. On days 10–14 after birth, 290 infants were classified as neurologically normal, 25 as mildly abnormal and two as definitely abnormal. Neurologically abnormal infants had significantly lower cord blood DHA levels than neurologically normal children. No significant association between umbilical cord blood DHA and quality of general movements at the age of three months was found (Bouwstra et al., 2006a). There were no significant associations between umbilical cord blood DHA and BSID MDI or PDI scores at 18 months. The Spearman rank correlation did not reveal a relationship between DHA content of the umbilical vein blood and NOS, but NOS scores were significantly lower in infants with umbilical vein blood DHA content in the lowest quartile as compared to infants in the other quartiles.

7.5.2.2. Prospective cohort studies conducted in Canadian Inuit

In a longitudinal cohort of 192 Inuit infants from Nunavik (Arctic Quebec, Canada), fatty acids and contaminants (polychlorinated biphenyls (PBCs), lead, and methylmercury) were measured in umbilical cord blood plasma samples. Neurodevelopment was assessed in 109 children using the Fagan Test of Infant intelligence and Teller cards visual acuity at 6 and 11 months of age, and the BSID-II at 11 months (Jacobson et al., 2008). At 11 years of age, cognitive function was assessed in 154 of the children with a continuous recognition task (CRT) which measured recognition memory, the digit span subtest from the WISC, and the California Verbal Learning Test-Children’s Version (CVLT). Electroencephalogram (EEG) recordings of brain activity were also obtained using event-related potentials (ERP), which provide a neurophysiologic measure of cognitive function (Boucher et al., 2011). EEG recordings were obtained during administration of the CRT, where shorter latency of the FN400 peak and larger amplitude of the late positive component (LPC) in the EEG indicates enhanced recognition memory. Visual function was also assessed at 11 years of age using visual evoked potentials (VEP) in 136 children (Jacques et al., 2011).

After adjusting for potential confounding variables (including contaminants) in step-wise multiple regression analyses, there was a significant positive relationship between infant cord plasma DHA and Fagan novelty preference test scores at six months, but not at 11 months. No statistically significant association was found with the Fagan fixation duration test at any age. There was also a significant positive association between infant cord plasma DHA and BSID-II MDI and PDI scores at 11 months. No significant relationships between cord plasma DHA and Teller cards acuity at either 6 or 11 months were observed.

No statistically significant relationship was found at 11 years of age between any cord plasma n-3 PUFAs and motion-onset VEP, but higher cord plasma DHA was significantly associated with shorter latencies in two components of the colour VEP, with shorter latency indicating faster and more efficient visual processing. However, there was no significant relationship between cord plasma DHA and visual acuity measured by the Functional Acuity Contrast Test. Significant positive associations were observed at the same age between cord DHA, digit span and CVLT recognition, but no significant association was found between cord DHA and CRT measures. ANOVA showed that children with higher cord DHA had shorter FN400 latency and larger LPC amplitude than children with lower cord DHA.

The Panel notes that no association was reported between umbilical cord blood DHA concentrations and children’s neurodevelopmental outcomes in the European studies available (all conducted in the Netherlands), whereas results in a cohort of Canadian Inuits were mixed. The Panel also notes that for the Canadian Inuit cohort, only results from models adjusted for PBCs, lead, and methylmercury were reported, and that Inuit have habitual seafood intakes much higher than current intakes (and current recommendations) in the majority of European countries.

7.5.3. Conclusion

The Panel considers that the results from observational studies investigating the association between biomarkers of DHA in maternal or umbilical cord blood samples and functional outcomes of children’s neurodevelopment are inconsistent and that they do not provide additional information on the relationship between seafood consumption during pregnancy and children’s neurodevelopment.

7.6. Intervention studies with n-3 LCPUFA supplementation during pregnancy

A systematic review and meta-analysis of randomised controlled trials (RCTs) examined the effects of maternal n-3 LCPUFA supplementation during pregnancy, or during pregnancy and lactation, on neurological (cognitive and motor) and visual development in early childhood (Gould et al., 2013). The primary outcome was the Developmental Standard Score (DSS) in infants (< 12 months), toddlers (13-24 months), and preschoolers (2-5 years), and the IQ in children (5-12 years) measured with a standardised psychometric test in which the mean is 100 and the SD is 15. Secondary outcomes included other aspects of neurodevelopment (such as language, behaviour, and motor development measured with standardised psychometric scales) and visual development. The literature search identified a total of 23 publications involving 11 trials, from which seven outcomes were included in the meta-analysis for the primary outcomes DSS or IQ, two were included in a meta-analysis on language, eight addressed visual development, and 10 evaluated other neurodevelopmental outcomes. All trials reported a double-blind, placebo-controlled, randomised design. The trials involved a total of 5 272 participants. The main inclusion criteria were women with a singleton pregnancy < 20 weeks of gestation, although two trials included only women with a history of allergic disease. All trials used an oral intervention including fortified foods, capsules, or liquid oil with n-3 LCPUFA from fish or algal oils. The dose of n-3 LCPUFAs was between 240 and 3 300 mg/day, whereas DHA ranged from 200 to 2 200 mg/day. Most trials used a vegetable oil containing no n-3 LCPUFA as control. Planned subgroup analyses by dose of DHA and type of supplement were not possible with the data available. The supplementation period started between 14 and 28 weeks of gestation and ended at birth in eight trials. Three trials supplemented breast-feeding women for 3 to 3.5 months after birth, and one of these also supplemented the formula-fed infants. Two trials that ended the study intervention at birth used DHA-supplemented formulae for infants who were not breast-fed. The planned meta-analyses involved separate comparisons of supplemented and control groups depending on the period of supplementation (pregnancy alone or pregnancy and lactation).

Nine trials reported 11 cognitive development outcomes measured with a global age-standardised assessment, although the means and SDs of three were not available and thus could not be incorporated into the meta-analysis. Six trials reported a total of seven outcomes for standardised assessments of cognitive development which included the BSID (2nd and 3rd Editions); the Griffiths Mental Development Scales; and the K-ABC (Helland et al., 2003; Tofail et al., 2006; Dunstan et al., 2008; Helland et al., 2008; Makrides et al., 2010; van Goor et al., 2010; Campoy et al., 2011). For supplementation during pregnancy or pregnancy and lactation, there were no significant differences between the study groups in the cognitive scores of infants, toddlers or schoolchildren. However, the cognitive scores of preschool children in the supplemented group were significantly higher than scores in the control group (mean difference in DSS compared with the control groups: 3.92; 95 % CI 0.77-7.08; n = 156; p = 0.01). There were no significant effects of supplementation during pregnancy alone for any age group, although no data were available for the meta-analysis in infants. One trial reported the proportion of toddlers with a cognitive score indicating developmental delay (i.e. < 85) and found significantly fewer children with developmental delay in the supplemented group (Makrides et al., 2010).

Two trials reported on two measures of language development (BSID 3rd Edition; PPVT). No significant effects of n-3 LCPUFA were observed in either toddlers (Makrides et al., 2010) or preschool children (Dunstan et al., 2008).

Several trials reported other measures of neurodevelopment which included BAEPs (Stein et al., 2012), the Fagan Test of Infant Intelligence (Helland et al., 2001; Judge et al., 2007), the Hempel neonatal neurologic examination (van Goor et al., 2010), and EEG recording (Helland et al., 2001). No significant differences between the treatment groups were observed on any of these measures. Two trials did report significant effects for other measures of neurodevelopment. Infants whose mothers were supplemented with DHA during pregnancy showed lower quality of general movements compared to infants of mothers supplemented with DHA plus ARA or placebo (van Goor et al., 2010). In contrast, infants of mothers supplemented during pregnancy with n-3 LCPUFAs (mean DHA intake of 214 mg/day) achieved significantly higher problem solving scores compared to infants whose mothers received placebo (Judge et al., 2007). In addition, infants of the supplemented women experienced fewer arousals during active sleep, and better infant sleep organisation is related to optimal neurocognitive and social-emotional outcomes (Judge et al., 2012).

Seven articles describing six RCTs reported the effects of n-3 LCPUFA supplementation during pregnancy on infant visual function. The dose of n-3 LCPUFAs varied from 240-900 mg/day, and the dose of DHA varied from 200–800 mg/day. The supplementation began at between 14 and 22 weeks of gestation, and ended at birth. The number of women in the trials ranged from 48 to 900. Visual function was measured by both electrophysiological methods (VEP; electroretinogram (ERG); and sweep VEP acuity) and behavioural methods (Teller acuity cards). The results from these studies could not be combined in a meta-analysis because of the variety of different assessments and age ranges. No significant differences between infants in the supplement and control groups were found for visual acuity measured by Teller cards at two months (Innis and Friesen, 2008) or six months (Judge et al., 2007), ERG at one week (Malcolm et al., 2003a), visual acuity measured by sweep VEP at four months (Smithers et al., 2011), flash or pattern VEP at 1-5 days, two months or six months (Malcolm et al., 2003b), or VEP latency or amplitude at two months (Broekaert et al., 2005), three months or six months (Stein et al., 2012). One trial reported better visual acuity measured by Teller cards at four months (Judge et al., 2007). One trial included two additional groups which received n-3 LCPUFA or placebo in combination with 400 μg 5-methyltetrahydrofolate (5-MTHF), and reported better visual acuity at two months when both n-3 LCPUFA supplemented groups (with or without 5-MTHF) were compared to both control groups (with or without 5-MTHF) (Broekaert et al., 2005).

The Panel notes that most of the studies included in the systematic review were underpowered with small sample sizes, high losses to follow-up and exclusions post-randomisation. The Panel also notes that this systematic review and meta-analysis of RCTs does not provide consistent evidence for any beneficial effects of n-3 LCPUFA supplementation during pregnancy on various measures of neurodevelopment in infants or children.

Five systematic reviews also considered the effects of n-3 LCPUFA on children’s cognitive development (Dziechciarz et al., 2010; Leung et al., 2011; Campoy et al., 2012; Larque et al., 2012; Lo et al., 2012), but none identified any additional studies that were not reviewed by Gould et al. (2013). The conclusions of these systematic reviews were similar. Although there are some positive findings, there is no clear evidence of any long-term beneficial effect of n-3 LCPUFA supplementation during pregnancy on children’s neurodevelopmental outcomes.

Two articles were published since these systematic reviews and this meta-analysis. Gustafson et al. (2013) supplemented 67 women with either 600 mg/day DHA or placebo from 14 weeks of gestation until birth. Measures of foetal heart rate and heart rate variability were obtained at 24, 32, and 36-week gestational age, and newborn behaviour was assessed with the Neonatal Behavioral Assessment Scale (NBAS). Maternal DHA supplementation produced significantly higher values on measures of foetal heart rate variability, with the greatest difference occurring in the third trimester. Increased heart rate variability indicates greater integrity of the autonomic nervous system, and has been linked to improved developmental and cognitive outcomes. Infants of women supplemented with DHA had significantly higher (i.e. more optimal) scores on the motor and autonomic clusters of the NBAS.

Gould et al. (2014) supplemented 160 women with either 800 mg/day DHA and 100 mg/day EPA or placebo from 20 weeks of gestation until birth. Multiple tests of children’s attention (distractibility) and working memory and inhibitory control were conducted at age 27 months. There were generally no effects of the n-3 LCPUFA supplement on any cognitive outcomes, apart from one minor result which suggested slightly reduced distractibility.

7.6.1. Conclusion

The Panel considers that there is no evidence for an effect of n-3 LCPUFA supplementation (mostly DHA) during pregnancy on any functional outcome of children’s neurodevelopment.

7.7. Quantification of the benefit

The data available do not provide evidence for an effect of n-3 LCPUFA supplementation during pregnancy on children’s neurodevelopmental outcomes, and the Panel did not consider in this Opinion the quantitative analysis by Cohen et al. (2005), in which results from RCTs using DHA-containing formula in infants and young children were used to quantify the potential benefits of DHA intakes during pregnancy on neurodevelopment.

Quantification of health benefits associated with seafood consumption during pregnancy in relation to child neurodevelopment have been undertaken by scientific and regulatory bodies in the context of risk-benefit analyses (FAO/WHO, 2010; FDA, 2014).

The FDA (2014) used summary data of 5 407 mother-child pairs from the ALSPAC study and built a multivariate regression model to assess the association between seafood consumption during pregnancy and children's neurodevelopmental outcomes. The analysis of a dose-response relationship was conducted using the ALSPAC-adapted DDST language scores assessed at 18 months and the full-scale and verbal IQ assessed by an abbreviated form of the WISC-III^{UK} at eight years of age. The model which yielded the best fit assumed that a plateau would be reached beyond seafood consumption of about 280 g per week. The test scores were converted to z-scores using the SD from the ALSPAC study, and z-scores were converted into an IQ scale assuming that one SD difference corresponded to a difference of 15 IQ points. Seafood consumption in the US was estimated by combining data on serving size (obtained from the US Department of Agriculture's Continuing Survey of Food Intake by Individuals (CSFII) using three-day dietary records) with data on frequency of seafood consumption and the types of seafood consumed over 30 days (obtained from the National Health and Nutrition Survey (NHANES) survey using a food frequency questionnaire). The variation in the seafood species consumed over a year was approximated by creating a rank order of popularity of commercial seafood from market share data. This allowed the modelling of a distribution of amounts and species of seafood consumed in the US. Owing to the fact that the exact combination of nutrients in seafood responsible for the beneficial effects on neurodevelopment is not fully understood, all commercial seafood was treated as being alike in terms of benefits conferred. Seafood consumption in women of childbearing age at the 50th percentile was estimated to be 7.8 g/day (95 % CI 6.8-8.7). At these amounts of maternal seafood intake during pregnancy, an increase in IQ as compared to no seafood consumption without adjusting for any potential effect of methylmercury on the outcome was predicted as follows (mean, 95 % CI): IQ derived from the ALSPAC-adapted DDST language scores at 18 months of age, 1.11 (0.26 to 2.49); full IQ at eight years, 0.05 (0.00 to 0.95); verbal IQ at eight years, 0.86 (0.06 to 1.37). At seafood intakes in the 25th percentile (3.0 g/day, 95 % CI 2.2 to 3.9), no statistically significant increase in IQ was predicted for any of the three outcomes. The effect levelled off around the 95th percentile (51 g/day, 95 % CI 46.4 to 56.3) with a gain of (mean, 95 % CI): 2.21 (1.37 to 3.03) for the DDST language scores-derived IQ at 18 months, 3.48 (2.66 to 4.55) for full IQ at eight years, and 5.76 (4.49 to 6.84) for verbal IQ at eight years.

FAO/WHO (2010) considered the results from both the ALSPAC study (Daniels et al., 2004; Hibbeln et al., 2007) and Project Viva (Oken et al., 2008a), and based its conclusions on the average increase in IQ scores observed in these two studies in relation to neurodevelopmental outcomes. In the ALSPAC study, children's (n = 5 449) IQ was estimated at eight years of age using an abbreviated form of the WISC-III^{UK} for full-scale, verbal and performance IQ. A non-linear dose-response relationship was obtained with each gram per day of maternal seafood consumption, improving child IQ by 0.152 points (95 % CI 0.104-0.212) until seafood intakes of 30.5 g per day (4.636 IQ points, 95 % CI 3.172-6.466), with no further gain thereafter. It is unclear from the document on which IQ measurement (full-scale, verbal or performance IQ) this estimate was based on. Average maternal DHA consumption from seafood in this study was estimated using seafood-specific DHA concentrations weighted by market shares in the USA and by using bootstrapping to account for data gaps. Assuming an average DHA content of 3.6 mg per gram of seafood, the observed effect translated into a gain of 4.2 points of verbal IQ (95 % CI 2.9-5.9) per 100 mg of DHA consumption. The effect levelled off at DHA intakes of 110 mg per day (maximum gain of 4.6 IQ points). In Project Viva (Oken et al., 2008a), receptive vocabulary was measured by the PPVT, and visual motor development was assessed by the WRAVMA at three years of age (n = 341). A 0.16 SD greater PPVT score and a 0.61 SD greater WRAVMA score were reported when maternal seafood consumption was more than two servings per week as compared with none. Assuming that one SD difference corresponded to a difference of 15 IQ points, a maximum gain of 5.8 IQ points from an average consumption of three servings of seafood per week was estimated, translating into an IQ gain of 3.8 points/100 mg of DHA. Combining the results from both studies, it was concluded that an average of 4.0 IQ points could be gained from an intake of 100 mg DHA per day, with a maximum attainable IQ gain of 5.8 points.

The Panel notes that the FDA (2014) assessment relied on data from one prospective cohort study only (ALSPAC) to quantify the benefit in terms of seafood consumption, that the FAO/WHO (2010) assessment combined the results from two studies (ALSPAC and Project Viva) to quantify the benefit in terms of DHA consumption only and not seafood, and that the largest prospective cohort study available (Danish National Birth Cohort study) was not considered in any assessment, possibly due to the lack of access to the original data (FDA, 2009). In addition, two extreme assumptions as regards to the role of nutrients in seafood on neurodevelopment were used, i.e. i) that all seafood is alike in terms of benefit, and ii) that the benefit is limited to DHA only.

The Panel also notes that different approaches have been used to model and quantify the health benefits of seafood consumption during pregnancy on children's neurodevelopment, that the comparability of results from individual studies that could be considered in quantitative benefit analyses is hampered by the use of different and heterogeneous neurodevelopmental tests, by testing at different ages, and by uncertainties in the estimation of seafood intakes, and that there is no agreement on an optimal model which would best predict the impact of maternal seafood consumption on children's neurodevelopment, or on a single test which would best predict performance later in life.

7.8. Conclusion

Two large and two smaller prospective cohort studies reported significant positive associations between fish/seafood consumption during pregnancy and children's neurodevelopment. These associations were observed for fish/seafood intakes of about 1-2 servings per week and up to 3-4 servings per week compared to no seafood intakes, and refer to fish/seafood *per se*, including nutrients and non-nutrients (such as methylmercury) contained in fish/seafood. These studies suggest that no additional benefit might be expected at higher intakes. Lower maternal DHA concentrations were associated with lower children's neurodevelopmental scores within the lowest quartile of maternal DHA, but not within the highest quartile, in one of these studies. Low maternal iodine status was associated with lower neurodevelopmental scores in that population. These data suggest that the observed health benefits of fish/seafood consumption during pregnancy may depend on the maternal status with respect to nutrients with an established role on the development of the CNS of the foetus, and on the independent contribution of seafood (relative to other food sources) to meeting the requirements of such nutrients during pregnancy.

Results from observational studies investigating the association between biomarkers of DHA in maternal or umbilical cord blood samples and children's neurodevelopment are inconsistent, and there is no evidence for an effect of DHA supplementation during pregnancy on children's neurodevelopmental outcomes, suggesting that maternal DHA has no effect on these outcomes when DHA requirements are met.

The comparability of results from individual studies that could be considered in quantitative benefit analyses is hampered by the use of different and heterogeneous neurodevelopmental tests, by testing at different ages, and by uncertainties in the estimation of seafood intakes from semi-quantitative FFQs.

8. Health benefits of seafood consumption on cardiovascular health

8.1. Beneficial effects

Since early ecological studies reported low rates of CHD death among Eskimos which were related to high consumption of EPA and DHA from seals and whales (Dyerberg and Bang, 1979), the cardiovascular effects of seafood consumption and n-3 LCPUFAs has been extensively investigated in a large number of human observational and intervention studies, as well as in animal experiments and *in* *vitro* studies. It has been hypothesised that n-3 LCPUFAs could modulate different factors playing a role in cardiovascular disease risk (e.g. arrhythmia, blood concentration of triglycerides, heart rate and heart rate variability, blood pressure, platelet aggregation, inflammation, and endothelial dysfunction), and that the doses of n-3 LCPUFAs needed to modify such factors and the time required for those factors to affect clinical cardiovascular events may vary widely (Mozaffarian and Rimm, 2006; Mozaffarian and Wu, 2011).

A wide range of cardiovascular outcomes has been investigated in relation to seafood and/or n-3 LCPUFA consumption in humans (e.g. coronary events, total cardiovascular mortality, CHD mortality, sudden death, ischemic and haemorrhagic stroke, atrial fibrillation (AF), recurrent ventricular tachiarrhythmias, congestive heart failure). The majority of human intervention studies available have investigated the effects of supplemental n-3 LCPUFA in diseased populations (secondary prevention), generally at doses beyond what is generally achieved only from food in the EU. In contrast, the majority of observational (prospective cohort, case-control, and cross-sectional) studies available have investigated the association between seafood consumption (and occasionally n-3 LCPUFA calculated from seafood) and/or biomarkers of n-3 LCPUFA and disease outcomes in healthy populations generally free of cardiovascular disease (CVD) at recruitment. Whereas results from observational studies are inconsistent and not supported by data from intervention studies for outcomes like arrhythmias (AF, recurrent ventricular tachyarrhythmias, heart rate variability), stroke (total, ischemic, haemorrhagic) and total cardiovascular events, there is strong evidence for an effect of n-3 LCPUFA from seafood on the reduction of CHD mortality.

Four (Frost and Vestergaard, 2005; Brouwer et al., 2006; Berry et al., 2010; Shen et al., 2011) of the five (Mozaffarian et al., 2004) prospective cohort studies available in subjects healthy at recruitment did not find an association between seafood or n-3 LCPUFA assessed by dietary questionnaires and risk of AF, whereas the only two studies which investigated circulating biomarkers of n-3 LCPUFAs (rather than estimated n-3 LCPUFA intakes) showed a significant decrease in the risk of AF in the highest quartiles of circulating n-3 LCPUFA concentrations (particularly DHA) compared to the lowest (Virtanen et al., 2009; Wu et al., 2012). RCTs which assessed the effect of n-3 LCPUFA supplementation on recurrent AF in patients with established paroxysmal or persistent AF, or on the prevention of postoperative AF after cardiac surgery, yielded inconsistent results. A meta-analysis of these RCTs found no significant overall effect of n-3 LCPUFAs on these outcomes (Li et al., 2011). Similarly, no effect of supplemental n-3 LCPUFAs was found in meta-analyses of RCTs on ventricular tachyarrhythmia (Brouwer et al., 2009), on number of defibrillator interventions in patients with implantable cardioverter defibrillators (León et al., 2008), or on measures of heart rate variability (Xin et al., 2013). It should be noted that the studies available were small and heterogeneous.

Most meta-analyses of prospective cohort studies (He et al., 2004b; Bouzan et al., 2005; Larsson and Orsini, 2011; Chowdhury et al., 2012; Xun et al., 2012), but not all (Hooper et al., 2004), report a consistent decreased risk of total stroke, and in particular of ischaemic stroke (He et al., 2004b; Larsson and Orsini, 2011; Xun et al., 2012) with increasing consumption of seafood as compared to no seafood consumption, whereas the association between n-3 LCPUFA intakes (either calculated from fish consumption or assessed using circulating biomarkers) and risk of total stroke is not significant (Chowdhury et al., 2012). Similarly, the majority of RCTs (primary and secondary prevention) do not show an effect of n-3 LCPUFA supplementation on the risk of total, ischaemic, or hemorrhagic stroke (Hooper et al., 2004; Chowdhury et al., 2012; Rizos et al., 2012).

The vast majority of prospective cohort studies and all meta-analyses of these studies report a significant inverse association between seafood consumption (and n-3 LCPUFAs calculated from seafood) and risk of CHD mortality compared to very little or no seafood intakes (He et al., 2004a; Whelton et al., 2004; König et al., 2005; Harris et al., 2008; Zheng et al., 2012), whereas this inverse association is not observed in studies where seafood intakes in the reference category for comparison are relatively high (Oomen et al., 2000; Iso et al., 2006; Manger et al., 2010). This observation is consistent with results from case control and prospective (nested case control and cohort) studies showing a significant inverse association between circulating and tissue markers of n-3 LCPUFAs (mostly DHA, either alone or in combination with EPA) and fatal CHD events (Harris et al., 2007; Mozaffarian et al., 2011). It is also consistent with results from RCTs using n-3 LCPUFA for secondary prevention (Chen et al., 2011; Rizos et al., 2012), particularly when conducted in patients which were not yet under pharmacological therapy for secondary prevention according to current guidelines (Chen et al., 2011); and with data from the only intervention study with fish as dietary intervention for secondary prevention (Ness et al., 2002). Data from both epidemiological studies and RCTs for non fatal cardiovascular events and for total cardiovascular events are less consistent (Mozaffarian and Wu, 2011; Kwak et al., 2012; Rizos et al., 2012).

In 2010, the NDA Panel proposed to set an Adequate Intake (AI) of 250 mg/day for EPA plus DHA based on considerations of cardiovascular health. This AI was set considering that prospective epidemiological and dietary intervention studies indicated that “oily fish” consumption or dietary n-3 LCPUFA supplements (equivalent to a range of 250-500 mg of EPA plus DHA daily) decreased the risk of mortality from CHD and sudden cardiac death. An intake of 250 mg daily appeared to be sufficient for primary prevention (EFSA NDA Panel, 2010a). Dietary recommendations for EPA and DHA intakes for European adults are between 250 and 500 mg/day (EFSA NDA Panel, 2012).

The Panel considers that seafood consumption decreases the risk of CHD mortality compared to no seafood consumption, and that the effect is likely attributable to its content in n-3 LCPUFA.

8.2. Quantification of the benefit

In the context of a risk and benefit assessment of seafood consumption in the general (healthy) population, the Panel considers that available data from epidemiological studies which have investigated the relationship between seafood (and n-3 LCPUFA from seafood) consumption and risk of CHD mortality in healthy subjects at recruitment are appropriate to explore whether the benefit of seafood on that outcome can be quantified. The Panel also considers that although intervention studies conducted with supplemental doses of n-3 LCPUFA in subjects with established CHD may support causality for a beneficial effect of seafood consumption on CHD mortality, these are not appropriate to quantify the benefit of seafood for the general population.

The Panel considered published meta-analyses of observational prospective cohort studies in adult populations without pre-existing CHD that aimed at quantifying the relationship between seafood (or n-3 LCPUFA from seafood) consumption and risk of CHD mortality (He et al., 2004a; Whelton et al., 2004; König et al., 2005; Mozaffarian and Rimm, 2006; Harris et al., 2008; Zheng et al., 2012). These meta-analyses are based on different combinations of the same cohort studies because their selection criteria and the studies available at the time of the literature search differed, so that some studies included in one analysis were excluded in (or not available for) another. **Appendix L** summarises the CHD-related outcomes addressed by each meta-analysis, the observational studies included in the analyses and the country in which the observational studies were conducted. The Panel also considered a quantitative benefit analysis performed by the FDA (2009) on the impact of seafood consumption on CHD mortality in the US adult population and the model proposed by the FAO/WHO (2010) to estimate the reduction in CHD mortality as a function of EPA plus DHA intakes from seafood. The Panel notes that the quantitative benefit analysis related to CHD mortality conducted by the FDA (2009) is still in a draft version, contrary to the quantitative benefit analysis on fetal neurodevelopment which has been published recently (FDA, 2014). However, the Panel considers that the description of the approach taken is relevant for the present Opinion.

8.2.1. Seafood

Four meta-analyses addressed the relationship between seafood consumption and risk of CHD mortality (He et al., 2004a; Whelton et al., 2004; König et al., 2005; Zheng et al., 2012).

The meta-analysis by He et al. (2004a) included 11 publications (published between 1985 and 2003) on 13 independent prospective cohort studies which included frequency of seafood intake, relative risks (RRs) and their corresponding 95 % CIs of CHD mortality in relation to each category of seafood consumption. Studies with only two levels of seafood intake (yes versus no; or high versus low) were excluded (Kromhout et al., 1995; Rodriguez et al., 1996). Seafood consumption was standardised and categorised into five intervals: never or < 1 per month, 1-3 times/month, once per week, 2-4 times/week and ≥ 5 times/week. The amount of seafood consumption (g/day) was estimated by multiplying the frequency of consumption (serving/day) by the corresponding serving size (g/serving) given in a particular study. When the range of seafood intake in a particular category was not available from the paper, the corresponding values were determined on the basis of data from the two largest cohort studies (Fraser et al., 1992; Ascherio et al., 1995). If the highest seafood intake category had an open upper bound (e.g. ≥ 5 times/week), one serving of seafood per day was assigned as the upper bound. As compared with the lowest category, the pooled RRs and 95% CIs of CHD mortality for all other categories of fish consumption were estimated by using both fixed effect and random effects models. The pooled RR for CHD mortality was obtained by averaging the regression coefficients weighted by the inverses of their variances and modelled as a linear function of fish intake. The median intake of fish for each category was used. During an average follow-up of 11.8 years, 3 032 fatal coronary events occurred in 222 364 participants. Subjects who ate seafood once per week, 24 times per week and ≥ 5 times per week had a significantly lower risk of CHD mortality than those who never ate seafood (RR = 0.85, 95 % CI 0.76-0.96; RR = 0.77, 95 % CI 0.66-0.89; RR = 0.62, 95 % CI 0.46-0.82, respectively). In an overall dose-response analysis, the pooled RR for each 20 g/day increase in seafood intake was estimated to be 0.93 (95 % CI, 0.87-0.99; p for trend = 0.03), corresponding to a 7 % decrease in the risk of CHD mortality. The Panel notes that this meta-analysis reports an inverse linear dose-response relationship between fish consumption and risk of CHD mortality, and that the beneficial effects of fish are already observed at a consumption frequency of once per week.

Another meta-analysis published in the same year (Whelton et al., 2004) included 19 observational studies published between 1985 and 2003 with a total of 228 864 participants. Fourteen were prospective cohort studies with a follow-up between 4 and 30 years, and five were case-control studies. Thirteen cohort studies investigated the association between fish consumption and CHD mortality, whereas six cohort studies and the five case-control studies assessed total CHD. Among the 19 studies included in the analysis, nine reported seafood consumption according to the number of servings of seafood, seven as the number of grams of seafood consumed, and three as grams of n-3 LCPUFA consumed. Seafood consumption was converted into number of servings per week assuming a serving size of 114 g and a content of 660 mg n-3 LCPUFA per serving. Fixed effect and random effects models were used to estimate pooled effect sizes, and yielded similar estimates. For case-control studies, OR was used as a surrogate of RR, because the absolute risk of CHD mortality was low. In six cohort studies (out of 13), seafood consumption was associated with a statistically significant reduction in fatal CHD, and in one (out of six) cohort and four (out of five) case control studies, seafood consumption was associated with a statistically significant reduction in total CHD. The overall pooled estimate (13 prospective cohort studies) of the RR of fatal CHD for those consuming any amount of seafood versus those consuming little to no seafood was 0.83 (95 % CI 0.76-0.90). The corresponding estimate (of six cohort and five case-control studies) for total CHD was 0.86 (95 % CI 0.81-0.92). Sensitivity analyses showed that, compared to little or no seafood consumption, seafood intakes of < 2 servings/week and of 2 to < 4 servings/week were associated with significantly lower risk of fatal and total CHD, whereas consumption of > 4 servings/week were not. The Panel notes that this meta-analysis shows a protective effect of seafood consumption on CHD and CHD mortality up to 4 servings/week, that the beneficial effects are already observed at < 2 servings/week, and that no benefit was reported for intakes > 4 servings/week on these outcomes.

The quantitative analysis by König et al. (2005) was based on a subset of prospective cohort studies identified in an earlier systematic review of the literature (Wang et al., 2004). Seven studies (five from the US, one from the Netherlands and one from Italy) met the following inclusion criteria: (a) reported RR for non fatal myocardial infarction (MI) or CHD-related mortality (sum of the risks for fatal MI and sudden cardiac death), (b) quantified the risk relative to a no intake or very low intake reference group (seafood consumption of less than one serving per month), (c) followed subjects approximately representative of the general population in terms of CHD risk factors, and (d) had a study design rated by Wang et al. (2004) as either “A” (least bias; results are valid) or “B” (susceptible to some bias, but not sufficient to invalidate the results). The number of participants per study ranged from 870 to 44 895 and follow-up was between 6 and 30 years. All studies included assessed CHD-related mortality. Seafood consumption data were converted into point estimates, and expressed as average servings per week, assuming a serving size of 100 g of seafood. Seafood consumption ranged from none to 6.5 servings per week. Data were analysed using a linear model in which adjusted RRs (weighted by the inverse of the study variance) were regressed against seafood consumption. As sensitivity analysis, a quadratic term was added to the model to account for a possible non-linear relationship at higher dose levels (levelling off of the effect). Using the linear model, seafood consumption (around 0.5 servings per week) was associated with a significant reduction in the risk of mortality from CHD of 17 % (95 % CI 8.8 to 25 %), with a further risk reduction of 3.9 % (95 % CI 1.1 to 6.6 %) for each additional serving of seafood per week. This corresponds to a 5.5 % decrease in the RR for CHD mortality for each 20 g seafood/day. The Panel notes that this meta-analysis shows an inverse linear dose-response relationship between seafood consumption and risk of CHD mortality, and that the beneficial effects of seafood are already observed at low dietary intakes (0.5 times/week).

The most recent meta-analysis (Zheng et al., 2012) considered the results from 17 independent prospective cohort studies (seven from the USA, two from Asia and eight from Europe) published until September 2010 (14 publications) which reported RRs or hazard ratios (HRs) with their 95 % CIs for CHD mortality taken from the most recent publication on the respective cohorts. HR was considered as RR directly. Studies were excluded if they had a cross-sectional, case-control or experimental design; reported on non-fatal outcomes; differentiated only between two categories of seafood intake (Kromhout et al., 1995; Rodriguez et al., 1996; Streppel et al., 2008); did not use as reference the lowest seafood intake group (Osler et al., 2003; Nakamura et al., 2005); or the reference category of seafood intake was too high to be compared with other studies (Iso et al., 2006; Manger et al., 2010). Ten out of the 17 studies investigated only male subjects. Seafood consumption data were gathered using a self-administered questionnaire (eight studies) or in an interview (nine studies). A serving of fish was assumed to be 105 g. For 315 812 participants, 4 472 fatal outcomes were reported after an average follow-up of 15.9 years (6-30 years). Compared to the very low (reference) category of seafood intake (either < 1 serving/month or 1-3 servings/month), the RRs for fatal CHD associated with low (one serving/week), moderate (2-4 servings/week) and high (≥ 5 servings/week) seafood consumption were 0.84 (95 % CI 0.75-0.95), 0.79 (95 % CI 0.67-0.92), and 0.83 (95 % CI 0.68-1.01), respectively. There was no linear relationship between the amount of seafood consumed and the risk of CHD mortality. In a restricted cubic-spline model, the suggested J-shaped relationship could not be confirmed statistically, possibly because too few data on high seafood consumers were available. A linear dose-response analysis showed that every additional 15 g of seafood/day lowered CHD mortality by 6 % up to four servings per week. The Panel notes that this meta-analysis shows a dose-response relationship between seafood consumption and risk of CHD mortality which is not linear, that the beneficial effects of seafood are already observed at low dietary intakes (one serving/week), and that the beneficial effect may be lost at high intakes (≥ 5 servings/week).

The Panel notes that these four meta-analyses show an inverse dose-response relationship between seafood intake and risk of CHD mortality, that the beneficial effects of seafood consumption on the risk of CHD mortality were observed for seafood intakes of about 1-2 servings per week and up to 3-4 servings per week compared to no seafood consumption, and that no benefit might be expected at higher intakes. The Panel also notes that the calculated benefits of seafood consumption in relation to CHD mortality refer to the net effects (i.e. combining beneficial and adverse effects) of nutrients and non-nutrients (i.e. including contaminants such as methylmercury) contained in seafood. However, the Panel also notes that the comparability of results from the studies pooled in the analyses may be hampered by the use of different tools to estimate seafood consumption (e.g. semi-quantitative FFQs, dietary history, 24-h recalls; type of questions asked in FFQs to gather seafood consumption data), to ascertain the cause of death (e.g. medical records, case registries, death certificates, family reports), and by the adjustment for different confounders. In addition, the number and type of individual studies included and the models used to combine data from individual studies differ, so that both linear and non-linear relationships between seafood intake and CHD mortality risk have been described.

The FDA (2009) made an attempt to overcome the assumptions made by meta-analyses regarding the comparability of results from the single studies pooled. Dose-response models were fit to data from 15 cohort studies **(Appendix L)** by generating 300 bootstrap data sets for each study, instead of using aggregated data from each study. The purpose of the dose-response analysis was to estimate the relationship between seafood consumption and risk of CHD mortality, rather than the contribution of specific nutrients in seafood. To that end, it was assumed that all commercial species of seafood are alike in relation to disease risk. A probability tree was used to integrate the results of each study, which were weighted by the square root of the sample size, into a single non-linear dose-response function assuming that the benefits of seafood consumption would peak at some point. Instead of calculating RRs and fixing the RR of the control group to one, adjusted group events were calculated to reflect sampling error in the control groups. This approach assumes that the relationship between seafood intake and risk of CHD mortality may differ among studies (e.g. owing to differences in confounding risk factors). Instead of assuming a common variance across all studies and dose groups, CIs were calculated using the sampling error for each individual data point. This led to wider CIs associated with the dose-response function compared to those calculated using a “conventional” meta-analysis approach, particularly at low doses.

Daily seafood consumption (based on data from yearly seafood consumption) for men and women 16-45 years and > 46 years was estimated from the CSFII, the NHANES and the National Marine Fisheries Service’s market share data, and included in the model. Estimated benefits of seafood consumption on CHD mortality at current median intakes and at intakes 50 % higher using both the “conventional” meta-analysis and the “pooled-analysis” approaches are depicted in Table 3.

Table 3: Estimated benefits of seafood consumption on CHD mortality.

	15-45 years (f)		**46+ years (f)**		**15-45 years (m)**		**46+ years (m)**	
Annual deaths from CHD	901		214,387		8,610		248,438	
Annual rate of CHD deaths	0.14/10,000		38/10,000		1.3/10,000		51/10,000	
	median	**90 % CI**	**median**	**90 % CI**	**median**	**90 % CI**	**median**	**90 % CI**
Baseline seafood intake (g/day)	7	6.1-7.7	8.2	7.2-8.9	9.3	8.1-10.4	10.7	9.1-11.6
Median change in CHD mortality at current intakes of seafood (deaths per year)								
Meta-analysis model	-43	-86 to -9	-12,498	-24,158 to -2,274	-589	-1,134 to -106	-18,104	-35,151 to -3,211
Pooled-analysis model	-69	-1,400 to 169	-15,906	-237,298 to 52,076	-728	-8,080 to 2,261	-22,922	-428,305 to 31,837
Additional median changes in CHD mortality at intakes of seafood 50 % higher (deaths per year)								
Meta-analysis model	-22	-43 to -4	-6,249	-12,079 to -1,137	-294	-567to -53	-9,052	-17,576 to -1,606
Pooled-analysis model	-11	-175 to 27	-2,306	-29,691 to 7,154	-124	-926 to 328	-5,243	-48,545 to 6,888

Median seafood intakes ranged from 7-10.7 g per day. Using the meta-analysis model, it was estimated that current seafood consumption prevents a median of 43 CHD deaths per year in women 15-45 years and a median of 18 104 CHD deaths per year in men > 46 years in the US. Mean estimates of CHD deaths prevented at current seafood intakes using the pooled analysis model were generally about 25 % higher than with the meta-analysis model, but 90 % CIs were wider and included zero. It was calculated using the meta-analysis model that increasing current seafood intakes by 50 % could further prevent a median of 22 CHD deaths per year in women 15-45 years and a median of 9 052 CHD deaths per year in men > 46 years in the US. Mean estimates using the pooled analysis model were generally about 50 % lower than with the meta-analysis model, again with much wider 90 % CIs which included zero.

The Panel notes that an attempt to fit intrinsic differences among studies into the dose-response model did not allow quantification of the benefit of seafood consumption on CHD mortality with sufficient certainty.

8.2.2. n-3 LCPUFA from seafood

Two meta-analyses addressed the relationship between the consumption of n-3 LCPUFA from seafood and risk of CHD mortality (Mozaffarian and Rimm, 2006; Harris et al., 2008).

A meta-analysis involving five cohort studies and one case-control study conducted in the US among subjects free of CHD at baseline was performed to identify the amount of EPA plus DHA derived from consumed seafood associated with the lowest risk for CHD mortality in order to set DRVs for these n-3 LCPUFA for the US population (Harris et al., 2008). Studies were included if risk for CHD death (including primary cardiac arrest and/or sudden cardiac death) was reported, if the risk was assessed as a function of quintiles of EPA plus DHA intakes, and if multivariate analysis was used to calculate RRs or ORs. In all studies but one (Albert et al., 2002), the RR for fatal CHD significantly decreased across quintiles of EPA plus DHA intakes. In the five studies showing an association, the EPA plus DHA intake with the lowest risk of CHD mortality ranged from 90-163 mg/day to 919 mg/day, with an average of 496 mg/day. Pooling the results, an overall 37 % reduction of the risk for CHD mortality was calculated for an average EPA plus DHA intake of 566 mg/day.

Mozaffarian and Rimm (2006) pooled the results of 16 prospective cohort studies and four RCTs (i.e. one study arm on secondary prevention) that evaluated the effect of seafood consumption and/or EPA and DHA intakes on CHD mortality. The model used to pool the results was not described. Whenever n-3 LCPUFA intakes were not reported in the studies, these were calculated from seafood consumption using surrogate data. For each 100 mg/day of EPA and DHA consumed, the risk of CHD mortality was lowered by 14.6 % (95 % CI 8-21 %) up to 250 mg/day, with an overall risk reduction of 36 % (95 % CI 20-50 %) when compared to no DHA and EPA. RCTs conducted with supplemental, higher doses of n-3 LCPUFAs did not show higher benefits than cohort studies, where n-3 LCPUFAs were consumed as seafood in lower amounts. The Panel notes that in this meta-analysis the amount of n-3 LCPUFAs needed to obtain a 36 % reduction in the risk for CHD mortality, and beyond which no additional benefit was observed, is less than half the amount calculated in the previous meta-analysis (Harris et al., 2008) to achieve a comparable risk reduction.

Based on the quantitative benefit analysis performed by Mozaffarian and Rimm (2006), the FAO/WHO (2010) derived an equation to estimate the deaths which could be prevented by reducing CHD mortality in a given population in a given period of time (e.g. per year) through intakes of EPA plus DHA, by assuming a 0.014 % reduction in CHD deaths for each mg of EPA and DHA consumed daily (36 % for a consumption of 250 mg/day of DHA and EPA vs. no consumption). A serving size of 100 g seafood was assumed. The Panel notes that even though it was acknowledged that the estimated mean proportional reduction in CHD mortality derived by Mozaffarian and Rimm (2006) was based on a linearity assumption up to an intake of 250 mg DHA and EPA per day only, the derived equation did not take into account any potential levelling off of the effect at intakes beyond 250 mg DHA and EPA per day.

The Panel notes that the amount of n-3 LCPUFAs needed to observe a comparable reduction (36-37 %) in the risk for CHD mortality, and beyond which no additional benefit could be expected, varied widely (250-566 mg/day) from one meta-analysis to another (Mozaffarian and Rimm, 2006; Harris et al., 2008), possibly due to differences in study selection and the mathematical model used to combine data from individual studies. The Panel also notes that, in addition to the limitations described above in relation to the comparability of results from the pooled individual studies, the type of questions asked in FFQs to gather seafood consumption data and the assumptions made to calculate n-3 LCPUFA intakes from seafood varied widely among the individual studies, even if conducted in the same country (Harris et al., 2008) (**Appendix M**), and that imputing data on n-3 LCPUFA intakes when not reported in the individual studies adds to the uncertainties of the data base (Mozaffarian and Rimm, 2006).

8.3. Conclusion

The beneficial effects of seafood consumption on the risk of CHD mortality are observed at intakes of about 1-2 servings per week and up to 3-4 servings per week compared to no seafood consumption, and no benefit might be expected at higher intakes (> 4-5 servings per week). Such benefits refer to the overall effect of beneficial and adverse effects of nutrients and non-nutrients contained in seafood (i.e. including contaminants such as methylmercury). However, the comparability of results from the individual studies pooled in quantitative benefit analyses may be hampered by the use of different tools to estimate seafood consumption, the use of different tools to ascertain the cause of death, and differences in the adjustment for different confounders. An attempt to fit intrinsic differences among studies into a dose-response model did not allow the benefit of seafood consumption on CHD mortality to be quantified with sufficient certainty. Quantitative benefit analyses using n-3 LCPUFA intakes from seafood introduce an additional level of uncertainty in the benefit estimate.

CONCLUSIONS

On the basis of the data available, and in relation to the four objectives listed in section 2, the Panel concludes that:

a) Seafood is a source of energy and protein with high biological value and contributes to the intake of essential nutrients, such as iodine, selenium, calcium, and vitamins A and D, with well-established health benefits. Seafood also provides n-3 LCPUFA, and is a component of dietary patterns associated with good health. Most European FBDG recommend (a minimum of) two servings of fish per week for older children, adolescents, and adults to ensure the provision of key nutrients, especially n-3 LCPUFA, but also vitamin D, iodine and selenium. Recommendations for children and pregnant women refer to the type of fish and are also based on safety considerations, i.e. presence of contaminants. Available data suggest a big variation in the amount of fish and other seafood consumed across European countries and age groups, as well as in the type of seafood and species eaten, although data from European surveys are difficult to compare, the type of seafood consumed is largely unknown in some countries, and data are particularly scarce for infants. Seafood provides the recommended amounts of n-3 LCPUFA in most of the European countries considered and contributes to the needs of other essential nutrients, such as vitamin D, iodine or selenium, in some countries.

b) Consumption of about 1-2 servings of seafood per week and up to 3-4 servings per week during pregnancy has been associated with better functional outcomes of neurodevelopment in children compared to no seafood. Such amounts have also been associated with a lower risk of CHD mortality in adults and are compatible with current intakes and recommendations in most of the European countries considered. These associations refer to seafood *per se* and include beneficial and adverse effects of nutrients and non-nutrients (i.e. including contaminants such as methylmercury) contained in seafood. No additional benefits on neurodevelopmental outcomes and no benefit on CHD mortality risk might be expected at higher intakes.

c) The observed health benefits of seafood consumption during pregnancy may depend on the maternal status with respect to nutrients with an established role in the development of the CNS of the foetus (e.g. DHA and iodine) and on the contribution of seafood (relative to other food sources) to meeting the requirements of such nutrients during pregnancy. No effect of these nutrients on functional outcomes of children’s neurodevelopment is expected when maternal requirements are met. The health benefits of seafood consumption in reducing the risk of CHD mortality are probably owing to the content of n-3 LCPUFA in seafood.

d) Quantitative benefit analyses of seafood consumption during pregnancy and children’s neurodevelopmental outcomes, and of seafood consumption in adulthood and risk of CHD mortality, have been conducted, but are generally hampered by the heterogeneity of the studies which have investigated such relationships. Such studies differ in the tools used to estimate seafood consumption, in the tools used to measure (or ascertain) the outcomes of interest, and in the adjustment for confounding variables.

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APPENDICES

Appendix A. Questionnaire on national recommendations for fish consumption

NAME:

COUNTRY:

AFFILIATION:

E MAIL:

DATE:

In contrast to dietary reference values or recommended nutrient intakes, Food-Based Dietary Guidelines (FBDG) are the expression of the principles of nutrition education mostly as foods. They represent the form in which advice is provided to people to assist them in selecting a diet to meet their needs for health.

The aim of this questionnaire is to get more information on the availability and the type of FBDG used in the EU (candidate) Member States, particularly in relation to recommendations for the consumption of fish, including finfish (fish meat) crustaceans and molluscs, and the way of coming to these FBDG.

To answer this questionnaire, please tick the relevant boxes.

We kindly ask you to send back the filled survey by e-mail to: silvia.valtuenamartinez@efsa.europa.eu

1. Does your country have Food-Based Dietary Guidelines (FBDG)?

☐ yes ☐ no

2. In which year were (the most recent) FBDG established:

3. In which year were they most recently updated?

4. Are the FBDG evaluated and monitored?

☐ yes ☐ no

if yes, please specify:

5. Who was involved in the development of FBDG in your country?

☐ Government bodies. Please specify

☐ Scientific societies. Please specify

☐ Industry. Please specify

☐ Non profit organisations. Please specify

☐ Other. Please specify

6. What is the origin of the FBDG used in your country?

6.1 ☐ fully translated from the CINDI dietary guide, WHO

6.2 ☐ fully translated FBDG from other country, please specify the country:

6.3 ☐ specially developed for your country

6.4. ☐ adapted for your country, please specify which FBDG were taken as basis:

If the answer is 6.1 or 6.2 → please go directly to question No. 18

7. To which population groups are the FBDG in your country directed?

☐ General population
☐ Elderly
☐ Adults
☐ Adolescents
☐ Others
☐ Schoolchildren
☐ Pre-school children
☐ Infants
☐ Pregnant women
☐ Lactating women

8. Which food (groups) are included in your national FBDG?

☐ bread, cereals
☐ rice, pasta, potatoes
☐ vegetables
☐ fruit
☐ legumes
☐ milk and dairy products
☐ meat
☐ fish
☐ eggs
☐ oil/fats
☐ nuts, seeds
☐ Other food groups:

9. Are amounts of foods quantified (recommended servings, portions or amounts)?

☐ yes ☐ partly ☐ no

if yes or partly, please specify:

10. Do the FBDG include specific recommendations for **fish** consumption in your country?

☐ yes ☐ no (if “no” ⟶ go directly to question No. 22)

11. The recommendations for **fish** consumption are made for (please tick as many as apply):

a. Fish and seafood (crustaceans and molluscs) ☐

b. Fish only ☐

d. Specific for fatty fish ☐

e. Specific for seafood ☐

f. Other. Please specify ☐

12. The recommendations for fish consumption are made for:

☐ General population ☐ Schoolchildren

☐ Elderly ☐ Pre-school children

☐ Adults ☐ Infants

☐ Adolescents ☐ Pregnant women

☐ Others ☐ Lactating women

13. Please specify the recommendations for fish consumption in your country in the context of FBDG. If available, please indicate the amount (servings, serving size if defined, grams) per unit of time, the type of fish (if different recommendations are made for different types of fish), and the population subgroup whenever the recommendations differ from those for the general population, when appropriate, e.g.:

- Population subgroup: General population

servings/portions ☐ day ☐ week serving size

(if applicable) of which servings/portions of (indicate type of fish)

OR

grams ☐ day ☐ week

(if applicable) of which servings/portions of (indicate type of fish)

- Population subgroup (please specify):

servings/portions ☐ day ☐ week serving size

(if applicable) of which servings/portions of (indicate type of fish)

OR

grams ☐ day ☐ week

(if applicable) of which servings/portions of (indicate type of fish)

- Population subgroup (please specify)

servings/portions ☐ day ☐ week serving size

(if applicable) of which servings/portions of (indicate type of fish)

OR

grams ☐ day ☐ week

(if applicable) of which servings/portions of (indicate type of fish)

- Population subgroup (please specify)

servings/portions ☐ day ☐ week serving size

(if applicable) of which servings/portions of (indicate type of fish)

OR

grams ☐ day ☐ week

(if applicable) of which servings/portions of (indicate type of fish)

Comments:

Questions related to the development of FBDG in general and in particular to fish consumption

14. Are diet-related health problems in your country taken into account when developing FBDG?

☐ yes ☐ no

15. Are diet-related health problems in your country taken into account when developing FBDG in relation to **fish** consumption?

☐ yes ☐ no (if “no” ⟶ go directly to question No. 17)

16. Which diet-related health problems were considered to develop recommendations for **fish** consumption? (please tick as many as needed)

☐ Cardiovascular diseases

☐ Dyslipidemia

☐ Hypertension

☐ Type 2 diabetes

☐ Overweight/obesity

☐ Osteoporosis

☐ Cancer

☐ Brain function

☐ Mental health

☐ Iron deficiency anaemia

☐ Iodine deficiency disorders

☐ Dental caries

☐ Malnutrition

☐ Pregnancy outcomes

☐ Others (please specify)

17. What information was used to review food consumption patterns in your country?

☐ National food supply data

☐ Household data

☐ Individual food consumption data: please provide name and year of the survey:

☐ Were any other data used? Please specify:

18. Which dietary reference values (nutrient based recommendations, recommended daily intakes etc.) were used in assessing adequacy of the diet?

☐ Not assessed

☐ Values from own country

☐ Values from other country: please specify

19. Recommendations on **fish** consumption were intended to:

a) fulfil intake of key nutrients:

☐ yes ☐ no (if “no” ⟶ please ignore No. 20a)

b) replace foods/nutrients with adverse health effects

☐ yes ☐ no (if “no” ⟶ please ignore No.20b)

(if “no” to both a and b⟶ go directly to question No. 22)

20. a) The key nutrients to be fulfilled by **fish** consumption in your country were:

☐ Energy

☐ Water

☐ Animal Protein

☐ Total Fat

☐ Saturated fatty acids

☐ Monounsaturated fatty acids

☐ Polyunsaturated fatty acids

☐ Omega-6 fatty acids

☐ Omega-3 fatty acids

☐ Trans fatty acids

☐ Cholesterol

☐ Other food components:

☐ Calcium

☐ Iron

☐ Phosphorus

☐ Magnesium

☐ Selenium

☐ Iodine

☐ Zinc

☐ Other minerals

☐ Vitamin A

☐ Thiamine (Vitamin B1)

☐ Riboflavin (Vitamin B2)

☐ Niacin (Vitamin B3)

☐ Vitamin D

☐ Vitamin E

☐ Other vitamins:

b) The food/nutrients to be replaced by **fish** consumption in your country were:

☐ Other animal protein

☐ Saturated fat

☐ Cholesterol

☐ Other (please specify):

21. a. Were food safety aspects taken into account to develop FBDG for **fish**?

☐ yes ☐ no

if yes, please specify:

if no, please go to question 22

b. Which food safety aspects were taken into account to develop FBDG for **fish**?

☐ biological hazards, please specify:

☐ chemical hazards, please specify:

22. Please indicate were the FBDGs in your country can be found (e.g. recommendations, reports, websites…):

If possible, please attach a copy of the FBDG for your country or provide the link to a website where they can be downloaded from. Please also provide and a link to an English translation if available.

23. Please add any general or specific comment, you might have:

Appendix B. Summary of the recommendations for fish consumption and frequency of their inclusion in national Food Based Dietary Guidelines from 21 European countries.

Question Number	Recommendations on fish included in national FBDG	Frequency
8	National FBDG includes the food (group) fish	19
10	National FBDG include specific recommendations for **fish**	18
11	The recommendations for **fish** consumption are made for:	
a)	Fish and seafood (crustaceans and molluscs)	7
b)	Fish only	11
d)	Specific for fatty fish	6
e)	Specific for seafood	0
f)	Other	1
12	The recommendations for fish consumption are made for:	
	General population	17
	Elderly	4
	Adults	9
	Adolescents	6
	Others	2
	Schoolchildren	7
	Pre-school children	6
	Infants	5
	Pregnant women	7
	Lactating women	6
13	Specified recommendations for fish consumption in the context of FBDG as regards the amount (servings, serving size) per unit of time, the type of fish and the population subgroup	19
16	Diet-related health problems considered to develop recommendations for **fish** consumption:	
	Cardiovascular diseases	15
	Dyslipidemia	6
	Hypertension	4
	Type 2 diabetes	3
	Overweight/obesity	5
	Osteoporosis	3
	Cancer	5
	Others	3
	Brain function	1
	Mental health	1
	Iron deficiency anaemia	2
	Iodine deficiency disorders	2
	Dental caries	2
	Malnutrition	2
	Pregnancy outcomes	5
19	Recommendations on **fish** consumption intended to:	
a)	fulfil intake of key nutrients	17
b)	replace foods/nutrients with adverse health effects	8
20 a)	The key nutrients to be fulfilled by **fish** consumption in your country were:	
	Animal Protein	6
	Saturated fatty acids	1
	Monounsaturated fatty acids	1
	Polyunsaturated fatty acids	8
	Omega-6 fatty acids	3

Question Number	Recommendations on fish included in national FBDG	Frequency
	Omega-3 fatty acids	18
	Cholesterol	1
	Calcium	1
	Phosphorus	1
	Selenium	4
	Iodine	6
	Zinc	1
	Vitamin A	2
	Niacin (Vitamin B3)	1
	Vitamin D	10
	Vitamin E	1
	Other vitamins	1
20 b)	The food/nutrients to be replaced by **fish** consumption were:	
	Other animal protein	7
	Saturated fat	10
	Cholesterol	2
	Other	1
21 b)	Safety aspects taken into account to develop FBDG for **fish:**	
	a) as regards biological hazards	2
	b) as regards chemical hazards	11

Appendix C. Surveys included in the EFSA Comprehensive European Food Consumption Database for calculating "chronic" dietary intakes.

Country	Survey	n[(a)]	Method	Days	Age	Year
Belgium	Regional Flanders	661	Dietary record	3	2-6	2003
Belgium	Diet National 2004	3 245	24-h dietary recall	2	15-105	2004
Bulgaria	NUTRICHILD	1 723	24-h dietary recall	2	0.1-5	2007
Cyprus	Childhealth	303	Dietary record	3	11-18	2003
Czech Republic	SISP04	1 751	24-h dietary recall	2	4-64	2004
Germany	DONALD 2006	303	Dietary record	3	1-10	2006
Germany	DONALD 2007	311	Dietary record	3	1-10	2007
Germany	DONALD 2008	307	Dietary record	3	1-10	2008
Germany	National Nutrition Survey II	13 926	24-h dietary recall	2	14-80	2006
Denmark	Danish Dietary Survey	4 118	Dietary record	7	4-75	2001
Spain	enKid	382	24-h dietary recall	2	1-14	2000
Spain	NUT INK05	760	24-h dietary recall	2	4-18	2005
Spain	AESAN	418	24-h dietary recall	2	18-60	2009
Spain	AESAN FIAB	1 068	Dietary record	3	17-60	2001
Finland	DIPP	1 448	Dietary record	3	1-6	2005
Finland	STRIP	250	Dietary record	4	7-8	2000
Finland	FINDIET 2007	2 038	48-h dietary recall	2	25-74	2007
France	INCA2	4 079	Dietary record	7	3-79	2006
United Kingdom	NDNS	1 724	Dietary record	7	19-64	2001
Greece	Regional Crete	874	Dietary record	3	4-6	2005
Hungary	National Representative Survey	1 360	Dietary record	3	18-96	2003
Ireland	NSIFCS	958	Dietary record	7	18-64	1998
Italy	INRAN SCAI 2005/06	3 323	Dietary record	3	0.1-98	2006
Latvia	EFSA TEST	2 070	24-h dietary recall	2	7-66	2008
the Netherlands	VCP kids	1 279	Dietary record	3	2-6	2006
the Netherlands	DNFCS 2003	750	24-h dietary recall	2	19-30	2003
Sweden	NFA	2 495	24-h dietary recall	4	3-18	2003
Sweden	Riksmaten 1997/98	1 210	Dietary record	7	18-74	1997

(a) n: number of participants.

Appendix D. Age classes considered in the EFSA Comprehensive European Food Consumption Database.

Age class	Age range	Surveys[(a)]	Member States[(b)]
Infants	0-11 months	2	2
Toddlers	12-35 months	9	7
Other children	36 months-9 years	16	12
Adolescents	10-17 years	12	10
Adults	18-64 years	15	14
Elderly	65-74 years	7	7
Very elderly	≥ 75 years	6	6

[(a)] Number of surveys available for each age class.

[(b)] Number of Member States providing at least one survey per age class.

Appendix E. Dietary intakes of "fish and other seafood" by age group and country.

E1. Children and adolescents.

Age group	Country	Survey	n	Fish category			
Infants (0-11 months)				**Fish meat**	**Fish products**	**Crustaceans**	**Molluscs**
	BG	Nutrichild	860	0.5	-	-	-
	IT	INRAN_SCAI	16	-	-	-	-
Toddlers (12-35 months)							
	BE	Regional Flanders	36	-	-	-	-
	BG	Nutrichild	428	5.2	-	-	-
	FI	DIPP	497	6.2	-	-	-
	DE	DONALD 2006	92	3.2	-	-	-
		DONALD 2007	85	1.8	2.6	-	-
		DONALD 2008	84	2.5	-	-	-
	IT	INRAN_SCAI	36	29.0	-	-	-
	NL	VCP_kids	322	1.2	1.9	-	-
	ES	enKid	17	22.4	-	-	-
Other children (36 months - 9 years)							
	BE	Regional Flanders	625	5.9	2.7	0.3	-
	BG	Nutrichild	433	6.7	-	-	-
	CZ	SISP04	389	10.6	1.0	-	-
	DK	DDS	490	10.1	-	0.8	-
	FI	DIPP	933	10.1	-	0.2	-
		STRIP	250	7.8	-	-	-
	FR	INCA2	482	11.3	6.4	0.6	0.6
	DE	DONALD 2006	211	4.2	3.9	-	-
		DONALD 2007	226	3.5	4.5	-	-
		DONALD 2008	223	4.1	4.2	-	-
	GR	Regional Crete	839	10.7	-	0.2	2.0
	IT	INRAN_SCAI	193	21.6	7.4	2.4	8.8
	LV	EFSA_TEST	189	4.9	-	-	-
	NL	VCP_kids	957	2.2	2.9	0.2	-
	ES	NUT_INK05	399	30.8	2.4	0.8	2.4
		enKid	156	21.5	-	-	3.1
	SE	Riksmaten_barn	1473	7.9	6.6	0.4	-
Adolescents (10-17 years)							
	BE	DN 2004	584	9.8	2.2	1.6	1.1
	CY	Childhealth	303	12.6	-	-	5.7
	CZ	SISP04	298	14.0	2.0	-	-
	DK	DDS	479	10.4	-	0.9	-
	FR	INCA2	973	12.6	5.2	1.0	0.9
	DE	NNS II	1011	4.6	1.8	-	-
	IT	INRAN_SCAI	247	26.7	3.6	4.8	13.8
	LV	EFSA_TEST	470	4.4	1.1	-	-
	ES	AESAN_FIAB	86	30.5	-	5.6	9.4
		NUT_INK05	651	36.4	2.3	1.3	4.2
		enKid	209	31.1	-	1.1	4.8
	SE	Riksmaten_barn	1018	7.5	7.4	0.7	-

NB: Mean intakes (g/day) for each age group and food category are shown. Only values for age groups and fish categories with at least 10 subjects who reported any consumption are indicated.

E2. Adults and elderly.

Age group	Country	Survey	n	Fish category			
Adults (18-64 years)				**Fish meat**	**Fish products**	**Crustaceans**	**Molluscs**
	BE	DN 2004	1304	16.9	2.4	4.1	1.9
	CZ	SISP04	1666	15.7	0.9	-	-
	DK	DDS	2822	15.3	-	2.1	0.1
	FI	FINDIET 2007	1575	24.7	-	0.9	0.2
	FR	INCA2	2276	21.3	3.3	1.6	2.5
	DE	NNS II	10419	13.3	2.2	0.6	0.4
	HU	NRS	1074	8.8	-	-	-
	EI	NSIFCS	958	20.3	-	0.8	0.2
	IT	INRAN_SCAI	2313	31.1	1.0	4.4	9.9
	LV	EFSA_TEST	1306	15.7	2.2	-	-
	NL	DNFCS_2003	750	4.8	2.7	1.2	0.4
	ES	AESAN	410	47.7	2.4	4.2	9.8
	ES	AESAN_FIAB	981	57.3	0.6	5.2	12.0
	SE	Riksmaten_97_98	1210	16.6	5.3	4.2	-
	UK	NDNS	1724	22.1	1.7	2.6	0.5
Elderly (65-74 years)							
	BE	DN 2004	518	21.8	0.5	2.4	2.4
	DK	DDS	309	21.3	-	1.9	-
	FI	FINDIET 2007	463	35.5	-	-	-
	FR	INCA2	264	26.7	2.5	1.2	3.5
	DE	NNS II	2006	19.7	1.9	0.2	-
	HU	NRS	206	5.5	-	-	-
	IT	INRAN_SCAI	290	35.0	-	2.5	8.0
Very elderly (≥ 75 years)							
	BE	DN 2004	712	18.3	0.8	1.9	1.6
	DK	DDS	20	25.8	-	1.9	-
	FR	INCA2	84	26.3	-	2.0	2.3
	DE	NNS II	490	19.8	1.6	-	-
	HU	NRS	80	-	-	-	-
	IT	INRAN_SCAI	228	22.2	-	1.8	4.0

NB: Mean intakes (g/day) for each age group and food category are shown. Only values for age groups and fish categories with at least 10 subjects who reported any consumption are indicated.

Appendix F. Dietary intakes of "fish and other seafood" by age group and country (consumers only).

F1. Children and adolescents.

Age group	Country	Survey	n (%)	Fish category			
Infants (0-11 months)				**Fish meat**	**Fish products**	**Crustaceans**	**Molluscs**
	BG	Nutrichild	15 (1.7)	-	-	-	-
	IT	INRAN_SCAI	2 (12.5)	-	-	-	-
Toddlers (12-35 months)							
	BE	Regional Flanders	12 (33.3)	-	-	-	-
	BG	Nutrichild	62 (14.5)	25.3 (10.5-90.9) n=62; 14.5%	-	-	-
	FI	DIPP	221 (44.5)	9.4 (3.2-35.7) n=221; 44.5%	-	-	-
	DE	DONALD 2006	24 (26.1)	16.4 (3.6-54.0) n=17; 18.5%	-	-	-
		DONALD 2007	23 (27.1)	9.1 (4.0-39.3) n=12; 14.1%	17.3 (9.3-38.9) n=11; 12.1%	-	-
		DONALD 2008	22 (26.2)	10.0 (1.3-46.4) n=16; 19.0%	-	-	-
	IT	INRAN_SCAI	23 (63.9)	50.0 (5.8-102.0) n=20; 55.6%	-	-	-
	NL	VCP_kids	44 (13.7)	13.5 (3.8-32.4) n=23; 7.1%	26.1 (6.6-125.0) n=17; 5.3%	-	-
	ES	enKid	6 (35.3)	-	-	-	-
Other children (36 months - 9 years)							
	BE	Regional Flanders	197 (31.5)	27.0 (8.3-54.2) n=133; 21.3%	26.3 (15.0-63.1) n=60; 9.6%	8.3 (2.7-17.0) n=18; 2.9%	27.0 (8.3-54.2) n=133; 21.3%
	BG	Nutrichild	69 (15.9)	25.3 (10.5-90.9) n=62; 14.5%	-	-	-
	CZ	SISP04	105 (27.0)	40.0 (2.3-87.5) n = 95.0; 24.4	25.0 (5.0-56.3) n= 14; 3.6	-	-
	DK	DDS	408 (83.3)	9.7 (1.7-39.8) n=379; 77.3%	-	0.3 (0.1-11.8) n=146; 29.8	-
	FI	DIPP	452 (48.4)	16.7 (2.6-56.2) n=443; 47.5%	-	4.4 (1.0-22.8) n=32; 3.4%	-
		STRIP	96 (38.4)	15.0 (2.5-48.8)	-	-	-

			n=94; 37.6%			
FR	INCA2	424 (88.0)	13.6 (2.3-40.2) n=336; 69.7%	12.9 (3.6-28.6) (n=215; 44.6%	2.1 (0.3-7.7) n=96; 19.9%	5.0 (2.1-18.6) (n=38; 7.9%
DE	DONALD 2006	75 (35.5)	19.0 (2.0-40.4) n=46, 21.8%	23.5 (4.9-65.0) n=30; 14.2%	-	-
	DONALD 2007	77 (34.1)	15.0 (4.7-36.5) n=41; 18.1%	27.6 (13.4-57.0) n=34; 15.0%	-	-
	DONALD 2008	71 (31.8)	17.9 (6.8-59.5) n=40; 17.9%	30.2 (13.7-50.0) n=30; 13.5%	-	-
GR	Regional Crete	302 (36.0)	33.3 (6.7-76.7) n=252; 30.0%	-	10.0 (2.0-33.3) n=13; 1.5%	33.3 (10-83.3) n=45; 5.4%
IT	INRAN_SCAI	138 (68.4)	40.0 (4.6-89.4) n=103; 53.4%	38.1 (9.5-91.4) n=35; 18.1%	24.5 (2.7-88.1) n=14; 7.3%	37.5 (4.8-108.9) n=37; 19.2%
LV	EFSA_TEST	32 (16.9)	22.5 (12.5-80.0) n=27; 14.3%	-	-	-
NL	VCP_kids	144 (15.5)	23.2 (5.0-83.3) n=69; 7.2%	26.1 (10.8-76.1) n=72; 7.7%	3.2 (0.5-123.8) n=15; 1.6%	-
ES	NUT_INK05	275 (68.9)	48.5 (6.6-105.2) n=230; 57.6%	27.8 (9.0-63.0) n=32; 8.0%	7.8 (2.9-21.6) n=31; 7.8%	15.0 (3.0-51.0) n=54; 13.5%
	enKid	80 (51.3)	50.0 (10.-115.0) n=65; 41.7%	-	-	25.0 (10.-100.0) n=15; 9.6%
SE	Riksmaten_barn	894 (60.7)	20.0 (5.8-50.0) n=488; 33.1%	18.8 (6.3-59.0) n=413; 28.0%	7.8 (2.2-25.0) n=50; 3.4%	-
Adolescents (10-17 years)						
BE	DN 2004	188 (32.2)	38.5 (7.5-106.0) n=127; 21.7%	15.0 (3.8-121.5) n=40; 6.8%	13.0 (2.3-45.0) n=55; 9.4	40.0 (4.8-160.0) n=13; 2.2
CY	Childhealth	121 (39.9)	40.0 (20.0-78.3) (n=88; 29.0	-	-	40.0 (20.0-68.3) n=42; 139.%
CZ	SISP04	71 (23.8)	66.3 (4.5-172.7) n=60; 20.1	56.3 (10-84.4) n=12; 4.0	-	-
DK	DDS	406 (84.8)	9.2 (1.8-38.3) n=394; 82.3%	-	0.6 (0.1-14.4) n=149; 31.1%	-
FR	INCA2	785 (80.7)	15.4 (1.4-50.8) n=617; 63.4%	14.3 (3.6-32.1) n=310; 31.9%	3.9 (0.7-14.3) n=192; 19.7%	8.6 (1.1-38.6) n=82; 8.4%
DE	NNS II	120 (11.9)	48.5 (3.3-142.5) n=80; 7.9%	45.0 (22.5-135.0) n=33; 3.3%	-	-
IT	INRAN_SCAI	171 (69.2)	48.9 (4.8-108.0) n=140; 56.7%	38.1 (20.3-61.0) n=22; 8.9%	35.7 (1.6-134.6) n=29; 11.7%	45.9 (10.4-120.9) n=60; 24.3%

LV	EFSA_TEST	58 (12.3)	45.0 (12.3-100.0) n=44; 9.4%	38.8 (12.5-90.0) n=14; 3.0%	-	-
ES	AESAN_FIAB	66 (76.7)	54.5 (6.7-109.8) n=48; 55.8%	-	12.3 (2.0-37.8) n=29; 33.7%	15.5 (4.2-87.5) n=34; 39.5%
	NUT_INK05	432 (66.4)	53.1 (6.0-154.0) n=364; 55.9%	31.5 (10.0-121.5) n=36; 5.5%	8.4 (1.7-33.8) n=64; 9.8%	17.9 (2.7-95.1) n=93; 14.3%
	enKid	111 (53.1)	60.0 (12.5-175.0) n=96; 45.9%	-	12.5 (2.5-60.0) n=10; 4.8%	30.5 (10.0-87.5) n=24; 11.5%
SE	Riksmaten_barn	527 (51.8)	25.0 (4.5-57.8) n=290; 28.5%	25.0 (6.3-68.8) n=238; 23.4%	12.5 (2.0-63.8) n=37; 3.6%	25.0 (4.5-57.8) n=290; 28.5%

NB: Median intakes (g/day) and P5-P95 (in parenthesis) for each age group and food category, as well as the number of consumers in each category and the percentage they represent from the total sample are shown. Only values for age groups and fish categories with at least 10 subjects who reported any consumption are indicated.

F2. Adults and elderly.

Age group	Country	Survey	n (%)	Fish category			
Adults (18-64 years)				**Fish meat**	**Fish products**	**Crustaceans**	**Molluscs**
	BE	DN 2004	544 (41.7)	47.5 (5.3-142.5) n=385; 29.5	16.5 (2.5-90.0) n=90; 6.9	20.0 (2.3-120.3) n=172; 13.2	41.4 (10.0-100.5) n=50; 3.8
	CZ	SISP04	350 (21.0)	75.0 (7.5-179) n=331; 19.9	56.3 (10-150) n=24; 1.4%	-	-
	DK	DDS	2527 (89.5)	13.4 (2.1-51.0) n=2392; 84.8%	-	1.7 (0.1-15.9) n=1357; 48.1%	9.5 (3.5-70.6) n=14; 0.5%
	FI	FINDIET 2007	651 (41.3)	51.0 (10.0-139.6) n=620; 39.4%	-	15.0 (3.8-53.8) n=60, 3.8%	8.3 (2.3-80.0) n=15; 1.0%
	FR	INCA2	1935 (85.0)	22.9 (2.9-74.6) n=1716, 75.4%	14.3 (3.6-40.7) n=420; 18.5%	4.3 (4.7-15.3) n=620; 27.2%	11.7 (2.9-36.9) n=387; 17.0%
	DE	NNS II	2652 (25.5)	51.0 (7.4-150.0) n=2192; 21.0%	67.5 (13.5-142.5) n=322; 3.1%	12.5 (1.2-100.0) n=246; 2.4%	30.0 (3.5-187.5) n=81; 0.8%
	HU	NRS	137 (12.8)	50.0 (20.0-150.0) n=136; 12.7%	-	-	-
	IE	NSIFCS	631 (65.9)	24.9 (6.6-78.1) n=609; 63.6%	-	7.0 (0.9-19.8) 87; 9.1%	11.8 (1.4-35.8) n=19; 2.0%
	IT	INRAN_SCAI	1602 (69.3)	50.0 (4.6-118.4) n=1432; 61.9%	40.6 (20.3-81.3) n=58; 2.5%	30.0 (2.7-108.8) n=253; 10.9%	44.6 (11.1-120.9) n=465; 20.1%
	LV	EFSA_TEST	364 (27.9)	50.0 (10.0-150.0) n=337; 25.8%	67.5 (22.5-180.0) n=38; 2.9%	-	-
	NL	DNFCS_2003	135 (18.0)	30.4 (4.0-110.0) n=86; 11.5%	72.5 (14.5-150.0) n=26; 3.5%	8.8 (3.2-119.7) n=37; 4.9%	26.7 (8.3-45.2) n=10; 1.3%
	ES	AESAN	329 (80.2)	56.0 (8.3-168.8) n=279; 68.0%	22.5 (7.5-100.0) n=30; 7.3%	14.0 (3.0-84.0) n=75; 18.3%	28.0 (2.0-133.6) n=93; 22.7%
	ES	AESAN_FIAB	885 (90.2)	59.2 (10.0-166.9) n=796; 81.1%	20.0 (5.0-66.7) n=21; 2.1%	12.3 (3.0-46.3) n=325; 33.1%	16.7 (6.1-86.4) n=415; 42.3%
	SE	Riksmaten_97_98	1027 (84.9)	21.4 (8.6-66.1) n=725; 59.9%	17.1 (8.6-38.6) n=311; 25.7%	17.1 (3.6-42.9) n=287; 23.7%	-
	UK	NDNS	1280 (74.2)	26.4 (6.4-81.3) n=1136; 65.9%	13.6 (3.3-40.7) n=181; 10.5%	8.0 (1.3-28.6) n=409; 23.7%	10.6 (2.9-32.3) n=58; 3.4%
Elderly (65-74 years)							
	BE	DN 2004	198 (38.2)	72.5 (13.5-144.5) n=159; 30.7	11.5 (0.9-54.0) n=17; 3.3	20.0 (2.3-66.7) n=49; 9.5	80.0 (5.1-250.0) n=17; 3.3

	DK	DDS	284 (91.9)	20.0 (3.1-53.9) n=279; 90.3%	-	2.3 (0.1-17.9) n=131; 42.4%	-
	FI	FINDIET 2007	222 (47.9)	60.0 (9.9-180.5) n=220; 47.5%	-	-	-
	FR	INCA2	241 (91.3)	27.1 (5.3-72.3) n=224; 84.8%	14.3 (4.3-28.6) n=41; 15.5%	3.0 (0.5-11.4) n=82; 31.1%	15.4 (2.3-40.0) n=54; 20.5%
	DE	NNS II	613 (30.6)	60.0 (7.5-156.9) n=544; 27.1%	67.5 (15.0-135.0) n=62; 3.1%	7.2 (1.8-50.0) n=33; 1.7%	-
	HU	NRS	20 (9.7)	50.0 (7.0-133.3) n=20; 9.7%	-	-	-
	IT	INRAN_SCAI	198 (68.3)	50.0 (6.7-124.2) n=180; 62.1	-	18.7 (2.2-50.0) n=30; 10.3%	34.2 (10.8-111.4) n=59; 20.3%
Very elderly (≥ 75 years)							
	BE	DN 2004	251 (35.3)	52.3 (13.1-147.5) n=202) 28.4	16.5 (2.5-75.5) n=24; 3.4	20.3 (1.5-61.3) n=56; 7.9	41.9 (5.1-90.0) n=23; 3.2
	DK	DDS	19 (95.0)	19.0 (2.1-120.8) n=19; 95.0	-	1.3 (0.1-11.7) n=11; 55.0%	-
	FR	INCA2	72 (85.7)	25.5(6.8-76.4) n=69; 82.1%	-	4.3 (1.4-34.9) n=17; 20.2%	10.0 (1.7-34.3) n=15; 17.9%
	DE	NNS II	156 (31.8)	59.4 (9.7-149.5) n=141; 28.8%	54.0 (2.0-99.5) n=16; 3.3%	-	-
	HU	NRS	7 (8.8)	-	-	-	-
	IT	INRAN_SCAI	131 (57.5)	50.0 (5.0-98.9) n=118; 51.8%	-	31.2 (8.7-107.7) n=10; 4.4%	35.7 (12.8-100.5) n=21; 9.2%

NB: Median intakes (g/day) and P5-P95 (in parenthesis) for each age group and food category, as well as the number of consumers in each category and the percentage they represent from the total sample are shown. Only values for age groups and fish categories with at least 10 subjects who reported any consumption are indicated. The P95 may not be statistically robust when the number of consumers is < 60.

Appendix G. Contribution of different species of fish to the consumption of "fish meat" (%) by country (adults).

	BE	CZ	DE	DK	IE	ES	ES_2	FI	FR	HU	IT	LV	NL	SE	UK
Fish meat (unspecified)	22	64	24	1	13	14	15	33	33	2	13	49	20	86	14
Anchovy (*Engraulis*)	0	0	0	-	0	8	5	1	1	-	7	0	-	-	0
Bass (*Marone*)	-	-	-	-	0	-	-	-	1	-	8	-	-	-	-
Bream (*Charax*)	0	-	0	-	-	0	0	-	1	2	10	-	-	-	-
Carp (*Cyprinus*)	-	5	1	-	-	-	-	-	0	26	-	6	-	-	-
Cod and whiting (*Gadus* spp.)	17	-	15	16	34	18	10	-	12	20	23	4	13	1	31
Eels (*Apodes*)	0	-	0	2	-	0	0	-	0	-	0	0	2	0	0
Flounder (*Platichthys flesus*)	-	-	-	-	-	0	0	0	-	-	-	2	-	0	-
Hake (*Merluccius*)	-	-	0	-	0	29	20	-	1	-	-	2	-	-	-
Halibut (*Hippoglossus* spp.)	1	-	1	-	-	-	-	-	1	-	-	-	-	-	0
Herring (*Clupea*)	4	9	17	20	1	0	0	3	1	9	0	19	7	6	2
Lophiiformes (*Pediculati*)	-	-	-	-	-	5	4	-	-	-	-	-	-	-	0
Mackeral (*Scomber*)	4	6	2	5	2	0	4	0	3	-	1	5	4	2	4
Perch (*Perca*)	3	-	7	-	-	-	-	1	2	-	2	3		0	-
Plaice (*Pleuronectes*)	1	-	2	17	6	-	-		0	-		-	1	-	3
Rays (*Hypotremata*)	-	-	-	-	-	-	-	-	1	-	2	-	-	-	-
Salmon and trout (*Salmo* spp.)	22	8	22	20	31	6	7	42	21	1	7	6	32	2	19
Sardine and pilchard (*Sardina*)	2	4	1	-	1	4	2	1	3	11	2	0	1	0	4
Sole (*Limanda*; *Solea*)	7	-	0	-	1	5	7	-	3	-	10	-	-	-	1
Sprat (*Sprattus sprattus*)	-	1	0	-	-	-	-	-		-		3	-	0	-
Tuna (*Thunnus*)	15	3	8	19	10	10	25	13	14	31	15	-	18	3	21
Whitefish (*Coregonus*)	-	-	-	-	-	-	-	6	-	-	-	-	2	0	-

NB: Only species with at least 1 % consumption in at least two countries or with at least 2 % consumption in one country are shown. A dash (-) denotes no consumption, whereas 0 % illustrates consumption < 0.5%.

Appendix H. Contribution of different species to the consumption of "crustaceans" and "water molluscs" (%) by country (adults).

	BE	CZ	DE	DK	EI	ES	ES_2	FI	FR	HU	IT	LV	NL	SE	UK
Crustaceans (unspecified)	1	100	13	-	-	18	37	-	-	-	-	-	39	77	14
Crab (*Cancer* spp.)	18	-	-	-	14	5	2	-	10	-	1	-	8	1	6
Crayfish (*Astacus* spp.)		-	3	-	-	-	-	20	1	100	-	-	-	8	-
Lobster (*Homarus vulgaris*)	10	-	2	-	1	3	-	-	6	-	1	-	-	0	2
Norway lobster (*Nephrophs norvegicus*)	1	-	-	-	-	31	20	-	-	-	-	-	-	-	-
Prawns (*Palaemon serratus*)	8	-	32	-	84	43	40	-	-	-	-	-	-	14	77
Shrimps (*Crangon crangon*)	63	-	48	100	2	-	-	80	83	-	97	100	53	0	1
Water molluscs (unspecified)	-	-	2	-	-	0	1	-	1	-	-	-	-	-	-
Clam (*Mya arenaria*)	-	-	-	-	5	8	3	-	0	-	10	-	-	-	1
Cockle (*Cardium edule*)	-	-	-	-	-	0	0	-	-	-	-	-	-	-	11
Cuttlefish (*Sepia officinalis*)	-	-	65	-	-	13	12	-	1	-	26	-	-	-	-
Mussel (*Mytilus edulis*)	76	-	26		52	16	15	79	22	-	9	-	90	17	32
Octopus (*Octopus vulgaris*)	-	-	-	-	-	9	29	21	-	-	19	-	-	-	-
Oyster (*Ostrea edulis*)	2	-	2	-	-	-		-	21	-	0	-	-	-	6
Scallop (*Pecten* spp.)	9	-	5	-	37	0	0	-	36	-	0	-	-	-	10
Squid (*Loligo vulgaris*)	13	-	-	100	6	53	41	-	17	-	35	-	10	83	32
Whelk (*Buccinum undatum, Fusus antiquus*)	0	-	-	-	-	-	-	-	1	-	-	-	-	-	7

NB: Only species with at least 1 % consumption in at least two countries or with at least 2 % consumption in one country are shown. A dash (-) denotes no consumption, whereas 0 % illustrates consumption < 0.5%.

Appendix I. Exemplary composition of fish species most consumed in the EU.

	Anchovy	Carp	Cod and whiting	Hake	Herring	Mackerel	Plaice	Atlantic Salmon	Tuna	Trout
Energy (kJ)	680 (403-1076)	498 (484-584)	320 (296-337)	350 (295-387)	778 (491-972)	350 (295-387)	363 (336-393)	787 (750-870)	682 (566-939)	447 (359-526)
Protein (g)	21.1 (13-28.9)	18.1 (18.0-18.9)	17.6 (17.0-18.3)	17.4 (16.5-18.0)	17.3 (14.8-23.0)	17.4 (16.5-18)	17.6 (16.7-19)	19.7 (18.4-20.2)	23.5 (22-26)	18.7 (14.7-20.0)
Fat (g)	6.6 (2.3-13)	5.1 (4.8-7.1)	0.6 (0.3-0.7)	1.5 (0.4-2.2)	13 (6.5-17.8)	1.5 (0.4-2.2)	1.7 (1.4-1.9)	12.1 (11.0-14.2)	7.6 (4.0-15.5)	3.5 (2.7-5.2)
n-3 LCPUFA (mg)	500 (n.a)	296 (n.a)	238 (196-265)	679 (n.a)	2515 (1602-3128)	679 (n.a)	403 (304-442)	1817 (1758-1875)	2523 (163-3467)	632 (n.a)
EPA (mg)	210 (n.a)	193 (n.a)	66 (57-71)	236 (n.a)	1720 (925-2038)	236 (n.a)	224 (162-249)	728 (697-760)	992 (10-1385)	139 (n.a)
DHA (mg)	290 (n.a)	103 (n.a)	172 (139-194)	443 (n.a)	795 (677-1090)	443 (n.a)	179 (143-193)	1088 (1061-1115)	1531 (153-2082)	493 (n.a)
Vitamin D (µg)	12.5 (5.7-20)	0.5 (n.a)	3.2 (0-7)	1.4 (n.a)	18.1 (15-25)	1.4 (n.a)	2.1 (0.0-3.0)	6.0 (3.8-9.2)	7.5 (3.0-11.8)	10.1 (2.1-18.0)
Calcium (mg)	99 (56-148)	61.4 (34-66)	21.2 (9-28)	24.8 (14.0-41.0)	135.1 (34-327)	24.8 (14-41)	47.7 (16-61)	15.0 (0.0-27.0)	21.3 (11.0-40.0)	47.1 (12-127)
Iodine (µg)	34.3 (30-45)	1.7 (n.a)	158 (105-360)	110.0 (n.a)	34.4 (29.0-47.1)	110.0 (n.a)	42.2 (33-53.2)	30.7 (5.0-43.8)	33.4 (14-50)	12.4 (4.6-25.0)
Selenium (µg)	35.5 (20-68)	27.7 (n.a)	27.1 (16.5-37.0)	25.3 (n.a)	27.9 (17.4-35.0)	25.3 (n.a)	31.2 (28.9-37)	26.6 (22.3-32.2)	75.2 (36-116)	20.6 (18.0-26.0)
Zinc (mg)	2.2 (0.96-4.2)	0.7 (n.a)	0.7 (0.4-2.0)	0.3 (n.a)	1.1 (0.6-2.1)	0.3 (n.a)	0.5 (0.5-0.7)	0.4 (0.0-0.6)	0.7 (0.1-1.1)	0.6 (0.4-1.2)

NB: Values are given in units/100 g edible parts (raw) as means (minimum-maximum). n.a = not applicable (only one value available).

Appendix J. Exemplary composition of selected species of crustaceans and molluscs most consumed in the EU

	Crustaceans					Molluscs				
	Crab	**Crayfish**	**Lobster**	**Prawns**	**Shrimps**	**Clam**	**Cuttlefish**	**Mussel**	**Octopus**	**Squid**
Energy (kJ)	416 (343-475)	308 (278-437)	358 (349-395)	351 (275-385)	342 (286-382)	319 (299-322)	312 (300-372)	334 (291-372)	327 (237-352)	330 (284-344)
Protein (g)	20.4 (18.0-22.9)	15.1 (15-17)	17.3 (13.6-18.6)	17.6 (13.6-18.6)	16.7 (13.6-18.6)	11 (10.2-11.1)	16 (14-18)	12.6 (10.5-15.6)	16.1 (11.0-17.9)	15.1 (13-16)
Fat (g)	1.5 (1.1-1.8)	1.2 (0.5-4.1)	1.5 (0.9-1.9)	1.1 (0.6-1.4)	1.2 (0.6-1.4)	1.1 (0.9-2.5)	0.9 (0.7-1.5)	2.1 (1.8-2.7)	1.1 (0.9-1.3)	1.6 (1.0-1.7)
n-3 LCPUFA (mg)	379 (n.a)	63 (n.a)	515 (n.a)	366 (n.a)	379 (n.a)	161 (n.a)	350 (n.a)	244 (n.a)	292 (265-354)	350 (n.a)
EPA (mg)	218 (n.a)	51 (n.a)	350 (n.a)	206 (n.a)	218 (n.a)	95 (n.a)	110 (n.a)	132 (n.a)	113 (110-114)	110 (n.a)
DHA (mg)	161 (n.a)	12 (n.a)	165 (n.a)	160 (n.a)	161 (n.a)	66 (n.a)	240 (n.a)	112 (n.a)	179 (155-240)	240 (n.a)
Vit. D (µg)	0.3 (0.0-0.5)	0.2 (0.1-0.5)	0.1 (0-0.2)	0.5 (n.a)	0.4 (0.1-0.5)	5 (n.a)	0 (n.a)	4.9 (0-8)	0.4 (0.0-1.0)	0 (n.a)
Calcium (mg)	104 (89-120)	46.9 (33-58)	55.3 (48-61)	92 (79-118)	84.9 (58-110)	69 (n.a)	44 (18-59)	49.4 (24-88)	46.3 (27-144)	33.7 (13-144)
Iodine (µg)	84.4 (21-130)	64.4 (6-100)	357 (100-700)	70.6 (21.090.5)	153 (130-210)	120 (n.a)	20.3 (20-21)	138 (116-150)	20 (n.a)	20 (n.a)
Selenium (µg)	75.6 (37-82)	44.9 (14-70)	41.3 (38.1-54.0)	19.3 (16-39)	22.3 (21-30)	51.8 (n.a)	65.1 (65-66)	51.1 (46-60)	59.7 (26-75)	66 (n.a)
Zinc (mg)	6.5 (2.2-11.9)	1.3 (n.a)	2.5 (1.6-4.6)	1.8 (0.9-2.2)	1.9 (1.3-2.2)	2 (n.a)	2 (1.1-4.2)	2.5 (1.8-3.4)	1.8 (0.7-5.1)	1.4 (1.1-3.1)

NB: Values are given in units/100 g edible parts (raw) as means (minimum-maximum). n.a = not applicable (only one value available).

Appendix K. Mean daily intakes of selected nutrients from fish in different EU countries (adults) in amounts per day and percentage of DRV.

	Energy (kJ)	Protein (g)	Fat (g)	n-3 LCPUFA[(a)] (mg)	n-3 LCPUFA[(a)] (%)	Vit D (µg)	Vit D (%)	Ca (mg)	Ca (%)	I (µg)	I (%)	Se (µg)	Se (%)	Zn (mg)	Zn (%)
DRV	**-**	**-**	**-**	**250**	**100**	**5**	**100**	**800**	**100**	**150**	**100**	**55**	**100**	**10**	**100**
All subjects (n)							**Mean**								
Denmark (2527)	106.4	3.5	1.3	270.3	108.1	1.3	26.0	10.1	1.3	12.1	8.1	6.1	11.1	0.2	1.7
Hungary (137)	46.1	1.7	0.4	122.3	48.9	0.6	11.4	4.4	0.5	5.0	3.4	3.6	6.6	0.1	0.7
Italy (1602)	209.7	8.5	1.6	349.9	140.0	2.0	39.4	22.5	2.8	31.2	20.8	18.2	33.1	0.5	5.1
Spain (885)	327.8	13.6	2.3	585.7	243.3	2.9	58.8	29.1	3.6	62.6	41.7	28.8	52.4	0.8	7.7
UK (1280)	149.5	5.1	1.6	327.1	130.8	1.4	27.6	10.1	1.3	23.7	15.8	9.6	17.5	0.2	2.5
Consumers (%)							**Median**								
Denmark (89.5)	84.2	2.9	0.8	214.8	85.9	1.0	20.0	7.4	0.9	9.3	6.2	5.3	9.6	0.1	1.0
Hungary (12.8)	297.3	11.7	2.7	591.0	236.4	4.5	90.0	26.6	3.3	17.0	11.3	21.9	39.8	0.5	5.0
Italy (69.3)	250.9	10.8	1.5	365.3	146.1	2.4	48.0	21.5	2.7	28.3	18.9	19.5	35.5	0.4	4.0
Spain (90.2)	289.0	12.3	1.5	513.9	205.6	2.2	44.0	22.7	2.8	48.3	32.2	25.8	46.9	0.5	5.0
UK (74.2)	151.3	5.6	1.3	294.3	117.7	1.4	28.0	9.3	1.2	22.4	14.9	10.2	18.5	0.2	2.0

[(a)] Value refers to EPA + DHA combined

Appendix L. Meta-analysis of observational studies on fish consumption and coronary heart disease-related outcomes.

			Meta-analyses						
Individual studies	**Country**	**Study design**	**Zheng et al. (2013)**	**FDA (2009)**	**Harris et al. (2008)**	**Mozaffarian and Rimm (2006)**	**König et al. (2005)**	**He et al. (2004b)**	**Whelton et al. (2004)**
Kromhout et al. (1985)	The Netherlands	PC	X	X	-	X	X	X	X
Gramenzi et al. (1990)	Italy	CC	-	-	-	-	-	-	X
Fraser et al. (1992)	United States	PC	-	X	-	X	-	X	-
Dolecek and Granditis (1991)	United States	RCT	-	-	X	X	-	-	X
Siscovick et al. (1995)	United States	CC	-	-	X	-	-	-	X
Ascherio et al. (1995)	United States	PC	X	X	-	-	X	X	X
Kromhout et al. (1995)	United States	PC	-	X	-	X	-	-	X
Salonen et al. (1995)	Finland	PC	-	-	-	-	-	-	X
Rodriguez et al. (1996)	United States	PC	-	-	-	-	-	-	X
Daviglus et al. (1997)	United States	PC	X	X	-	X	X	X	X
Pietinen et al. (1997)	Finland	PC	-	-	-	-	-	-	X
Mann et al. (1997)	United Kingdom	PC	X	X	-	-	-	X	
Albert et al. (1998)	United States	PC	X	X	X	X	X	X	X
Gillum et al. (2000)	United States	PC	-	-	-	-	-	-	X
Oomen et al. (2000)	Finland	PC	X	X	-	X	-	X	X
Oomen et al. (2000)	Italy	PC	X	X	-	X	X	X	X
Oomen et al. (2000)	The Netherlands	PC	X	X	-	X	-	X	X
Sasazuki and the Fukuoka Heart Study Group (2001)	Japan	CC	-	-	-	-	-	-	X
Tavani et al. (2001)	Italy	CC	-	-	-	-	-	-	X
Yuan et al. (2001)	China	PC	X	X	-	X	-	X	X

Albert et al. (2002)	United States	PC	-	-	X	-	-	-	-
Hu et al. (2002)	United States	PC	X	X	X	X	X	X	X
Martinez-Gonzalez et al. (2002)	Spain	CC	-	-	-	-	-	-	X
Mozaffarian et al. (2003)	United States	PC	X	X	X	X	X	X	-
Osler et al. (2003)	Denmark	PC	-	X	-	X	-	X	X
Folsom and Demissie (2004)	United States	PC	X	X	-	X	-	-	-
Mozaffarian et al. (2005)	United States	PC	-	-	-	X	-	-	-
Nakamura et al. (2005)	Japan	PC	-	X	-	X	-	-	-
Iso et al. (2006)	Japan	PC	-	X	-	X	-	-	-
Jarvinen et al. (2006)	Finland	PC	X	-	-	-	-	-	-
Yamagishi et al. (2008)	Japan	PC	X	-	-	-	-	-	-
de Goede et al. (2010)	The Netherlands	PC	X	-	-	-	-	-	-
Tomasallo et al. (2010)	Canada	PC	X	-	-	-	-	-	-

CC = cross-sectional study; PC = Prospective cohort study; RCT = randomised controlled trial.

Appendix M. Dietary assessment of n-3 LCPUFA intakes from seafood in some prospective cohort studies conducted in the US.

Study	Type of questionnaire	Seafood items assessed	Serving size (g)	Mean content of n-3 LCPUFA (g/serving)
Hu et al. (2002)	Semi-quantitative FFQ	(1) dark-meat fish such as mackerel, salmon, sardines, bluefish, or swordfish	84-140	1.5
		(2) canned tuna	84-112	0.42
		(3) other fish	84-140	0.48
		(4) shrimp, lobster, or scallops as the main dish	98	0.32
Albert et al. (1998); Albert et al. (2002)	Semi-quantitative FFQ	(1) dark-meat fish such as mackerel, salmon, sardines, bluefish, or swordfish	84-112	1.37
		(2) canned tuna	NR	0.69
		(3) other fish	84-112	0.17
		(4) shrimp, lobster, or scallops as the main dish	NR	0.46
Mozaffarian et al. (2003)(a)	Semi quantitative FFQ	(1) fried fish or fish sandwich (fish burger)	NR	NR
		(2) tuna fish/tuna salad/tuna casserole	NR	NR
		(3) other fish (broiled or baked)	NR	NR

(a) Authors report that estimated dietary EPA plus DHA content of these fish meals were derived from US commercial landings and Department of Agriculture data (National Marine Fisheries Service. Fisheries of the United States, 2000. Silver Spring, MD: US Dept of Commerce; 2001; US Department of Agriculture, Agricultural Research Service. 2002 USDA National Nutrient Database for Standard Reference, Release 15. Available at: http://www.nal.usda.gov/fnic/foodcomp. Accessed August 1, 2002), but it is unclear how this was done and which EPA plus DHA values were effectively attributed to each category.

GLOSSARY AND ABBREVIATIONS

5-MTHF	5-methyltetrahydrofolate
AF	atrial fibrillation
AI	Adequate Intake
ALA	α-linolenic acid
ALSPAC	Avon Longitudinal Study of Parents and Children
ANOVA	analysis of variance
ARA	arachidonic acid
ASQ	Ages and Stages Questionnaire
b.w.	body weight
BAEP	brainstem auditory evoked potential
BMDL_{05}	95% lower confidence limit of the Benchmark Dose
BSID	Bayley Scales of Infant Development
CBCL	Child Behaviour Checklist
CC	cross-sectional study
CHD	coronary heart disease
CI	confidence interval
CINDI	WHO Countrywide Integrated Non-communicable Disease Intervention
CNS	central nervous system
CONTAM	EFSA Panel on Contaminants in the Food Chain
CRT	Continuous Recognition Task
CSFII	US Department of Agriculture’s Continuing Survey of Food Intake by Individuals
CVD	cardiovascular disease
CVLT	California Verbal Learning Test-Children’s Version
DDST	Denver Developmental Screening Test
DHA	docosahexaenoic acid
DNBC	Danish National Birth Cohort

DPA	docosapentaenoic acid
DRV	Dietary Reference Value
DSS	Developmental Standard Score
EEG	electroencephalogram
EFSA	European Food Safety Authority
EPA	eicosapentaenoic acid
ERG	electroretinogram
ERP	event-related potential
FADS	fatty acid desaturase
FAO	Food and Agriculture Organization of the United Nations
FBDG	Food-Based Dietary Guidelines
FFQ	food frequency questionnaire
Fish	vertebrate aquatic animals (see seafood), synonym of finfish
FUQ	food use questionnaire
GCI	General Cognitive Index
GOS	Groningen Development Scale
HOME	Home Observation for Measurement of the Environment
HR	hazard ratio
IQ	intelligence quotient
ISSCAAP	International Standard Statistical Classification of Aquatic Animals and Plants
JECFA	Joint FAO/WHO Expert Committee on Food Additives
K-ABC	Kaufman Assessment Battery for Children
KBIT	Kaufman Brief Intelligence Test
KBIT-M	KBIT - matrices scores
KBIT-VK	KBIT - verbal knowledge scores
LPC	Late Positive Component
MCDI	MacArthur Communicative Development Inventory

MDI	Mental Development Index
MI	myocardial infarction
MMT	Maastricht Motor Test
MSCA	McCarthy Scales of Children’s Abilities
n-3 LCPUFA	omega-3 long chain polyunsaturated fatty acids
n-3 PUFA	omega-3 polyunsaturated fatty acids
NBAS	Neonatal Behavioral Assessment Scale
NEPSY	development NEeuroPSYchological assessment
NES2	Neuropsychological Examination System
NHANES	National Health and Nutrition Survey
NOEL	No Observed Effect Level
NOS	Neurologic Optimality Score
OR	odds ratio
P	percentile
PBC	polychlorinated biphenyl
PC	prospective cohort study
PDI	Psychomotor Developmental Index
PLS	Preschool Language Scale
PLS-AC	PLS auditory comprehension
PLS-TL	PLS total language
PLS-VA	PLS verbal ability
PMT-5	Peg Moving Task 5
PPVT	Peabody Picture Vocabulary Test
PTWI	Provisional Tolerable Weekly Intake
RBC	red blood cell
RCT	randomised controlled trial
RR	relative risk
SD	standard deviation

SDQ	Strengths and Difficulties Questionnaire
Seafood	vertebrate and invertebrate aquatic animals whether of marine or freshwater origin, whether farmed or wild, except aquatic mammals (e.g. whales, dolphins), aquatic reptiles (e.g. turtles, crocodiles), echinoderms (e.g. sea urchins, starfish), and jellyfish; it does not include aquatic plants.
Shellfish	invertebrate aquatic animals (see seafood); it includes crustaceans and molluscs
TWI	Tolerable Weekly Intake
VEP	visual evoked potential
VRM	Visual Recognition Memory
WAIS-R	Wechsler Adult Intelligence Scale-Revised
WASI	Wechsler Abbreviated Scale of Intelligence
WHO	World Health Organization
WISC	Wechsler Intelligence Scale for Children
WISC-R	WISC-Revised
WMS	Wechsler Memory Scale
WRAVMA	Wide Range Assessment of Visual Motor Abilities