

**OPINION OF THE SCIENTIFIC PANEL ON CONTAMINANTS IN THE
FOOD CHAIN ON A REQUEST FROM THE EUROPEAN PARLIAMENT RELATED TO
THE SAFETY ASSESSMENT OF WILD AND FARMED FISH**

Question N° EFSA-Q-2004-22

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SUMMARY

EFSA was requested by the European Parliament to conduct a scientific assessment of the health risks related to human consumption of wild and farmed fish. An EFSA Interpanel working group was set up to conduct this assessment. The opinion focused on the following finfish species as being marketed to a significant amount in the European Union: salmon, herring, anchovies, tuna, mackerel, pilchards, rainbow trout and carp. A special focus was also given to Baltic herring at the request of the European Parliament.

Of the selected fish, salmon, rainbow trout and carp are predominantly or exclusively farmed. The other species are predominantly caught from the wild. About two-thirds of fish consumed in the EU is caught from the wild.

Species, season, diet, location, lifestage and age have a major impact on both the nutrient and contaminant levels of fish. These levels vary broadly within species and between species in both wild and farmed fish. There is a need for standardisation of sampling procedures before a robust comparison of wild and farmed fish can be made. From the limited data available it seems that if there are any differences between farmed and wild fish, they are small when taking into account the above mentioned factors. However, regional differences exist, e.g. in the Baltic Sea.

Contaminants in fish derive predominantly from their diet, and levels of bioaccumulative contaminants are higher in fish that are higher in the food chain. Whilst it is not possible to control the diet of wild fish, the levels of contaminants, and of some nutrients, in farmed fish may be modified by altering their feed. Fish meal and fish oil, are the most important sources of contamination of farmed fish feed with dioxin-like compounds. EU regulations on polychlorinated dibenzo-*p*-dioxins and furans (PCDD/F) in fish feed were introduced in 2002; the planned inclusion of the dioxin-like polychlorinated biphenyls (DL-PCBs) in the regulations may help to reduce levels of these contaminants in farmed fish.

Fatty fish is an important source of long chain n-3 polyunsaturated fatty acids (LC n-3 PUFA). Other substantial natural sources of LC n-3 PUFA are human milk and marine algae. Farmed fish tend to have higher total lipid levels with lower proportions of LC n-3 PUFA than wild fish. Together, these differences mean that the amount of LC n-3 PUFA per portion of fish is similar. Replacement of fish products by vegetable protein and oils in fish feed or decontamination procedures may be a possible means of reducing some contaminant levels. However modification of the fish oil inclusion rate may change the fatty acid composition and in particular reduce the LC n-3 PUFA levels in farmed fish.

There is evidence that fish consumption, especially of fatty fish (one to two servings a week) benefits the cardiovascular system and is suitable for secondary prevention in manifest coronary heart disease. There may also be benefits in foetal development, but an optimal intake has not been established.

Fish can contribute significantly to the dietary exposure to some contaminants, such as methylmercury, persistent organochlorine compounds, brominated flame retardants and organotin compounds. The most important of these are methylmercury and the dioxin-like compounds, for which high level consumers of certain fish may exceed the provisional tolerable weekly intake (PTWI) even without taking into account other sources of dietary exposure. Such exceedance is undesirable and may represent a risk to human health if repeated frequently. However, eating for example meat instead of fish will not necessarily lead to decreased exposure to dioxin-like compounds. Intakes of the other contaminants in fish reviewed in this opinion were not a health concern, because they do not contribute significantly to total dietary exposure and/or it is very unlikely that even high level consumers of fish exceed the health-based guidance values, if available.

The greatest susceptibility to the critical contaminants, e.g. methylmercury and the dioxin-like compounds occurs during early development. Exposure during this life stage results from the total amount in the mother's body. For methylmercury it is possible for a woman to decrease the amounts in her body by decreasing intake in the months preceding and during pregnancy, whereas this is not possible for the PCDD/Fs and DL-PCBs because it would take many years to decrease the levels in the body significantly.

This evaluation focussed on fish that are widely available in the EU, and likely to be consumed most frequently. Of these, the highest levels of methylmercury are found in tuna, which is mostly caught from the wild. The fish with the highest levels of PCDD/F and DL-PCBs are herring which are caught from the wild and salmon which are mostly farmed.

Frequent consumers of Baltic herring and wild Baltic salmon are more likely to exceed the PTWI for PCDD/F and DL-PCBs than other consumers of fatty fish

Overall the Panel concluded that with respect to their safety for the consumer there is no difference between wild and farmed fish.

Key words: fish contamination, evaluation overview, PCDD/F, PCB, methylmercury, polybrominated flame retardants, quality of feed, wild and farmed fish, Baltic herring, nutritional composition, beneficial effects, fish consumption

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BACKGROUND

Consumption of fish is considered to be an important element of a balanced human diet. The aquatic environment, from which it is derived, however, is also the ultimate repository for a considerable range of natural and anthropogenic contaminants. These may be found in the waters themselves, in the sediments, or within the components of the natural web of productivity, and can concentrate in the tissues of fish and shellfish used for human consumption which feed within that ecosystem. Awareness of possible risks associated with such contamination has led to greater controls over release of many contaminants into the ecosystem and levels in the biota are falling for some but not all of these contaminants in many areas.

Following a proposal presented by the Commission on 28 August 2001, the Council adopted on 29 November 2001 a Council Regulation amending Commission Regulation (EC) No 466/2001 of 8 March 2001, setting maximum levels for certain contaminants in foodstuffs. This Regulation establishes maximum levels for dioxins and furans in several foodstuffs, including fish and fishery products and products thereof. For a temporary period ending in 2006, the Council granted Finland and Sweden derogation from the application of the maximum levels for wild fish originating from the Baltic region intended for consumption in their territory. The Commission accepted this derogation taking into account that a significant part of the Baltic fatty fish will not comply with the maximum level and would thus be excluded from the Swedish and Finnish diet and such an exclusion of fish from the diet may have negative health impacts. In Sweden and Finland governmental authorities have issued dietary recommendations regarding the consumption of fatty fish from the Baltic and a few big lakes. In Sweden, girls and women of childbearing age are advised not to eat certain fatty fish species from a well defined area more often than once a month. The rest of the population is advised not to eat the same species from the same areas more often than once a week. In Finland children, young people and people at fertile age are advised not to eat large herring or wild salmon from the Baltic more than one or twice a months. Denmark has recently introduced restrictions on the landing of wild salmon over 4.4 kg because of elevated levels of dioxins above EU limits. (Danish national order number 1145 of 25/11 2004).

In the lay press and in a small number of scientific publications, concerns have been expressed that levels of compounds such as dioxins and heavy metals such as mercury represent a health hazard to human consumers even at the current levels found in the tissues of fish. For example, Hites *et al.* (2004a,b) claimed that consumption of farmed salmon may pose serious risks due to contamination which would detract from any beneficial effects of consuming it. In all of these discussions, the main weight of emphasis has been on the chemical assessment and putative health risk of consumption of wild and farmed fish, but no consideration was given to the nutritional value of fish consumption.

TERMS OF REFERENCE

The European Parliament requests the EFSA to conduct a scientific assessment of the health risks related to the human consumption of wild and farmed fish (salmon and other carnivorous fish species farmed in substantial quantities) marketed in the European Union. The assessment should focus on the presence and adverse effects in these fish species of persistent organochlorine pollutants (POPs) and other contaminants for which adequate analytical data exists, and on the methodologies for setting safety limits. The European

Parliament also requests the EFSA that the scientific assessment should cover an overall impact and risk assessment of the consumption of Baltic herring.

Interpretation of the terms of reference by the Panels

The EFSA notes that methodology for setting limits for contaminants in fish is a risk management function of the European Commission. Therefore the EFSA provides a scientific opinion on the health risks related to the human consumption of wild and farmed fish. The opinion concentrates on some fish species (farmed, wild, marine, freshwater, lean, and oily) marketed to a significant amount in the European Union. The assessment focuses on those chemicals generally considered most relevant in the context of health risks of fish consumption and for which substantial analytical data exist.

An EFSA Interpanel working group consisting of members of the Panels on Contaminants in the Food Chain (CONTAM Panel), Dietetic Products, Nutrition and Allergies (NDA Panel), Additives and Products or Substances used in Animal Feed (FEEDAP) and Animal Health and Welfare (AHAW Panel) was set up to prepare this opinion.

The scientific opinion addresses:

- The influence of season and life history stage of fish on the nutrient levels and the contaminant levels in fish (selecting the right fish comparator)
- The quality of feed and feeding practices and its impact on the pattern of contaminants in fish
- A comparison of the nutritional composition of wild and farmed fish
- The beneficial effects associated with fish consumption
- An evaluation of relevant contaminants in fish and comparison with health based guidance values for risk characterisation
- An overall impact and risk assessment of the consumption of Baltic herring.

Chapter 5 on nutritional composition of wild and farmed fish and chapter 6 on beneficial effects associated with fish consumption were endorsed by the NDA Panel. The opinion was adopted by the CONTAM Panel.

ASSESSMENT

1. General introduction

Food obtained from the aquatic environment may be of plant (seaweeds) or animal origin. Animals however comprise by far the larger component. They may be representatives of the Invertebrata or the Vertebrata and represent a much greater range of taxonomic diversity than is found in food from terrestrial sources (Bone *et al.*, 1995). They all inhabit a continuous medium and there is some redistribution of energy and anthropogenic contaminants from oceans back to rivers with migratory fish and birds. Generally, however, the flow of energy, and any contaminants, is from land and rivers to the oceans.

Although certain forms of shellfish culture and extensive finfish culture have existed for 3,000 years or more, food from the aquatic environment has traditionally been derived from a hunter-gatherer process involving totally wild populations. Recently this process has become increasingly efficient with the advent of high-powered vessels, sophisticated fishing gears and advanced fish finding equipment. As a result, over-fishing has led to a situation where, despite

increased catching effort, e.g. by deep sea fishing, world total annual catches have been virtually stable or declining since 1986 ranging between 88 and 98 million metric tons (FAO, 2003a).

Any consideration of fish consumption in Europe has to take account of the international nature of fish production and trading, since fish is one of the most widely traded commodities. While EU fish catches have been declining, consumption has been increasing by at least 1 % per annum for more than a decade (FAO, 2003a). The difference has been met by increased imports of wild caught fish and, particularly, by the huge increases in both EU derived and imported farmed fish. There is not, however, much direct substitution, as Atlantic salmon, trout and sea bass are the principal products of the expansion of European aquaculture whereas it is the whitefish and in particular the gadoids, that represent the main reduction in production from the capture sector. Production of all species from aquaculture has risen from about 1 million tonnes in 1960 to 46 million tonnes in 2000, the latest date for which statistics are available (FAO, 2003a). At last count, 210 species of finfish, shellfish, molluscs and aquatic plants were cultivated for human consumption (Tacon, 2003). This total included 131 species of finfish, 42 mollusc species, 27 crustacean species, 8 plant species and two amphibian and reptile species. Global finfish production from aquaculture was 23 million tonnes, mollusc production was 11 million tonnes, crustacean production was 1.6 million tonnes and aquatic plant production was 10 million tonnes in 2000 (FAO, 2003a).

China is the largest aquaculture producer, at 32 million tonnes, followed by India (12 million tonnes), Japan (1.3 million tonnes), Philippines and Indonesia (1 million tonnes) and Thailand (0.7 million tonnes) (FAO, 2003a). Norway is the largest salmon producer (488,000 tonnes), with the US and Chile not far behind (428,000 and 425,000 tonnes). US production of Pacific salmon, included in this figure, however, is a mix of wild, farmed and ranched production.

Traditionally in Western Europe, marine species such as cod, haddock, mackerel and herring, and canned Pacific salmon, have been consumed in significant quantities, with fish such as the Atlantic salmon and rainbow trout being much less available. In Mediterranean countries, a wider range of species including sea bass, bream and tunas, have been consumed, while in Germany and eastern countries of Europe, freshwater carp from pond culture has traditionally been of significance. Imports of frozen or canned fish have also been considerable, particularly tunas, white fish such as hake and cod and Pacific salmon.

The importance of fish and in particular the fatty fishes such as herring, mackerel, tuna and salmon, as a dietary source of long chain n-3 polyunsaturated fatty acids (LC n-3 PUFA), is well recognised. Unfortunately it is also within the lipid component of the fish that lipid-soluble contaminants such as dioxins and polychlorinated biphenyls (PCBs) are stored. 'Fish' is a generic term and there are wide differences in both nutritional value and in potential contaminant levels, depending not only on the origin and the fish species, but also on the tissue sampled, the season of harvest and, for farmed fish, the content of the diet. The diet of wild fish is totally beyond human control (apart from global measures to decrease the release into the environment) and it is only with the development of formulated diets, for farmed species such as salmon and sea bass that the opportunity to directly control tissue contaminant levels has become available. Such considerations are essential in relation to any assessment of comparative contaminant levels and their significance. Comparisons between farmed and wild fish are particularly difficult in this context, and it is essential to compare like with like. Hites *et al.* (2004a,b) for example compared farmed Atlantic salmon (*Salmo salar*) harvested in mid reproductive cycle from Atlantic Oceanic waters and wild Pacific salmon (*Oncorhynchus* spp), of different species, captured in pre-spawning condition, from Pacific coastal waters.

The inter-relationship between lipid levels and any possibly harmful contaminant levels in fish is one which has to be addressed in any dietary recommendations. Such recommendations require understanding of the factors involved and the comparative risks of recommending an increase or reduction in fish consumption. This assessment focuses on selected wild and farmed fish species consumed in Europe (chapter 2). Chapter 3 describes the factors that may influence the levels of contaminants in fish, which would need to be standardised to allow a robust comparison of nutrients and contaminants in wild and farmed fish. Chapter 4 addresses the contribution of diet to levels of contaminants in and the opportunities for reducing contaminant levels in farmed fish.

The assessment also reviews the nutrients in fish (chapter 5) and the specific beneficial effects of eating fish (chapter 6).

A wide variety of contaminants may be present in fish. In particular, there are numerous lipophilic organochlorine contaminants in the environment including polychlorinated dibenzo-*p*-dioxins and furans (PCDD/F), polychlorinated biphenyls (PCBs), camphechlor, hexachlorocyclohexane, dichlorodiphenyltrichloroethane (DDT) and its metabolites (DDD, DDE), chlordane, dieldrin, aldrin, endrin, heptachlor and hexachlorbenzene. Most of these compounds are no longer produced, levels in the environment are generally decreasing and they tend to occur in parallel. It is beyond the scope of this opinion to review all of them in detail, or to evaluate the toxicological data. The assessment therefore focuses on some examples of heavy metals and lipophilic contaminants for which adequate data are available and for which previously established health-based guidance values may be exceeded by some consumers (chapter 7).

2. Selection of fish species

The amount of fish on the EU market was used as the criterion for the selection of relevant fish species to be considered in this opinion.

This was based on the statistics from FAO (FAO, 2003a), see Table 1. It should be noted that calculations give an overall picture of the landings but cannot take into account local situations in which a group of consumers would eat a single species not considered as significant at the EU level or which would be imported from third countries.

Table 1. Landings of Fish Species in EU (FAO, 2003a)
(<http://www.fao.org/fi/statist/fisoft/FISHPLUS.asp>)

Fish species	Catches/produced Tonnes/year	Production/inhabitant** kg/year
Salmon	674,000*	1.50
Herring	651,000	1.45
Anchovy	387,000	0.86
Tuna	353,000	0.79
Mackerel	357,000	0.79
Pilchard	336,000	0.75
Rainbow trout	227,000	0.50
Carp	131,000	0.29
Cod	76,000	0.17
Sea bream	71,000	0.16
Haddock	53,000	0.12
Sea bass	48,000	0.11
Perch	13,000	0.03
Pike	11,000	0.02
Eel	6,000	0.01

* For salmon the figure includes Norway

** Assuming 450 million inhabitants

According to the terms of references, species produced in significant amounts in the European Union should be considered in this opinion. For practical reasons this was defined as species produced in amounts higher than 100,000 tonnes/year. In consequence the selected fish species for consideration in this opinion are rainbow trout, salmon, tuna, herring, mackerel, pilchard anchovies and carp. However the Panel noted that on a national level some other species such as cod, seabream, haddock and seabass may also be consumed in significant amounts. Nevertheless, the list of those species considered significant for this opinion, includes three which are almost entirely produced by culture (Atlantic salmon, rainbow trout and common carp). The remainder are derived from commercial fisheries.

Fish species are often categorized into carnivore, omnivore, detritivore and herbivore (De Silva and Anderson, 1995). This has an influence on the concentration of contaminants which derive from the food chain. Of the eight species compared in this study, four (salmon, trout, tuna and mackerel) are carnivores, at the top of the food chain; the others would generally be classified as omnivores.

Table 1 of Annex 1 lists common and Latin names of some fish species.

The panel noted that from the available statistics it would appear that about two thirds of the fish consumed in Europe is from wild sources and one third is farmed.

3. Influence of species, life-stage and season on nutrient and contaminant levels in fish

Comparing chemical contaminant levels in fish is complicated by the variation that is induced by the age of the fish, the geographic origin and the season the fish is harvested or caught. In the wild older fish are generally larger, and will eat larger prey species. Thus they have the opportunity to accumulate higher levels of contaminants over a longer period than their

younger, smaller peers in the same population. Influences of the fish species, environment and where relevant, the farming system, on possible contaminant levels are many and varied. It is generally accepted that most contaminants are derived from the aquatic food chain rather than directly from the water.

Because storage lipids, the main repository tissue for lipophilic contaminants in fish, vary widely with season and life stage, this leads to significant inconsistencies in recorded level of contaminants depending on species, age and tissue sampled. For example, all species store lipid prior to maturation and subsequently transfer maternal nutrients to developing ovaries. Consequently, fish captured or harvested in the early stages of maturation will have high lipid contents in tissues and organs, whereas those captured or harvested after spawning will have low lipid contents in tissues and organs. Cod are very fatty in the spring and summer months when plankton and/or prey levels are high, and most of this fat is stored in the liver. In winter they have lower levels of liver lipid.

Salmon on the other hand store most fat in the abdominal peritoneal lipid tissue, the intermyotomal fascia and particularly in the dermis of the skin, not in the liver. Mackerel and tuna, other oily fish, are particularly likely to store fat in skeletal muscle fascia. Thus the lipid and contaminant levels to be recorded from fish are critically dependent on which tissue is sampled and generally at what time of year.

Differences in life stage also complicate comparisons between farmed and wild salmon contaminant levels for the same species. Farmed salmon are generally harvested well before reaching sexual maturity, whereas wild salmon are generally captured just before ascending rivers to spawn. At this stage wild fish have ceased to eat and much of their lipid will already have been transferred from muscle fascia to gonad. This will influence the comparison between levels of contaminants in edible tissues of farmed and wild fish. Among salmonids the situation is also dependent on the species of salmon. For example, there are five species of Pacific salmon that are captured or farmed in significant numbers. Chinook and coho salmon are farmed as well as being captured as wild fish, whereas sockeye, pink and chum salmon are only captured (although a proportion of these are sea-ranched in Alaska). The five species of Pacific salmon have different life histories and also exploit different ecological niches and target different trophic levels and species as their principal prey. Sockeye salmon are planktivores, whereas chinook and coho salmon are carnivorous, consuming mainly small forage fish along with squid or shrimp (krill) (Groot *et al.*, 1995). Pink and chum salmon are somewhat between sockeye and the top predator salmon species in terms of target prey. Interpretation of comparisons of contaminant levels found in farmed and wild salmon should take this into account. Some of the Pacific species which have been claimed to have lower contaminant levels than farmed Atlantic salmon (Hites *et al.*, 2004a,b) are omnivores, and should not be compared with carnivores at the top of the food chain.

Thus there is clearly a need for standardization of sampling procedures as well as analytical protocols if any valid comparisons of contaminant levels are to be made between different fish species and between fish derived from farmed and wild sources.

Aquaculture

Finfish aquaculture may be carried out in cages in fresh water lakes or in ponds, tanks or raceway systems supplied by ground water (springs or wells) or surface water (reservoirs or rivers). Some freshwater facilities re-use water (recirculation systems) and many have sophisticated water filters, heating systems and waste water effluent controls. Such systems are only suitable for producing high-value products, such as salmon smolts or exotic species for specialized live markets. Aquaculture is also carried out in seawater, often called

mariculture, where fish are often hatched in sophisticated pumped seawater hatcheries, on land, but grown on in cages in the sea. Because of the limits of freshwater availability it is likely that any major future expansion of aquaculture will be in the sea.

Aquaculture, irrespective of medium, is of three basic types: extensive, semi-intensive and intensive. Extensive aquaculture, where fish are held in low density earthen ponds and expected to forage for themselves, with possibly some fertilization of the pond to enhance eutrophication, was a long-standing method of production of considerable tonnages of carp in China and also Eastern Europe. Production from extensive systems typically ranges from 300 - 500 kg/hectare/crop. Cost of land and limited sources of water have, however, driven production towards semi-intensive culture, where feed is given to supplement the natural feeding and, increasingly, towards intensive production, where all of the nutrients required for growth are provided by means of an extraneous formulated diet. Production from semi-intensive systems ranges from 1,000 - 3,000 kg/hectare/crop, whereas in intensive production, yields of 5,000 - 10,000 kg/hectare/crop can be achieved. Higher inputs increase the cost of production, but proportionally higher yields make such systems economical to operate.

Another form of aquaculture practised in some regions is sea ranching. This makes use of the homing instinct of fish such as the salmonids. Very large numbers of fry are reared intensively in hatcheries and released into the sea, where they feed on their natural prey species. They may then be captured at sea, as happens in the Baltic, where almost all fish are originally derived from hatcheries, or when they return to the estuary of their home river, as in the case of Alaskan salmon, some 30 % of which are hatchery derived. Sea ranching is a relatively low-input form of aquaculture, but only suitable for homing species or those that remain close to the vicinity of their release point.

In mariculture, the largest tonnages of finfish in production terms are Atlantic salmon (0.9 million tonnes) produced principally in Norway, Chile, Scotland, Ireland and Canada. Japanese amberjack is the next largest marine production species at 137,000 tonnes. Sea bass/sea bream produced in Spain, France, Italy, Greece and tuna produced in Australia, Croatia and Malta are growing but still small mariculture crops compared to production of established species. Newer species now in culture include cod, halibut, turbot and barramundi. All mariculture species are produced in floating cages at sea, although the Atlantic salmon has to have a fresh water stage of 6-12 months in fresh water before going to sea.

4. Feeding practices, quality of feed and transfer of contaminants into fish

4.1. Introduction

In 2000, an estimated 15 million tonnes of feedstuffs were produced for aquaculture, using approximately 2.4 million tonnes of fish meal and 550,000 tonnes of fish oil produced from marine forage species not used to supply seafood for human consumption. Approximately one-third of all seafood landings are used to make fish meal and fish oil, with the remaining two-thirds consumed directly.

All of the key economic species farmed for the Western market are top trophic-level predators which in their major growth phases, hunt and consume prey species, principally finfish and crustaceans. They derive metabolic energy from protein metabolism and consequently carbohydrate plays little part in their energy supply (Halver and Hardy, 2002). They also require significant levels of certain PUFAs. In the wild, they acquire these from the prey species that concentrate them from marine plankton but they must be supplied in farmed fish

diets, generally from fish oils. Salmonids, e.g., Atlantic salmon, Pacific salmon, trout and char, are an exception to this requirement as they can to some degree synthesise their own longer chain PUFAs from 18-carbon, n-3 precursors found in plant oils. This ability to synthesise LC n-3 PUFA is severely reduced in wild fish following migration to seawater.

Tuna farming at present depends on capturing young tuna at sea and holding them in very large cages off shore for a year or more. They are fed by-catch species and do not currently receive formulated diets.

The contribution of different feedstuffs to compound feed varies broadly with the category of fish because of the species dependent adaptation of digestive systems to dietary substrates like starch, crude fibre and fat. In general, the majority of relevant fish species needs mixed diets, but varying ingredients in the compound feed. Table 2 lists typical diets for omnivorous (emphasizing plant feedstuffs) and carnivorous fish species.

Table 2: Typical composition of omnivorous and carnivorous fish diets (EC SCAN, 2000)

Feed materials	Omnivorous fish	Carnivorous fish
	(fish diet expressed in %)	
Cereals (wheat, corn)	30	11
Oilseed meals (soybean meal)	56	7
Corn gluten meal	-	5
Fish meal	10	50
Fish oil	2	25
Premix*	2	2

* Includes minerals, trace elements, vitamins, single cell proteins and other feed additives

4.2 Transfer of contaminants into fish

Diet is a main source of exposure to a wide range of contaminants in fish, although uptake also occurs via the gills. In farmed fish the level of contaminants in feed materials can be monitored and controlled, whereas in wild fish exposure remains unknown and will vary considerably in different geographical regions.

4.2.1. Dioxins and dioxin-like PCBs (DL-PCBs)

The concentrations of persistent organic contaminants are highly variable as demonstrated for PCDD/Fs and DL-PCBs in Table 3 and depend primarily on the inclusion level and type of lipid in the diet.

Table 3. Variation of dioxin (PCDD/F), dioxin-like PCB (DL-PCB) and total (PCDD/F + DL-PCB) contamination of selected feedstuffs in fish nutrition (ng WHO-TEQ/kg 12% moisture content) for different feedingstuffs sampled between 1997 and 2004 (upperbound concentrations) (adapted from Gallani *et al.*, 2004).

FEEDINGSTUFFS	N	DIOXINS + FURANS			DIOXIN-LIKE PCBs			TOTAL TEQ		
		Low 5 th %ile	Median	High 97.5 th %ile	Low 5 th %ile	Median	High 97.5 th %ile	Low 5 th %ile	Median	High 97.5 th %ile
Feed materials of plant origin	169	0.03	0.12	0.72	0.02	0.07	0.35	0.06	0.22	0.95
Vegetable oils	17	0.12	0.20	0.35	0.08	0.17	0.81	0.30	0.39	1.03
Premixes*	28	0.03	0.11	0.58	0.02	0.03	0.81	0.06	0.23	1.07
Animal fat, incl. milk and egg fat	42	0.08	0.22	0.81	0.03	0.18	0.81	0.12	0.41	1.62
Fish oil	222	0.87	3.17	6.48	2.10	6.75	16.84	3.2	9.95	22.56
Fish and other aquatic animals and their products (fish meal)	104	0.10	0.46	1.98	0.13	1.01	4.23	0.33	1.48	5.85
Feedingstuffs for fish	188	0.1	0.63	2.01	0.04	1.34	3.30	0.16	2.01	5.09

* Two data have been removed from the database as they are clearly to be considered as outliers.

Data (EC SCAN, 2000) indicate that fish oil and fish meal from European production contain higher levels of PCDD/F and DL-PCBs than fish oil and fish meal of the South Pacific origin. In omnivorous diets the contribution of fish products to the total contamination with PCDD/F and DL-PCBs was above 55 % and may be up to 98 % in carnivorous diets (EC SCAN, 2000). The final feed will comply with the actual regulatory limit (2.25 ng/kg) if the individual components also comply with their respective limits (fish meal, 1.25 ng/kg; fish oil, 6 ng/kg).

Karl *et al.* (2003) reported data on the transfer of PCDD/F from commercial fish feed produced in Norway into the edible part of rainbow trout (*Oncorhynchus mykiss*). In muscle tissue PCDD/F increased continuously up to the end of the experimental period (0.914 ng PCDD/F WHO-TEQ/kg meat). The mean transfer rate ranged from 11.1 % at 6 months to 30.7 % at 19 months. The transfer rate in females appeared lower possibly as a result of distribution into the eggs. A direct correlation ($r^2 = 0.98$) between concentration in the lipid fraction of feed and in fish was observed.

The transfer rate for DL-PCBs was shown to be higher than that of PCDD/F in Atlantic salmon (Isosaari *et al.*, 2004; Lundebye *et al.*, 2004) and rainbow trout (Isosaari *et al.*, 2002). Table 4 shows dioxin and DL-PCBs contamination matter of Atlantic salmon due to feeding differently contaminated feed. Considerable variation was observed in transfer rates of dioxins (40 – 65 %) and DL-PCBs (78 – 93 %) because of different transfer of the congeners from feed to fillet in Atlantic salmon over an entire production cycle (Berntssen *et al.*, 2005). Among PCDD/Fs, tetra- and pentachlorinated congeners were found to be preferentially accumulated in salmon, while hepta- and octachlorinated dibenzo-*p*-dioxins were excreted into the feces. Congener patterns that were associated with a preferential accumulation of PCBs in salmon included non-*ortho* and tetrachloro congeners. Non-*ortho* tetrachloro congeners were preferentially accumulated compared with other non-*ortho* and mono-*ortho* PCBs (Isosaari *et al.*, 2004).

Table 4. Dioxin and DL-PCBs in Atlantic salmon following feeding of differently contaminated mixed feed (A-D) over 30 weeks (Lundebye *et al.*, 2004)

Diet		A	B	C	D
Dietary contaminants from different fish oil sources (pg WHO-TEQ/g dry matter)	PCDD/F	0.71	1.7	3.89	4.89
	DL-PCBs	2.79	3.65	4.99	5.40
	Total	3.5	5.35	8.88	10.3
Contaminants in whole fish* (pg WHO-TEQ/g wet weight)	PCDD/F	0.58	1.13	2.02	2.37
	DL-PCBs	2.35	2.97	3.67	4.01
	Total	2.93	4.10	5.69	6.29

* At the end of the experimental period (30 weeks)

A model calculation based on different assumed transfer rates, indicates that even with a very high transfer rate (80 %), the total PCDD/F can be expected to be about 50 % of the current maximum permitted EU level for PCDD/F in fish, i.e. 4 pg WHO/TEQ/g wet weight (Commission Regulation (EC) No 466/2001).

There is only a limited database for a reliable comparison of wild and farmed fish and data on the related dietary contamination are mostly not available.

A recent overview on dioxins and DL-PCBs in fish in the EU is provided in Table 5, but this does not allow a comparison of wild and farmed fish as it includes only very partially the same species. The farmed fish is mainly dominated by salmon and trout, whilst the wild fish

covers a wider range of fish species, and wild salmon and trout are very minor contributors in the data base of wild fish.

The Swedish Food Administration is monitoring basic data regarding the concentration of non-readily biodegradable environmental organic contaminants in fatty fish from Sweden (Baltic Sea, lakes Vänern and Vättern, waters along Sweden west coast). PCDD/F levels in fatty fish from Sweden 2000 - 2003 including wild and farmed fish species are provided in Table 2 of Annex 1. However conclusions based on comparison of these results should be made with care because of differences in size of fish and the season and location that they were caught.

Table 5. Occurrence data on dioxins, furans and dioxin-like PCBs in food based on Gallani *et al.* 2004¹. Data are expressed as ng WHO-TEQ/kg fresh weight

FOOD	N SAMPLES	DIOXINS + FURANS				DIOXIN-LIKE PCBs				TOTAL TEQ				
		Average	Median	90 th %ile	95 th %ile	Average	Median	90 th %ile	95 th %ile	Average	Median	90 th %ile	95 th %ile	99 th %ile
FISH Total – wild + farmed except fish from the Baltic region	(426)	0.55	0.34	1.12	1.76	1.39	0.91	3.20	3.81	1.93	1.23	4.37	5.16	12.09
- wild	(215)	0.59	0.18	1.63	2.19	1.41	0.47	3.16	5.58	2.00	0.70	4.67	7.31	19.09
- farmed	(211)	0.50	0.40	0.89	1.06	1.36	1.01	3.18	3.61	1.87	1.44	4.03	4.60	6.15
- Herring	(53)	1.29	1.06	2.29	2.64	1.90	1.70	3.01	3.74	3.20	2.83	5.29	5.91	9.17
- Fish other than herring	(373)	0.44	0.29	0.88	1.11	1.31	0.82	3.21	3.81	1.76	1.10	4.03	4.71	14.10
BALTIC FISH (total)	(340)	5.44	3.1	14.53	18.14	3.48	2.3	7.52	10.01	8.92	5.39	22.58	27.28	33.73
- Baltic herring	(173)	7.67	4.76	17.70	20.46	3.79	2.85	7.77	9.79	11.46	7.71	25.95	29.80	35.60
- Baltic processed herring	(14)	10.75	11.26	16.28	18.25	5.20	5.28	7.70	8.65	15.96	16.54	23.98	26.89	29.00
- Baltic pike-perch	(39)	1.09	0.72	2.38	3.55	1.24	0.91	2.52	3.15	2.34	1.62	4.90	6.68	9.58
- Baltic salmon	(22)	7.23	6.27	14.17	15.61	9.24	7.8	14.29	15.25	16.47	13.55	27.38	29.85	32.35
- Baltic other ²	(89)	1.67	1.20	3.60	3.88	2.10	1.76	4.18	5.72	3.77	3.2	7.72	8.86	10.74
Herring from other regions compared to herring from Baltic region														
- Herring – other regions	(53)	1.29	1.06	2.29	2.64	1.90	1.70	3.01	3.74	3.20	2.83	5.29	5.91	9.17
- Baltic herring	(173)	7.67	4.76	17.70	20.46	3.79	2.85	7.77	9.79	11.46	7.71	25.95	29.80	35.60

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¹ Based on data contained in the report “Dioxins and PCBs in Food and Feed: Data available to DG SANCO – Joint Report DG SANCO / DG-JRC-IRMM” and data submitted after 31 January 2004 by the EU-Member States (only data from 1998/1999 onwards have been taken into account. NB: farmed fish is mainly dominated by salmon and trout; wild fish covers a wider range of fish species with few wild salmon and trout.

² Burbot, sprat, bream, white fish, roach, vendace, flounder, smelt, eel, brown trout, cod. The three samples of River lamprey are not included (high levels).

4.2.2. Non-dioxin-like polychlorinated biphenyls (NDL-PCB)

Data on the level of contamination of fish feed with NDL-PCB (expressed as the sum of the 6 indicator PCB) presented in table 6 show that mean concentrations vary from 10.7 to 54.7 ng/g depending on the type of feed product.

Table 6. NDL-PCB (Σ 6) contamination of selected feeding stuffs in fish nutrition (ng/g product)

Feedingstuff	N	Mean	Median	Min	Max
Feed material of plant origin	55	10.7	10.0	0.99	19.6
Fish oil	29	54.7	48.6	24.9	94.9
Fish and fishery products	17	25.6	7.47	0.41	66.7
Feedingstuffs for fish	46	19.4	13.9	5.48	40.1

Based on the information presented in the opinion of the Scientific Panel on Contaminants in the Food Chain on the Presence of NDL-PCB in Food and Feed (EFSA 2005, in preparation) it can be concluded that transfer of NDL-PCB from fish feed into fish is comparable to that in other animal species. Higher chlorinated indicator PCB (PCB 138, 153, 150) shows a greater transfer than the lower chlorinated congeners.

Just as for dioxins and DL-PCB, also for NDL-PCB there is only limited information allowing a comparison between levels in farmed and wild fish. An overview on levels of NDL-PCB in fish from EU Member States is given in table 7, showing particularly high levels of NDL-PCB in Baltic fish.

Table 7. Occurrence of NDL-PCB (Σ 6) in fish and fish products in ng/g fish.

Fish	N	Mean	Median	Min	Max
Fish and fishery products (including Baltic fish)	1620	12.5	3.98	0.42	30.6
Baltic fish	275	32.9	16.7	3.54	83.3
- all fish					
- herring	152	38.8	28.4	9.17	82.5
- salmon	18	92.0	109	12.3	14.5

4.2.3. Polybrominated flame retardants

Recent market basket studies have detected polybrominated diphenyl ethers (PBDEs) in a wide range of food, including fish and other seafood species, and national food surveys have identified the diet as one of the main sources of human exposure to these brominated flame retardants (BFRs) (Bocio *et al.*, 2003; Ohta *et al.*, 2002; Schecter *et al.*, 2004).

Information related to fish feed contamination with PBDEs is limited (Bethune *et al.*, 2005, Table 3 of Annex 1; see also www.mattilsynet.no). Dietary accumulation of PBDEs has been investigated in feeding trials with Atlantic salmon (Isosaari *et al.*, 2005), zebra fish (Andersson *et al.*, 1999), juvenile common carp (Stapleton *et al.*, 2002, 2004a,b,c), pike (Burreau *et al.*, 1997, 2000), juvenile rainbow trout (Kierkegaard *et al.*, 1999) and juvenile lake trout (Tomy *et al.*, 2004). A wide range of congener-dependent accumulation was reported, ranging from less than 0.02 to 5.2 % for BDE 209 (Kierkegaard *et al.*, 1999; Stapleton *et al.*, 2002) to more than 90 % for BDE 47 (Burreau *et al.*, 1997, 2000; Stapleton *et al.*, 2004b). Isosaari (2005) found that Atlantic salmon accumulated PBDEs from feed as efficiently as non-*ortho* PCBs and more efficiently than other PCBs and PCDD/Fs (Isosaari *et al.*, 2004). Biotransformation and/or preferential accumulation of certain PBDE congeners led to differences in the congener patterns between feeds and fish (Isosaari *et al.*, 2005).

Summarized data from different studies on the occurrence of PBDEs in fish samples (Table 4 of Annex 1) show considerable variation in concentrations. PBDE concentrations up to 3 – 4 ng/g fresh weight have been reported in salmon fillets (with skin), and concentrations ranging from 0.49 to 10.9 ng/g wet weight have been found in fish feed (Hites *et al.*, 2004b). Farmed European Atlantic salmon were found to have the highest mean PBDE concentration and wild Pacific salmon had the lowest, (Hites *et al.*, 2004b). Similarly a study of 16 PBDE congeners in several fish species conducted by the FSAI (documentation provided to EFSA) found that farmed Atlantic salmon had the highest PBDE concentration of the fish species examined (mean 3.71 ng/g fish; range 2.4 - 5.0 ng/g fish), considerably higher than found in wild salmon (mean 0.86 ng/g fish; range 0.7 - 1.0 ng/g fish). Data from Sweden on wild Baltic salmon (ten pooled samples containing five fishes each) reveal levels similar to those found in farmed Irish Atlantic salmon (mean 2.95 ng/g fish; range 2.15 - 3.94 ng/g fish). However, these and other available data on PBDE concentrations in farmed and wild salmon (Haglund *et al.*, 1997; Manchester-Neesvig *et al.*, 2001; Easton *et al.*, 2002; Jacobs *et al.*, 2002; Bethune *et al.*, 2005) show that there is a large variation among salmon species, sampling locations and salmon individuals, with no consistent differences between wild and farmed salmon.

4.2.4. Camphechlor

Limited data on transfer rates from one study of three camphechlor congeners (#26, 50 and 62, Parlar nomenclature) from feed to rainbow trout are summarized in Table 5 of Annex 1. Average transfer rates seemed to be about 25 % (Karl *et al.*, 2002).

4.2.5. Mercury

The retention of dietary mercury by fish is dependent on dietary concentration and exposure duration. For example, Lock (1975) found that the retention of mercury from feed by rainbow trout decreased from 71 % to 38 % following exposure to 3.4 mg Hg/kg feed and 21.6 mg Hg/kg feed for 1 week respectively. Furthermore, accumulation decreased from 71 % to 31 % following 12 weeks exposure of Atlantic cod (*Gadus morhua*) to 3.4 mg Hg/kg. Mercury from feed appears to have a specific affinity for muscle tissue in fish. (Julshamn *et al.*, 1982). The maximum permitted level of mercury in fishery products in the EU is 0.5 mg/kg wet weight for most species, and 1 mg/kg for a limited list of fish species including tuna (EU, 2001). For fish feed the maximum permitted level for mercury is 0.1 mg/kg (European Community, 2002). Maage *et al.* (2005) reported mean mercury contents for 5 sampling time points between 1995 - 2003 between 0.015 - 0.039 mg/kg wet weight in farmed Atlantic salmon (*Salmo salar*) (range 0.008 - 0.052, n = 225). Average concentrations and ranges for methylmercury in 4 different tuna species are given in table 8.

Table 8. MeHg values (in µg/g fish) in different tuna species (French Authorities, DGAL, DGCCRF, IFREMER, FIAC)

Tuna Species	Skipjack	Albacore	Yellowfin	Bluefin
Number of samples	42	24	89	20
Average	0.15	0.49	0.3	0.49
Min	0.08	0.16	ND	0.26
Max	0.43	1.59	1.28	1.30

ND: Not Detected

4.2.6 Cadmium

Cadmium accumulates primarily in the viscera (intestine, liver and kidney) of fish (Kraal *et al.*, 1995; Berntssen *et al.*, 2001). Transfer of dietary Cd into fish muscle is low (2 – 6 %) (Cincier *et al.*, 1998). High dietary exposure of salmon to Cd concentrations up to 250 mg/kg for four months caused significant accumulation in several tissues including muscle (up to approximately 0.25 mg/kg), whereas there was no significant accumulation at dietary concentrations up to 5 mg/kg (Berntssen *et al.*, 2001). The EU maximum level for cadmium in fish feed is 0.5 mg/kg (European Community, 2002). The cadmium content reported for commercial salmon fillets (n = 225) is less than 0.001 mg/kg (Maage *et al.*, 2005), whereas the maximum permitted level of cadmium in fish fillets is 0.05 mg/kg for most species, and 0.1 mg/kg for certain species of fish (EC No 466/2001).

4.2.7 Lead

Although lead exists in many different forms in marine and fresh waters, most of the lead found in fish is inorganic in nature, and is bound to proteins. Bioaccumulation of lead in marine animals is low compared to mercury (Dietz *et al.*, 1996). Internal organs, and especially skin and bone appear to be the main sites of lead accumulation in fish, whereas lead does not appear to accumulate in fish muscle tissue (Somero *et al.*, 1977). The lead content reported for commercial salmon fillets is less than 0.01 mg/kg, (Maage *et al.*, 2005, n = 225), whereas the maximum permitted level of lead in fish fillets is 0.2 mg/kg for most fish species, and 0.4 mg/kg for selected species (EC No 466/2001). The EU maximum level for lead in fish feed is 5 mg/kg (European Community, 2002)

4.2.8 Arsenic

Marine organisms accumulate arsenic, predominantly as non-toxic arsenobetaine and arsenocholine. Products like fishmeal and fish oil have been identified as major sources of feed contamination with arsenic, and it is likely that the measured arsenic represents predominantly these organic compounds. The EU maximum level for arsenic in fish feed is 6 mg/kg and for inorganic arsenic is 2 mg/kg. (European Community, 2002).

4.3. Reducing levels of persistent organic contaminants

Different strategies in fish feeding are under investigation to reduce fish meal and fish oil in farmed fish diets, mainly due to limited availability of fish oils. The possibilities and limitations of such a “substitution strategy” are mainly related to different fish species and depend on physiological needs.

Between 25 and 90 % of the fish meal in fish feed can be replaced by plant proteins (Lim, 2004) depending on the target fish species, but plant feedstuffs have lower nutrient concentration, lower digestibility and occurrence of certain natural constituents, such as inhibitors of proteolytic enzymes, isoflavones or glucosinolates (Mambrini *et al.*, 1999; Burel *et al.*, 2000). Furthermore, a high concentration of plant phytates, primarily reducing mineral bioavailability in fish diets, is an important limiting factor for the maximum inclusion of plant feedstuffs (Liebert and Portz, 2005).

Substitution of fish oil by vegetable oil in fish feed significantly reduced PCDD/F and DL-PCBs concentration in farmed salmon, *Salmo salar* (Bell *et al.*, 2004; Berntssen *et al.*, 2005). Complete replacement of fish oil by a mixture of vegetable oils (55 % rapeseed, 30 % palm, 15 % linseed) did not lead to any adverse effects on growth, total muscle lipid content or organoleptic properties in rainbow trout (Corraze *et al.*, 2004). Dioxin levels were twenty times lower in salmon fillet following fish oil substitution by vegetable oil (Berntssen *et al.*, 2005). However, these studies indicate significant changes in the n3/n6 fatty acid ratio in fish products due to this substitution, which may have implications for the nutritional benefits of fish consumption. Genetic selection procedures are currently under way to increase the capacity in farmed fish to synthesize longer chain PUFAs from C 18, n-3-precursors found in plant oils.

Another possibility for reducing contaminant levels in feed is the selective use of marine feed ingredients with relatively low natural levels of persistent organic contaminants as previously mentioned. Using certain marine fish oils such as oil from Pacific Ocean species in fish feed can lead to reduced levels of dioxins and to a lesser degree dioxin-like PCBs in farmed Atlantic salmon (Isosaari *et al.*, 2004; Lundebye *et al.*, 2004).

A final option for reducing contaminant levels in feed is the technical removal of contaminants from fish meal and fish oil, e.g. by activated carbon treatment, combined with stripping at low pressure and low temperature (De Kock *et al.*, 2004) or short path distillation (Breivik and Thorstad, 2004). However, practicality of different procedures needs to be demonstrated as well as the efficiency for the removal of different contaminants/congeners and maintenance of the nutritional quality of the oil.

4.4. Conclusions

The level of contaminants in fish is related to the concentration in the diet, and the duration of exposure. Therefore the level of contaminants in carnivorous species irrespective of whether wild or farmed, may be higher compared to omnivorous species. In farmed fish, marine feed ingredients, primarily fish oil and to a lesser extent fish meal, are the main sources of organic contaminants. Maximum permitted levels are set for a range of contaminants in animal feed in the EU (Directive 2002/32/EC). Research and monitoring programmes indicate that these levels ensure that the concentrations of undesirable substances in fish are below maximum levels permitted for these contaminants in food, where such regulations exist. Risk assessments of undesirable substances in animal feed have recently been conducted by EFSA (EFSA, 2004c,d; EFSA, 2005a,b) and are still on-going. The replacement of fish products by plant feedstuffs in fish diets or decontamination procedures may be a possible means of reducing some contaminant levels. Lowering of fish oil inclusion rates of farmed salmon and trout diets could significantly reduce flesh lipid content and hence contaminant levels but this would also change the fatty acid composition of the fish.

5. Nutritional Composition of wild and farmed fish

5.1. General considerations

Data on the nutritional composition of the eight species selected are given in Table 10 (average and minimum and maximum values). The data in this table and in Table 11 are from one source only (Souci-Fachmann-Kraut, 2000) for practical reasons, because datasets from different sources differ widely. The ranges given in Table 10 include, as a rule, the average values of other databases, e.g. from USDA at <http://www.nal.usda.gov/fnic/foodcomp/> and from MAFF (1998) and FSA (2002). Databases on food composition give, in most cases, no indication of the region where the fish was harvested or caught, nor of sampling time, age of the fish and often not the number of samples analysed.

There is a high degree of interspecies variability for all nutrients, except protein, and a considerable degree of intraspecies variability as well, again with the exception of protein. Tables 6, 7 and 8 of Annex 1 demonstrate that under the designation of "salmon", "tuna" and "mackerel" four, three and three different species of fish are listed in the USDA database which varies in their average contents of energy and nutrients.

This physiological variability in nutrient content is due to many factors outlined in chapter 3, and moreover to analytical methods.

5.1.1. Energy

The energy value per weight of edible parts of the eight fish species varies by a factor of 2. This variability is due to the percentage of water in various species, but there is also intraspecies variability of the fat content, e.g. in herring from the Baltic or Atlantic sea, and variability dependent on life-stage. The percentage of water in fish varies inversely to the lipid content (Love, 1980; 1988). When making comparisons of proximate constituents of fish, e.g., lipid and protein, such comparisons should be made on a wet-weight, or as-is basis (Shearer, 1994).

5.1.2. Protein

Fish of all eight species have similar protein content on a weight basis (15 - 20 g/100 g), although the protein content decreases somewhat with age-related increases in the lipid content.

On an energy basis the protein content of fish varies between 7.8 and 20 g/100 kcal and protein provides between 30 and 80 % of the total energy value (Table 10 and 11).

Fish protein, which is essentially muscle tissue with less connective tissue than in terrestrial animals, has a high biological value and is easily digestible (Bohl, 1999). The amino acid content of fish fillets is similar among species. Compared to the amino acid score proposed by FAO/WHO (1991) the content of essential amino acids is high.

The protein content of the edible parts of fish is comparable to that of sheer animal muscle meat (mutton, lamb, veal, beef, pork, rabbit, horse, and goat). Further, the quality of fish fillets is uniformly high, in contrast to cuts from many animals. Fish provides less energy as protein than meat, with the potential exception of rainbow trout (76 %) and anchovy (80 %).

5.1.3 Lipids

Lipid content

The lipid content of fish is much more variable than the protein content, both between species but also in the same species. Fish can be divided into fatty (oily, blue) and lean (white) fish on the basis of the percentage of fat in muscle. Whereas oily or fatty fish accumulate fat in muscle tissue (edible parts of fish), white or lean fish, e.g. cod, accumulate fat predominantly in the liver, which normally is not eaten but is a good source of fish oil. However, the characterisation as lean or fatty is somewhat arbitrary and varies among authorities. Values for categorisation by different authors are given in Table 9.

Table 9. Categories of fish according to fat percentage of body weight

	Fatty (oily, blue)	Intermediate	Lean (white)
FSA, 2004a	5 - 20 %	–	1 – 2 %
Steffens, 1979	> 5 %	1 - 5 %	< 1 %
Danish Fish Assessment, 2003	> 8 %	2 - 8 %	< 2 %

This classification depends on many factors, especially the season. From regular analyses throughout the year for total lipids, protein, energy and fatty acids of 35 species of fish, shellfish and molluscs consumed in Spain a wide seasonal variability of the total lipid and also the n-3 fatty acid content is apparent. Mackerel, a fatty fish, e.g., is "lean" in spring (Soriguer *et al.*, 1997). Seasonal changes in the lipid content and composition were also observed in herring from the North Sea. There was an increase in the percentage of unsaturated fatty acids in summer (23 %) while the lowest values for polyunsaturated fatty acids (14 %) were found between January and March, which is the period after starvation and spawning (Aidos *et al.*, 2002; Grigorakis *et al.*, 2002). This variation in lipid content and especially lipid composition may be one of the reasons for divergent epidemiological observations on the health effects associated with fish consumption or intake of non-standardised fish oils. In general tuna is considered to be a fatty fish. However much of the lipid content including PUFA is lost during the canning process, and canned tuna is not classified as a fatty fish.

In addition, the distribution of stored lipid in fillets is not uniform. In general the lipid content of fillets decreases from head to tail and also from dorsal to ventral. Lipid levels are higher below the skin and in red muscle (Bell *et al.*, 1998).

In the eight species selected mean lipid values (sum of triglycerides and phospholipids) range from 2 - 18 g/100g and from 2.3 to 7.6 g/100 kcal corresponding to 21 – 68 % of the total energy value. The absolute phospholipids content of fish remains relatively constant within species, but triglycerides, the main form of storage lipids, vary greatly. The cholesterol content is 44 - 77 mg/100g or 22 - 54 mg/100 kcal. This is similar to the cholesterol content of mutton, lamb, veal, beef and pork.

Fatty acid composition

The fatty acid pattern of fish lipid varies with species, and is also influenced by dietary lipid intake and water temperature. In many fish species, the percentage of monounsaturated fatty acids (MUFA), of polyunsaturated fatty acids (PUFA) and of long-chain polyunsaturated fatty acids (LC n-3 PUFA) increases with lower temperature of the water, reportedly to maintain membrane fluidity.

There is no typical fatty acid profile which allows the identification of a fish species, but all fish lipids are high in long-chain n-3 polyunsaturated fatty acids (LC n-3 PUFA), with eicosapentaenoic acid, EPA (C 20:5 n-3) and docosahexaenoic acid, DHA (C22:6 n-3) predominating. In the eight fish species chosen for this opinion, between 18 and 28 % of total lipid may be LC n-3 PUFA. Per 100 g fresh weight the eight fish species provide the following average amounts of DHA plus EPA: rainbow trout 0.6 g (DHA/EPA ratio 3.4), salmon 1.29 g (DHA/EPA ratio 2.5), tuna 1.5 g (DHA/EPA ratio 1.5), herring 1.17 g (DHA/EPA ratio 0.3), mackerel 0.96 g (DHA/EPA ratio 1.8), pilchard 1.18 g (DHA/EPA ratio 1.4), anchovy 0.5 g (DHA/EPA ratio 1.4) and carp 0.26 g (DHA/EPA ratio 0.5). This illustrates that consumption of the same amount of different fish can result in different intakes of LC n-3 PUFA. Moreover, the relative contribution of DHA or EPA varies, with DHA dominating in decreasing order in rainbow trout, salmon, mackerel, tuna, pilchard, anchovy, carp and herring (Table 11).

Per 100 g fresh weight the eight fish species provide on average the following amounts of lipids, rainbow trout 2.7 g lipids of which 23 % are DHA plus EPA; salmon 13.6 g lipids of which 19.2 % are DHA plus EPA; tuna 15.5 g lipids of which 22.2 % are DHA plus EPA; herring 17.8 lipids of which 15.3 % are DHA plus EPA; mackerel 11.9 g lipids of which 14.8 % are DHA plus EPA; pilchard 4.5 g lipids of which 31 % are DHA plus EPA; anchovy 2.3 g of lipids of which 21.5 % are DHA plus EPA; carp 4.8 g lipids of which 6.1 % are DHA plus EPA (Table 11).

Per an energy equivalent of 100 kcal of the edible parts of fish the LC n-3 PUFA content is between 0.3 and 1.6 g and per 100 g of lipids between 6 and 30 g (Table 10 and 11). A small percentage of C-18n-3 PUFA, α -linolenic acid (LNA), can be measured. However, if fish are fed dietary lipids with high levels of LNA, tissue levels will be higher. The DHA to EPA ratio on a weight basis for the eight fish species is 0.3-3.4, highest in rainbow trout and lowest in Atlantic herring. Seawater fish require EPA and DHA in their diet (phytoplankton, zooplankton and other fish), whereas freshwater fish, e.g. trout and salmonids, have high 6- and 5-desaturase activity and can form LC n-3 PUFA from dietary LNA acid (Bohl, 1999; Henninger and Ulberth, 1997). Phospholipids are particularly rich in LC n-3 PUFA (Rueda *et al.*, 1997).

In contrast, n-6 PUFA are generally low in fish fat both in absolute amounts and in relation to the total fatty acid content, except in farmed fish that are fed diets containing high amounts of n-6 PUFA. Arachidonic acid (C20:4 n-6; AA) is relatively low in all species and the ratio EPA/AA is between 3.9 and 16.8, except in Atlantic herring where it is especially high (56.6), and in freshwater salmonids.

The ratio of total n-3 to n-6 fatty acids is typically far greater than 1 in most fish species: it is 3.0 in rainbow trout, 5.7 in salmon, 8.8 in tuna, 21.2 in herring, 6.7 in mackerel, 15.9 in pilchard, 9.6 in anchovy and 1.3 in carp (Table 10).

All these values are average content values covering a wide range and, therefore, cannot be considered as characteristic for all fish of the same species. However, the special nature of fish lipids is apparent when compared to fats and oils of other animal or vegetable origin, e.g. the n-3/n-6 ratios of olive oil are 0.07 - 0.4, of rapeseed oil 0.2 - 1.2, of soya bean oil 0.08 - 0.2, of lard (pork) 0.25 - 1.2, and of milk fat 0.28.

The fatty acid profile of fish oil produced from both wild and farmed fish reflects essentially the profile found in edible parts or in the liver and is dependent in the same species on environmental factors and, in the case of farmed fish, on the feed composition.

5.1.4. Vitamins

Fish are good sources of some vitamins, especially vitamin D, A and B₁₂. Some marine species contain high amounts of preformed retinol and provide the recommend daily intake per 100 g.

5.1.5. Minerals

Seawater fish, especially shellfish, are good sources of iodine (Table 10). Fresh water fish contains between 5 and 15 µg iodine per 100 g fresh weight of edible parts, sea-water fish between 8 and 1210 µg/100 g. Promoting marine fish consumption in a population with a low total iodine intake from the diet is an effective mean of increasing the iodine intake.

All species of fish contain considerable amounts of selenium (Table 10), and fish is, after beef, lamb, pork and veal the best dietary source of selenium (Schubert *et al.*, 1987). There are contradictory reports on the bioavailability of selenium from fish in the literature. Whereas fish consumption was found to have no apparent effect on the amount of selenium incorporated into functional selenoproteins and a low effect on plasma selenium levels in some studies (Meltzer *et al.*, 1993; Svensson *et al.*, 1992; Huang *et al.*, 1995), others have reported a high correlation between fish consumption and glutathione peroxidase activity and selenium protein-P plasma levels (Bergmann *et al.*, 1998; Hagmar *et al.*, 1998). A recent study with sea-water trout biosynthetically labelled with ⁷⁴Se demonstrated apparent selenium absorption and seven-day retention of 90 and 85 %, respectively in 35 male healthy men (Fox *et al.*, 2004).

Table 10. Exemplary composition of eight fish species most consumed in the EC [unit/100g edible parts; mean and minimum/maximum value] (Souci-Fachmann-Kraut, 2000)¹

	rainbow trout	salmon	tuna	tuna, canned in oil	herring	mackerel	pilchard	anchovy	carp
Scientific name	<i>Oncorhynchus mykiss</i>	<i>Salmo salar</i> L.	<i>Thunnus spp.</i>	-	<i>Clupea spp.</i>	<i>Scomber spp.</i>	<i>Sardina pilchardus</i>	<i>Engraulis encrasicolus</i> L.	<i>Cyprinus carpio</i> L.
Predator	-	+	+		-	-	-	-	-
Sea	-	+	+		+	+	+	+	-
Fresh water	+	+	-		-	-	-	-	+
Energy [kcal]	103	202	226	283	233	182	118	101	115
Protein (Nx6.25) [g]	19.5 (18-20.2)	19.9 (17.4-21.1)	21.5 (18-24)	23.8	18.2 (17.3-19.6)	18.7 (17.2-20.1)	19.4 (16.4-21.2)	20.1 (15.3-23.5)	18.0 (16.7-19.3)
Fat [g]	2.7 (1.9-4.6)	13.6 (12.5-16.5)	15.5 (4.2-24.0)	20.9	17.8 (9.9-19.4)	11.9 (5-20.2)	4.5 (1.2-9.8)	2.3 (1.7-3.6)	4.8 (2.0-7.1)
C20:4 n-6 [mg]	26 (25-30)	191 (60-650)	244 (176-290)	NA	37 (20-90)	170	8.4	10	119 (83-200)
Sum n-6 PUFA [mg]	258	621	477	NA	190	340	96	60	441
Sum n-3 PUFA [mg]	750 (487-870)	3570 (1100-5460)	4208 (2700-6330)	NA	4035 (870-5500)	2290	1523	575	574 (174-940)
n-3/n-6	3.0	5.7	8.8	NA	21.2	6.7	15.9	9.6	1.3
Sum LC n-3 PUFA [mg]	627 (429-750)	2991 (800-4520)	3725 (2410-5620)	NA	2824 (820-5150)	1880 (1600-2390)	1430	515 (230-380)	296 (154-590)
Retinol [µg]	32 (30-45)	41 (9-65)	450 (80-830)	152 (6-830)	38 (20-64)	100 (45-140)	20 (-)	NA	44 (10-140)
Vitamin D [µg]	1.7	16 (5-20)	4.5 (2.5-8.3)	NA	27 (25-38)	4 (0.5-16)	11 (8-14)	NA	NA
Vitamin B ₁₂ [µg]	NA	2.9	4.3	NA	8.5	9.0	0.14	NA	NA
Iodine [µg]	3.4 (3-3.6)	34	50 (40-50)	149	40 (24-65)	51 (40-106)	32 (18-54)	NA	1.7
Selenium [µg]	25 (18-140)	29 (20-34)	82 (66-130)	12	43 (25-143)	39 (22-130)	60 (50-85)	NA	- (7-130)

NA: Data not available

¹Data from different databases may differ, see chapter 5.1

Table 11. Exemplary composition of eight fish species¹, mean values, calculated from values in Table 10

	rainbow trout	salmon	tuna	herring	mackerel	pilchard	anchovy	carp
Scientific name	<i>Oncorhynchus mykiss</i>	<i>Salmo salar L.</i>	<i>Thunnus spp.</i>	<i>Clupea spp.</i>	<i>Scomber spp.</i>	<i>Sardina pilchardus</i>	<i>Engraulis encrasicolus L.</i>	<i>Cyprinus carpio L.</i>
Protein [g/100kcal]	18.9	9.9	9.5	7.8	10.3	16.4	19.9	15.6
Fat [g/100kcal]	2.6	6.7	6.9	7.6	6.5	3.8	2.3	4.2
Fatty acids/100kcal								
Sum n-3 [g]	0.73	1.77	1.89	1.73	1.26	1.29	0.57	0.5
Sum LC n-3 [g]	0.61	1.48	1.65	1.21	1.03	1.21	0.51	0.26
C20:4 n-6 [g]	0.025	0.095	0.110	0.016	0.093	0.007	0.010	0.1
C20:5 n-3 [g]	0.136	0.371	0.613	0.875	0.346	0.492	0.208	0.168
C22:5 n-3 [g]	0.056	0.190	0.114	0.046	0.070	0.034	0.015	0.043
C22:6 n-3 [g]	0.463	0.920	0.921	0.291	0.618	0.686	0.287	0.090
Fatty acids/100g lipids								
Sum n-3 [g]	28.0	26.4	27.4	22.8	19.4	33.9	24.8	12.0
Sum LC n-3 [g]	23.5	22.1	23.9	15.9	15.8	31.8	22.2	6.2
C20:4 n-6 [g]	1.0	1.4	1.6	0.2	1.4	0.2	0.4	2.5
C20:5 n-3 [g]	5.2	5.5	8.9	11.5	5.3	12.9	9.0	4.0
C22:5 n-3 [g]	2.2	2.8	1.7	0.6	1.1	0.9	0.7	1.0
C22:6 n-3 [g]	17.8	13.7	13.3	3.8	9.5	18.1	12.5	2.1

¹Data from different databases may differ, see chapter 5.1

5.2. Nutritional composition of farmed fish

5.2.1. Influence of fish farming (aquaculture)

From the eight species selected for this opinion only carp, rainbow trout and some salmon species are produced by aquaculture. Rainbow trout are farmed in both freshwater and seawater, whereas salmon only spend their juvenile stage in freshwater, and grow-out occurs e.g. in sea cages.

The efficiency of conversion of digested feed protein into body protein (percentage protein retention) in farmed fish varies greatly with species and can be more or-less equivalent to that observed in wild fish. Protein retention is highest when non-protein sources of dietary energy are sufficiently high to supply the bulk of metabolic energy. Feeding diets that are deficient in essential amino acids or greatly imbalanced lowers protein retention, and, if sufficiently severe, growth rate.

The fatty acid profiles of farmed fish reflect the lipid profiles of dietary lipids to a remarkable degree. The percentage of saturated fatty acids in fish lipids does not increase beyond a certain level, regardless of dietary lipid composition, but levels of monoenes and especially dienes are highly variable depending on diet. Marine fish require three highly unsaturated long-chain fatty acids for their normal growth and development, i.e. AA, EPA and DHA. Replacing EPA and DHA containing fish products in the feed of cultured fish by linoleic acid-rich vegetable oils will change also the content of these LC n-3 PUFA in fish (Sargent and Tacon, 1999, Hardy *et al.*, 1987). When Atlantic salmon were fed either 100 % fish oil, a mixture of 50 % fish oil and 50 % rapeseed oil or 100 % rapeseed oil in their feed, which in all cases satisfied the minimum requirement for n-3 PUFAs of salmonids, the fatty acid pattern of the feed was mirrored in the fatty acid pattern of the fish fillets (weight percentage of total lipids) as demonstrated in Table 12.

Table 12. Influence of feed lipid composition on the fatty acid spectrum in farmed fish¹ (Seierstad *et al.*, 2005).

	Fish feeds			Fish fillets		
	Fish oil	Fish oil + Rapeseed oil	Rapeseed oil	Fish oil	Fish oil + Rapeseed oil	Rapeseed oil
Oleic acids	9.2	30.9	50.4	12.4	31.7	48.0
Linoleic acid	2.7	11.3	19.4	3.3	10.5	15.9
α -Linolenic acid	1.0	4.7	8.0	1.0	3.9	5.4
Arachidonic acid	0.8	0.4	0.0	0.8	0.7	0.7
EPA	13.2	7.2	1.5	8.5	4.4	1.5
DHA	13.8	7.5	1.9	12.9	7.8	2.9
Sum n-3	35.5	23.4	11.8	30.2	20.5	11.7
Sum n-6	3.7	11.6	19.4	4.6	12.3	18.6
Ratio n-3/n-6	9.6	2.0	0.6	6.5	1.7	0.6

¹Data from different databases may differ, see chapter 5.1

5.2.2. Differences in nutritional composition of farmed and wild fish

Depending on the fish feed the nutritional composition of wild and farmed fish may differ, especially with respect to the total lipid content and to the fatty acid composition.

In 1990 van Vliet and Katan published analytical data on 58 trout, 51 eel and 5 salmon collected throughout Europe both from fishermen and hatcheries. Cultured eel and salmon contained 50 % more fat than wild counterparts, whereas the fat content of wild and farmed trout was not significantly different. A higher fat content of farmed over wild coho salmon was also seen in other studies. More importantly, the n-3/n-6 fatty acid ratio was two- to three-times higher in wild than in cultured fish. Linoleic acid was substantially higher in all cultivated species than in wild fish. On a weight basis LC n-3 PUFA were found to be higher in cultivated fish, therefore, on a per-serving basis, the LC n-3 PUFA levels of farmed and wild fish can be similar (Nettleton, 2000). Table 13 lists the fatty acid content per 100g wet weight of three species, either wild or farmed.

Table 13. Total fat and fatty acid content¹ (g/100g wet weight) of wild and farmed rainbow trout (*Oncorhynchus mykiss*), Atlantic salmon (*Salmo salar L.*) and coho salmon (*Oncorhynchus kisutch* (Walbaum)) <http://www.nal.usda.gov/fnic/foodcomp/>

Scientific name	Rainbow trout		Coho salmon		Atlantic salmon	
Latin name	<i>Oncorhynchus mykiss</i> (Walbaum)		<i>Oncorhynchus kisutch</i> (Walbaum)		<i>Salmo salar L.</i>	
	wild	farmed	wild	farmed	wild	farmed
Fat	3.46 ± 0.226	5.4 ± 0.306	5.93 ± 0.1616	7.67 ± 0.696	6.34 ± 1.772	10.85
Fatty acids						
18:3 n-3	0.119	0.058	0.157	0.075	0.295	0.094
20:5 n-3	0.167	0.260	0.429	0.385	0.321	0.618
22:6 n-3	0.420	0.668	0.656	0.821	1.115	1.293
22:5 n-3	0.106	0.000	0.232	NA	0.287	NA
18:2 n-6	0.239	0.710	0.206	0.349	0.172	0.586
PUFA	1.237	1.805	1.992	1.861	2.539	3.931
Sum of LC n-3 PUFA	0.693	0.928	1.327	1.206	1.723	1.911

ND: Data not available

¹Data from different databases may differ, see chapter 5.1

Provided that cultured fish are raised using appropriate feeds, their nutritional value is comparable to wild fish or even higher with respect to the content of LC n-3 PUFA (Cahu *et al.*, 2004; Nichols *et al.*, 2003; McKenzie, 2001).

5.2.3. Other differences between wild and farmed fish

Wild Atlantic and Pacific salmonids contains predominantly the 3S,3'S isomer of astaxanthin as the principal natural dietary pigment of their skeletal musculature, whereas the feed of cultured salmon may contain both synthetic astaxanthin (a mixture of 3R,3'R, 3R,3'S and 3S,3'S isomers in the ratio 1:2:1) and canthaxanthin as colouring agent (Ostermeyer and Schmidt, 2004).

5.3. Conclusion

Farmed fish tend to have higher whole body lipid levels than their wild counterparts. The fatty acid composition of body fat can be influenced by the lipid composition of the feed to resemble to that of wild fish. Even if the relative LC n-3 PUFA content of farmed fish tends to be lower than that of wild fish but the amount provided per portion is likely to be the same due to the higher fat content.

6. Beneficial effects associated with fish consumption

As apparent from the previous section fish is a valuable food in that it provides considerable amounts of easily digestible protein of high biological value and can be, especially in the case of marine species, a good source of iodine, selenium and vitamins A and D. In spite of its potentially high content of total fat, of up to 60 % of the total energy value, it has been shown to be likely of benefit to the health of consumers probably because of its content of long-chain polyunsaturated fatty acids of the n-3 variety (LC n-3 PUFA). While all other nutrients mentioned above can be derived satisfactorily from other food, fish is unique in containing substantial amounts of LC n-3 PUFA. Human milk and marine algae are other natural sources of LC n-3 PUFA besides foods and supplements enriched with LC n-3 PUFA, which have become available in recent years".

This special fatty acid composition of fish lipids results in both a low index of atherogenicity and thrombogenicity (indices based on the low content of saturated fatty acids C12, 14, 16 in relation to the content of n-6 and n-3 PUFA, oleic acid and other MUFA, calculated according to Ulbricht and Southgate (1991)), of around 0.4 and 0.2, respectively (Rueda *et al.*, 1997; 2001). This is more favourable than the atherogenicity indices of 0.7 for beef and 0.6 for pork and the thrombogenicity indices of > 1 for both beef and pork (Ulbricht and Southgate, 1991).

DHA (and also AA) are essential for the development of the central nervous system. They are preferentially incorporated into the cell membranes of neuronal cells and the retina, particularly during the last trimester of pregnancy and the first postnatal months. The foetal brain and retina do not synthesise LC n-3 PUFA, therefore placental transfer and supply via breast-milk or infant formula are crucial (Clandinin *et al.*, 1989, 1999; Martinez, 1992), considering that the production by the foetal liver (Rodriguez *et al.*, 1998) may be insufficient.

EPA and DHA have an immunosuppressive effect but they suppress inflammatory responses occurring in chronic degenerative diseases. The metabolic background for these effects is described in Annex 2.

Overall, LC n-3 PUFA affect numerous processes, including growth, neurological development, lean and fat mass accretion and immunity, and may be of influence in infections and both incidence and severity of chronic degenerative diseases.

6.1. National recommended intakes of fish or LC n-3 PUFA

The Scientific Committee on Food (SCF, 1993) has not formulated recommendations for the intake of single fatty acids, but has defined population reference intakes for total n-6 PUFA and n-3 PUFA, which are 2 and 0.5% of the daily energy intake of adults, respectively. The SCF has also recommended that the total n-3 PUFA intake per day should not exceed 5 % of the total energy intake.

A daily intake of 4 – 10 % of the energy intake as linoleic acid and a ratio of LA to LNA between 5:1 and 10:1 was advised by FAO/WHO (1994). Population nutritional goals were set at an intake of 6 – 10 % of energy as total polyunsaturated fatty acids (5 – 8 % as n-6 PUFA and 1 – 2 % as n-3 PUFA) and a regular fish consumption (1 - 2 servings per week providing the equivalent of 200 - 500 mg EPA plus DHA per serving) was recommended for the prevention of coronary heart disease and ischaemic stroke (WHO, 2003).

The Food and Nutrition Board of the American Institute of Medicine (IOM, 2002) has defined adequate intake (AI) values for both linoleic acid (LA) (17 and 12 g/day for men and women, respectively) and LNA (1.6 and 1.1 g/day for men and women, respectively) based on current intakes in the US population. FNB also recommended that the LA/LNA ratio should not be less than 5:1, particularly in infants, to avoid reduction in AA synthesis.

In France an intake of LA of 4 % of the energy intake and of LNA of 0.8 % of the energy intake is recommended for adults. The recommendation for LC n-3 PUFA is 0.2 % of the energy intake and for DHA it is 0.05 % of the energy intake, which corresponds to 120 mg and 100 mg of DHA per day for men and women, respectively (Martin, 2001).

The UK Scientific Advisory Committee on Nutrition (FSA, 2004a) has recently confirmed the previous UK recommendation that at least two portions of fish (of which one oily) should be eaten per week and that the intake of LC n-3 PUFA should be 0.45 g/d.

The Dutch Health Council (2001) has set adequate levels of intake for LA and LNA. For LA it is 0.6 g/kg/day for infants up to the age of 5 months, 2 % of the energy intake for all other age groups and 2.5 % of the energy intake for pregnant and breastfeeding women. For LNA it is 0.08 g/day for infants below six months of age and of 1 % of energy for all other age groups. An intake of 0.2 g DHA plus EPA/day was recommended. The tolerable upper level of intake for total PUFA has been set at 12 % of the energy intake.

A working group at the US National Institutes of Health on the essentiality of n-6 and n-3 fatty acids has recommended that with an energy intake of 2000 kcal/day the LA intake should be 4.4 g or 2 % of the energy, the intake of LNA should be 2.2 g/day or 1 % of the energy and 0.65 g/day of DHA plus EPA should be consumed or 0.3 % of the energy (Simopoulos *et al.*, 1999).

The International Society for the Study of Fatty Acids and Lipids (ISSFAL, 2004) has recommended the following dietary intakes for cardiovascular health: LA 2 % of energy, LNA 0.7 % of energy, and EPA/DHA 0.5 g/day.

For the purpose of this opinion a fish consumption equivalent to an LC n-3 PUFA intake of 0.5 g/day was chosen for comparison of intakes. The Scientific Committee for Food (EC, 2003) reviewed the available evidence and concluded that a minimum content of LC n-3 PUFA in infant formula could not be set.

The physiological background for the requirement of LC n-3 PUFA is given in Annex 2.

6.2. Health benefits from LC n-3 PUFA consumption

Studies on beneficial effects associated with fish consumption on health are mostly lacking details on the type of fish or of LC n-3 PUFA intake from fish and, therefore, rarely permit a dose-response assessment relating amounts of LC n-3 PUFAs to health effects. In many intervention studies fish oils of known composition are used. The methodological quality of studies using fish oil or supplements is generally better than those with dietary advice on fish (Wang *et al.*, 2004). Contaminants in fish were not looked at in most of the studies.

6.2.1. Risk of cardiovascular disease and stroke

Early observational studies reported that a high intake of fatty marine fish was associated with a lower mortality from cardiovascular disease and that Eskimos showed low serum levels of

total LDL and VLDL cholesterol and triglycerides and high levels of HDL-cholesterol despite a high dietary fat intake. Also their blood coagulation time was prolonged (Dyerberg *et al.*, 1978). These findings were followed up in a number of prospective epidemiological and some randomised controlled intervention studies both for primary and secondary prevention of coronary heart disease.

The evidence has recently been reviewed by the UK Scientific Advisory Committee on Nutrition (FSA, 2004a) of the Food Standards Agency of the UK, in a Cochrane Review (Hooper *et al.*, 2004) and by the US Agency for Healthcare Research and Quality (Wang *et al.*, 2004) and is summarised here.

Risk of stroke

SACN (FSA, 2004a) considered equivocal the results of prospective cohort studies on the relationship between fish consumption and the incidence of thrombotic stroke (Keli *et al.*, 1994; Zutphen study: reduced risk with more than 20 g fish/day compared to less; Morris *et al.*, 1995; Physician's Health Study: no association with fish consumption; Gillum *et al.*, 1996; NHANES I Epidemiologic Follow-up Study: reduced risk of stroke with fish consumption in black and white women and black men, but not in white men; Orenca *et al.*, 1996; Chicago Western Electric Study: 30-years follow-up no positive effect of fish consumption on incidence of stroke; Iso *et al.*, 2001; Nurses' Health Study: no association between fish consumption and risk of haemorrhagic stroke, reduced risk of thrombotic stroke with fish consumption > 2 times per week; He *et al.*, 2002; Health Professional Follow-up Study: eating fish once per month or more reduced the risk of ischaemic but not of haemorrhagic stroke). A recent metaanalysis of 8 observational studies calculated reduced relative risks for any type of stroke when fish consumption was once per week compared to no fish consumption or up to one to three times per month: RR 0.87 (95 % CI, 0.77 - 0.98; P for trend = 0.06). The effect of higher fish consumption was greater on risk of ischaemic stroke (P for trend = 0.24) than for haemorrhagic stroke (P for trend = 0.31) (He *et al.*, 2004a). Nonetheless, the overall evidence is not conclusive and does not permit definition of a dose response relationship.

Risk of coronary heart disease

Observational studies (fish)

SACN concluded from several prospective cohort studies (for details see FSA, 2004a) that evidence indicates an inverse relationship between fish consumption (and LC n-3 PUFA intake) and mortality from coronary heart disease (Dolecek and Granditis, 1991; Hu *et al.*, 2002; Kromhout *et al.*, 1985, 1995; Mozaffarian *et al.*, 2003; Rodriguez *et al.*, 1999; Yuan *et al.*, 2001) but not all studies were supportive (Albert *et al.*, 1998; Ascherio *et al.*, 1995; Gillum *et al.*, 2000; Kromhout *et al.*, 1996; Morris *et al.*, 1995; Osler *et al.*, 2003)

In a systematic review of eleven prospective cohort studies examining the relationship between fish consumption and coronary heart disease mortality and counting a total of 116 764 individuals it appeared that in two high quality studies with the largest population (n = 44 895 and n = 20 051) at low risk of coronary heart disease (Albert *et al.*, 1998; Ascherio *et al.*, 1995) fish consumption had no protective effect. In two smaller studies of high quality (n = 852 and n = 1182) in individuals at high risk for coronary heart disease (Daviglius *et al.*, 1997; Kromhout *et al.*, 1985), however, an inverse relationship between fish consumption and death from coronary heart disease was apparent. Fish consumption of 40 - 60 g/day was associated with a risk reduction of 40 - 60 % (Marckmann and Gronbaek, 1999).

However, Yuan *et al.* (2001) found in a prospective study in 18 244 men aged between 45 - 64 years between 1986 and 1998 a significantly reduced risk for death from acute myocardial infarction in those consuming ≥ 200 g of fish/shellfish per week compared to men with an intake of less than 50 g/week (relative risk 0.41; 95 % CI, 0.22, 0.78). Overall a 20 % reduction in total mortality was observed in the study population with weekly fish/shellfish intake. Discrepancies in the outcome of prospective studies may be related to the length of the follow-up period: the initial report on the Honolulu Heart Program found no relationship between fish intake and risk of coronary heart disease whereas a positive effect was demonstrated after a follow-up of 23 years (Rodriguez *et al.*, 1996).

High mercury content in fish was reported to attenuate the beneficial effect associated with fish consumption (Rissanen *et al.*, 2000) or high levels of DHA in adipose tissue (Guallar *et al.*, 2002) on mortality from coronary heart disease. In the prospective Kuopio Ischaemic Heart Disease Risk Factor Study which involved 1871 Finnish men aged 42 - 60 years and followed on average 13.9, years men with the highest tertile in mercury hair content had an adjusted 1.6-fold (95 % CI, 1.24 - 2.06) risk of acute coronary event, 1.68-fold (95 % CI, 1.15 - 2.44) risk of cardiovascular disease, 1.56-fold (95 % CI, 0.99 - 2.46) risk of coronary heart disease, and 1.38-fold (95 % CI, 1.15 - 1.66) risk of any death compared with men in the lower two tertiles. A high mercury content in hair attenuated the protective effects of high serum DHA and DPA concentration (Virtanen *et al.*, 2005). This study suggests that failure to adjust for contaminant exposures may explain some of the apparent differences between studies.

Cardiac benefits of fish consumption may also vary with type of fish meal consumed. Whereas in a prospective cohort study which involved 3910 adults above the age of 65 years and free of known cardiovascular disease at baseline only consumption of tuna or other boiled or baked fish 3 or more times/week reduced the risk of total mortality from ischaemic heart disease ($p = 0.001$ for trend) and for mortality from arrhythmic ischaemic heart disease ($p = 0.001$) but not for non-fatal myocardial infarction compared with less than one fish meal per month, no such association was found for fried fish or fish sandwiches (Mozaffarian *et al.*, 2003).

High fish consumption (two servings or more per week or one or more serving of oily fish/week) was also reported to slow the progression of coronary artery diameter decrease in a prospective cohort study involving 229 postmenopausal women with coronary stenosis compared to women consuming less than two servings of fish per week. This association was only significant in diabetic women (Erkkilä *et al.*, 2004).

A metaanalysis of eleven observational studies, involving 13 cohorts including 222 364 individuals with an average of 11.8 years of follow-up reported that individuals who consumed fish had a lower mortality from coronary heart disease than individuals not consuming fish or less than once per month. The pooled multivariate relative risks for coronary heart disease mortality were 0.89 (95 % CI, 0.79 - 1.01) for fish intake 1 - 3 times per months, 0.85 (95 % CI, 0.76 - 0.96) for fish once per week, 0.77 (95 % CI, 0.66 - 0.89) for fish two to four times per week, and 0.62 (95% CI, 0.46 - 0.82) for fish five or more times per week. Each 20 g/day increase in fish intake was related to a 7 % lower risk of mortality from coronary heart disease (P for trend = 0.03) (He *et al.*, 2004b).

Intervention studies (fish)

There are no randomised controlled trials available which address the primary prevention of coronary heart disease with consumption of defined amounts of fish or the application of fish oil.

Few secondary prevention trials have been performed to demonstrate that fish consumption or supplementation with fish oil or LC n-3 PUFA reduce the mortality in patients after a myocardial infarction.

One study investigated the effect of an increased consumption (200 - 400 g per week or 0.5 - 0.8 g LC n-3 PUFA/day) of fatty fish during two years in 2033 male survivors of a myocardial infarction (Diet and Reinfarction Trial, DART). The greatest benefit of increased fish consumption was seen in fatal myocardial infarction (29 % decrease). An analysis of those patients who received fish oil capsules (0.9 g EPA and DHA) instead of fish suggested that the effect was due to LC n-3 PUFA (Burr *et al.*, 1989; 1994).

However, when in a study of poor methodological quality 3114 men under the age of 70, who had angina pectoris were randomly allocated to four groups: (1) advised to eat two portions of oily fish each week or to take three fish oil capsules daily; (2) advised to eat more fruit, vegetables, oats; (3) advised to do both; (4) given no advice, and followed for 3 - 9 years, there was no effect on all-cause mortality in either group. The risk for cardiac death was higher in the fish group (RR 1.26; 95 % CI, 1.0 - 1.58; $p = 0.047$), especially for sudden cardiac death (RR 1.54; 95 % CI, 1.06 - 2.23; $p = 0.025$) (Burr *et al.*, 2003).

Fifty-eight patients with stable angina pectoris (angiographically verified) were randomised to consume during six weeks either 700 g/week of farmed salmon fed with fish oil, fish oil plus rapeseed oil or rapeseed oil. The amounts of LC n-3 PUFA thus provided were 2.9, 1.5 and 0.5 g/day, respectively. Patients on high LC n-3 PUFA salmon showed a decrease in serum triglycerides, of vascular cell adhesion molecule-1 and of interleukin-6. The intervention period was too short to assess other favourable clinical effects (Seierstad *et al.*, 2005).

Fish oil or pure EPA/DHA preparations

In one study 240 patients suspected of an acute myocardial infarction were randomised to receive either fish oil (1.8 g EPA and DHA) or mustard oil (2.9 LNA) or placebo. After one year, total cardiac events were 25 % and 28 % in the fish oil and mustard oil group, respectively, compared to 35 % in the placebo group ($p < 0.01$) (Singh *et al.*, 1997).

The largest prospective randomised controlled intervention trial (GISSI-Prevenzione trial) randomised 11 324 patients with pre-existing coronary heart disease to either 300 mg of vitamin E, 850 mg EPA and DHA as ethyl esters, both or neither. After 3.5 years of follow-up the group receiving LC n-3 PUFA showed a 15 % reduction in the primary endpoint of death, non-fatal myocardial infarct, and non-fatal stroke ($p < 0.02$). There was a 20 % reduction in all cause mortality ($p = 0.01$) and a 45 % reduction in sudden death ($P < 0.001$) compared with the control group. Vitamin E provided no additional benefit. Triglycerides decreased by 4 % and LDL-cholesterol levels increased by 2.5 % (GISSI, 1997; Marchioli *et al.*, 2002).

When 300 Norwegian post-infarct patients were randomised to 3.5 g DHA and EPA/day or corn oil, no beneficial effect on cardiac events was observed after 18 months. It was speculated, that the LC n-3 PUFA intake was habitually high in these patients and, therefore, no additional benefit could be observed (Nilsen *et al.*, 2001). Withdrawal of LC n-3 PUFAs after treatment for 12 - 24 months resulted in no differences in the prognosis for the two study groups after 45 months. Favourable effects on serum triglycerides and HDL-cholesterol were lost and total cholesterol decreased (Grundt *et al.*, 2004).

The overall analysis of these randomised controlled trials shows a beneficial effect of dietary and supplemental LC n-3 PUFA on CHD (Kris-Etherton *et al.*, 2002). However, several trials performed to investigate the effect LC n-3 PUFA on angiographic progression rates in patients with coronary artery stenosis or coronary bypasses (Cairns *et al.*, 1996; Eritsland *et*

al., 1996; Gapinsky *et al.*, 1993; Johansen *et al.*, 1999; Sacks *et al.*, 1995; von Schacky *et al.*, 1999) with LC n-3 PUFA amounts between 1.5 and 7 g/day have led to equivocal results, and most investigators have concluded that further trials are not indicated. The beneficial cardiovascular effects of LC n-3 PUFA and higher EPA and DHA contents of phospholipids in serum and (red blood) cells include a more favourable serum lipid pattern, lower blood pressure, lower inflammatory factors in serum and reduced blood clotting but are predominantly ascribed to the antiarrhythmic effects. These are attributed to a decreased myocyte excitability and a reduction in cytosolic calcium fluctuation via inhibition of Na⁺ and L-type Ca⁺⁺ channels (Albert *et al.*, 2002; Kang and Leaf, 2000; Kris-Etherton *et al.*, 2002; McLennan, 2001).

The beneficial effects observed in the secondary prevention trials were observed at LC n-3 PUFA intakes around 1 g/day.

For the induction of demonstrable effects on cardiovascular risk factors like the reduction of serum triglycerides (Finnegan *et al.*, 2003; Sacks and Katan, 2002), blood pressure (Geleijnse *et al.*, 2002), platelet aggregation (Hornstra, 2001) and the inflammatory response (Calder, 2005) amounts higher than 1 g/day are necessary. Doses between 1.5 and 3 g/day have been shown to reduce platelet aggregation but also to raise LDL-cholesterol levels in a significant proportion of subjects (Harris, 1997). An antiarrhythmic effect has been induced with amounts of 1 g/day.

The comprehensive reviews of the effects of n-3 PUFA in prevention and treatment of cardiovascular disease by Hooper *et al.* (2004) and by Wang *et al.* (2004) come to different conclusions. Hooper *et al.* (2004) state that it is not clear that dietary or supplementary n-3 fatty acids alter total mortality or combined cardiovascular events in people with or at high risk of cardiovascular disease or in the general population. Wang *et al.* (2004) state that consumption of n-3 fatty acids from fish or from supplements of fish oil reduces all cause mortality and various cardiovascular disease outcomes, such as sudden death, cardiac death and myocardial infarction, the evidence being strongest for fish or fish oil. SACN (FSA, 2004a) concluded that both randomised controlled trials in secondary prevention as well as prospective epidemiological evidence support that increased fish consumption or fish oil supplementation decrease mortality in patients with or at risk of cardiovascular disease, and that consumption of at least one portion of fatty fish per week would confer a public health benefit in terms of a reduced risk of cardiovascular disease. The Panel observed that the result of the metaanalysis by Hooper *et al.* (2004) maybe partly explained by the inclusion of one big randomised controlled trial on the use of fish oil (Burr *et al.*, 2003), which shows some methodological deficits, and concludes that there is sufficient evidence of beneficial effects associated with fish consumption for populations with or at risk of cardiovascular disease.

6.2.2. Effect of fish or LC n-3 PUFA supplementation in pregnancy on outcome

Fish consumption (or LC n-3 PUFA intake) of pregnant and breastfeeding women determines the DHA concentration in blood phospholipids (Olsen *et al.*, 1991; Connor *et al.*, 1996; van Houwelingen *et al.*, 1995; Williams *et al.*, 2001) and the DHA content of breast milk (Harris *et al.*, 1984; Koletzko *et al.*, 1992; Makrides *et al.*, 1996). Serum concentration of PCBs in 182 seafood consuming mothers in the Faroe islands have been shown to be associated with modest decreases in AA in phospholipids of maternal and umbilical cord serum. This could indicate an inhibitory effect of some PCB congeners on Δ -5 and Δ -6 desaturation of LC n-3 PUFA (Grandjean and Weihe, 2003).

Length of gestation and birth weight

Olsen *et al.* (1991) suggested that the higher fish consumption of women in the Faroe Islands was responsible for the longer gestation duration compared to Danish women. In 182 women with singleton births in 1994 - 1995 in the Faroe Islands an increased maternal seafood intake and consecutively increased level of DHA in umbilical cord serum was associated with an increased duration of gestation (1.5 days per 1 % increase of DHA in cord serum phospholipids). However, birth weight adjusted for gestational length decreased by 246 g for each increase by 1 % of the EPA concentration in cord serum. While the levels of mercury and PCB were associated with fatty acid levels, they did not affect the outcome parameters (Grandjean *et al.*, 2001). The dietary intake of marine n-3 fatty acids in 965 pregnant Danish women (mean intake 0.25 g/day), was not associated to gestation length, birth weight or birth length (Olsen *et al.*, 1995). But the occurrence of preterm delivery differed significantly across four groups of seafood intake, falling progressively from 7.1 % in the group never consuming fish to 1.9 % in the group consuming fish at least once a week. Estimates of risk for low birth weight (< 2500 g) were similar to those for preterm delivery: odds ratio 3.57 (95 % CI: 1.14 to 11.14) when the group with highest intake (44.3 g fish or 0.54 g LC n-3 PUFA/day) was compared with the group with the lowest intake (0.3 g fish or 0.04 g LC n-3 PUFA/day) (Olsen *et al.*, 2002).

In a double-blind study with 341 women randomised to either 10 ml of cod liver oil (2.1 g DHA plus DPA plus EPA) or 10 ml of corn oil at weeks 17 to 19 of pregnancy through three months after delivery no differences in gestational length or birth weight between the groups were observed. However, neonates with higher concentrations of DHA in umbilical plasma phospholipids had longer gestational length than neonates with low concentrations. Growth during the first two years of life did not differ between the two groups (Helland *et al.*, 2001).

Supplementation with 133 mg DHA as opposed to 33 mg DHA/day from eggs from 24 - 28 weeks of gestation until delivery in women of low social status resulted in an increase of gestation length by 6.0 ± 2.3 days ($p = 0.009$) (Smuts *et al.*, 2003).

Pregnancy-induced hypertension PIH

Epidemiologic observations in Inuit women had shown that a diet rich in marine n-3 fatty acids decreased the incidence of PIH 2.6 fold compared to a diet rich in terrestrial foods (Popeski *et al.*, 1991), however when fish oil was supplemented in a double-blind, randomised, placebo-controlled trial in high-risk pregnancies the rate of PIH was not influenced (Onwude *et al.*, 1995). The alterations observed in cases of PIH at delivery in the fatty acid patterns of maternal phospholipids (increases of LC n-3 and n-6 PUFAs) is considered as a consequence and not as a cause of the disease (Al *et al.*, 1995b).

Visual function and cognitive development

A neurological examination of 317 apparently healthy term infants was performed at 10 - 14 days of age and related to the fatty acid composition of umbilical artery and vein as a surrogate biomarker for prenatal fatty acid status. Neurologically abnormal infants had lower indices of foetal DHA and essential fatty acid status, while the neurological optimality score correlated positively with AA status and indices of DHA and essential fatty acid status and negatively with LA and *trans*-fatty acids (Dijck-Brouwer *et al.*, 2005).

Supplementation with purified cod liver oil (2.1 g LC n-3 PUFA) during the second half of pregnancy did not lead to differences in EEG scores at age 2 days and 3 months or novelty preference (Fagan test) at age 6 and 9 months in infants born to supplemented mothers

compared to infants from mothers supplemented with corn oil. More mature EEG scores on the second day of life were associated with significantly higher ($p = 0.004$ for DHA) LC n-3 PUFA concentrations in umbilical plasma phospholipids (Helland *et al.*, 2001). An intelligence test was performed in 76 children of the study cohort at the age of four years. Children whose mothers had been supplemented with cod liver oil scored higher on the Mental Processing Composite of the Kaufman Assessment Battery for Children ($p = 0.049$) than children from mothers supplemented with corn oil. The concentrations of n-6 docosapentaenoic (Osbond) acid (C22:5 n-6) and n-9 eicosatrienoic (Mead) acid (C20:3 n-9) in umbilical plasma phospholipids correlated negatively with intelligence at 4 years (Helland *et al.*, 2003).

These results are supported by a study in 73 healthy breastfed infants. Length of breastfeeding was positively associated with a higher IQ at the age of 6 ½ years. There were no significant single correlations between PUFA in breast milk and measures of cognitive development, but length of gestation, duration of breastfeeding and the quotient DHA/AA in colostrum explained 76 % of the variation in total IQ (Gustafsson *et al.*, 2004).

Assessment of visual function development is often used as an outcome marker for neurodevelopment. Breastfed infants, both preterm and term, were shown to have better visual acuity, more advanced retinal development, and visual function up to 3.5 years of age (Birch *et al.*, 1992a,b; Williams *et al.*, 2001) in some but not all studies. Higher fish consumption by 135 US mothers during pregnancy was associated with higher scores of their infants at six months of age on visual recognition memory testing as a surrogate measure for cognition (increase by 4.0 points (95 % CI: 1.3 to 6.7) per each additional weekly fish serving). However, an increase of 1 µg/g in mercury in the hair of the mothers was associated with a decrement in the visual recognition memory score of 7.5 (95 % CI: -13.7 to -1.2). The scores were highest in infants whose mothers consumed more than 2 fish meals per week but had hair mercury levels below 1.2 µg/g hair (Oken *et al.*, 2005).

Bakker *et al.* (1999; 2003) could not demonstrate an association between feeding modus (breast-milk or infant formula) or DHA concentrations in umbilical venous plasma and plasma phospholipids at age 7 years on the one side and visual acuity at 7 months of age nor with intelligence at age 7 years on the other side.

Stereoacuity was assessed at the age of 3.5 years in 435 children born at term. Breastfeeding ($p = 0.002$), maternal age > 25 years ($p = 0.017$), fatty fish consumption by the mother during pregnancy ($p = 0.012$) and fatty fish consumption by the child at three years ($p = 0.039$) were associated with increased likelihood of the child having foveal stereoacuity. The variable most associated with an increased likelihood of foveal stereoacuity in multiple logistic regression analysis was breastfeeding. The other variable was consumption of fatty fish by the mother during pregnancy at least once every two weeks (adjusted odds ratio 1.57, 95 % CI: 1.00 to 2.45) compared to mothers who never ate fatty fish. There was a correlation between the child's stereoacuity score and the mother's antenatal DHA concentration in erythrocyte phospholipids ($p < 0.05$) (Williams *et al.*, 2001). These findings may indicate that not only breastfeeding and the DHA concentration of breast milk (which depend on the maternal diet) but also the availability of DHA to the foetus have consequences on the development of visual function and cognition.

In a double-blind randomised controlled prospective trial 100 pregnant women were supplemented with either fish oil capsules (200 mg DHA/day) or sunflower oil capsules devoid of DHA starting in the 15th gestational week until delivery. There was no effect of supplementation on gestation length, birth weight or DHA concentration in umbilical cord red

blood cells, neither on measures of visual evoked potential (VEP) shortly after birth. However, there was a significant correlation between infant DHA status and maturity of the retina as assessed by electroretinography within the first week of life and with the maturity of the pattern-reversal VEP at 50 and 66 weeks postconceptional age (Malcolm *et al.*, 2003a,b).

Immunology and allergy

Supplementation with fish-oil (3.7 g LC n-3 PUFA) during pregnancy in atopic women has been shown to influence the concentration of these fatty acids in breast milk and to decrease the AA content, and this was associated with changes in some immunological parameters (IgA, soluble CD 14, and some cytokines) in breast-milk and in cord blood cells (haemopoietic progenitor phenotype) which might be of influence on the infant's immune system and the development of subsequent allergic disease (Dunstan *et al.*, 2004; Denburg *et al.*, 2005).

6.2.3. Other beneficial effects associated with fish (fish oil) consumption

Inconsistencies in the results from different studies with different fish, different fish oils and different LC n-3 PUFA are potentially explained by different doses but also by variability in the ratio of DHA to EPA. The Panel noted that purity and contamination levels of fish (oils), which may have an impact on the outcome of the study, was not always known.

Hyperlipidaemia

The most consistent and important effect of fish or fish oil consumption is the very rapid decrease in serum triglycerides, which, when elevated, constitute an independent risk factor for myocardial infarction (Stavenow and Kjellström, 1999) In the epidemiological studies conducted in Greenland Eskimos in the 1970s (Dyerberg *et al.*, 1978) it was observed that the high fish consumption was associated with low cholesterol and triglyceride levels in serum. Experimental studies showed that LC n-3 PUFA from fish and fish oil reduced triglycerides (VLDL, chylomicrons) as well as LDL, apo B and apo E in both normal subjects and in patients with hypertriglyceridaemia (type V) and, to a lesser extent total cholesterol, whereas the change in HDL was variable. Very high doses were used in these studies: 20 g LC n-3 PUFA from fish in normal subjects and 30 g LC n-3 PUFA from fish oil in hyperlipidaemic patients (Connor *et al.*, 1993). Finnegan *et al.* (2003) compared the effects of 5 different regimes in 150 moderately hyperlipidaemic subjects who were randomised to receive during six months 1) 0.8 g EPA/DHA or 2) 1.7 g EPA/DHA or 3) 4.5 g LNA or 4) 9.5 g LNA per day or 5) no supplement. There was no effect on plasma fasting or postprandial lipids, except that triglycerides decreased by $7.7 \pm 5\%$ in group 2) and increased by $10.9 \pm 4.5\%$ in group 4). The difference was significant ($p < 0.05$). *Ex-vivo* susceptibility of LDL to oxidation increased in group 2). Addition of fish (LC n-3 PUFA 0.7 % of total energy intake) to a National Cholesterol Education Program step-2 diet for 24 weeks lowered significantly medium and small VLDL compared with the same diet without fish. And it reduced the atherogenic HDL fractions, while the low-fish diet reduced HDL particle size (Li *et al.*, 2004).

The triglyceride lowering effect of LC n-3 PUFA is effective only when the intake of saturated fatty acids is reduced at the same time. EPA can, in contrast to DHA, cause an increase in LDL cholesterol (Nelson *et al.*, 1997a; Harris, 1997) and, therefore, should not be given to patients with combined hyperlipidaemia (Stone, 1996).

Hypertension

A blood-pressure lowering effect of fish oil has been observed in many small intervention trials of mostly short duration and with high doses: average dose of 31 trials 4.3 g LC n-3 PUFA/ day, maximum dose 15 g/day. The effect is mostly moderate with mean reductions of the systolic and diastolic value by 3.0 and 1.5 mmHg, but a dose-response of -0.66/-0.35 mm Hg per g of LC n-3 PUFA can be calculated. The effect is greater with higher baseline pressure and in subjects with clinical atherosclerotic disease or hypercholesterolaemia. It can be enhanced by a concomitant restriction of the sodium intake (Morris *et al.*, 1994; Howe, 1995). The potential blood pressure lowering mechanism is discussed by Das (Das, 2004).

Blood coagulation and thrombosis

Fish consumption and intake of fish oil in high amounts decreases the aggregation of platelets, increases bleeding time and the levels of tissue plasminogen activator. It correlates negatively with fibrinogen, factor VIII and von Willebrand factor levels in blood. The result is an antithrombotic effect (Barcelli *et al.*, 1993), which is not observed when DHA (6 g/day) is administered without EPA (Nelson *et al.*, 1997b).

Cancer

The evidence for a protective role of fish consumption against major cancers is not clear, partly due to lack of properly designed studies (Hjartåker, 2003). Available epidemiological studies show inconsistent results, but overall there seems to be either no association or an inverse association between fish consumption and risk of breast, colorectal and prostate cancer.

Observational epidemiological studies suggest that high fish intake is associated with lower incidences of both breast and colorectal cancer in Inuit. Prospective and case-control studies either do not show an association between fish intake and cancer risks or show reduced risks at high fish intakes. In population, case-control and prospective studies, fish and LC n-3 PUFA were not found to increase cancer risks (Hooper *et al.*, 2004). Clinical studies on markers of colorectal cancer indicate that fish n-3 PUFA may reduce cancer risk. Effective doses in such studies were between 2.5 g of EPA plus DHA and 1.4 - 4.2 g EPA and 1.2 - 3.6 g DHA (de Deckere, 1999).

In a large nation-wide case control study in Sweden, there was a weak and statistically non-significant association between fish consumption (both lean and fatty) and breast cancer risk. With multivariate adjustment the odds ratio for women with the highest consumption (> 3.5 servings per week) compared with women with the lowest, virtually no consumption was 0.88 (95 % CI: 0.6 to 1.29). The association was similar for lean and fatty fish (Terry *et al.*, 2002a). In another Swedish case-control study involving 709 cases and 2888 controls, the consumption of fatty fish was found to be inversely associated with endometrial cancer risk: multivariate odds ratio for women with the highest fish intake (2 servings/week) compared with women with the lowest intake (median 0.2 servings/week) was 0.6 (95 % CI: 0.5 to 0.8, p for trend 0.0002). There was no such association for the consumption of lean fish (Terry *et al.*, 2002b).

In the Health Professionals Follow-up Study, which started with 51,529 men aged 40 - 75 years in 1986, the relationship between fish consumption/intake of marine fatty acids and the occurrence of prostate cancer during 12 years was assessed. Eating fish more than three times per week was associated with a reduced risk. This association was strongest for metastatic cancer (multivariate relative risk 0.56 (95 % CI: 0.37 to 0.86). Each additional daily intake of

0.5 g marine fatty acids from food was associated with a 24 % decreased risk of metastatic cancer (Augustsson *et al.*, 2003).

Immunology, inflammation, allergy

Greenland Eskimos and Norwegian fishers with high fish consumption were found to be prone to infections but rarely afflicted by autoimmune diseases. This was attributed to the inhibition of the formation of proinflammatory eicosanoids (prostaglandins, leukotrienes, hydroxy fatty acids, lipoxines and isoprostanes) from AA by high concentrations of LC n-3 PUFA and the production of anti-inflammatory eicosanoids from EPA. The intake of EPA and DHA is negatively correlated with inflammatory markers in plasma (CRP, IL-6, TNF receptors) and is not influenced by n-6 fatty acid intake. The combination of both types of fatty acids is associated with the lowest levels of inflammation (Pischon *et al.*, 2003; Adam, 2004; James *et al.*, 2000). Because of this observation LC n-3 PUFA have been used in the therapy of rheumatoid arthritis with good results when the doses were high enough (e.g. 54 mg/kg EPA and 36 mg/kg DHA/day) and the administration period e.g. > 24 weeks (Kremer *et al.*, 1990; Remans *et al.*, 2004).

The inhibitory effect of EPA and DHA on cyclooxygenase 2 activity and consequently reduced production of PGE₂ from AA is probably related to the modulation of the immune response away from allergic reactions. In addition, LC n-3 PUFA can possibly regulate T-cell function directly via membrane effects. Children who regularly consumed oily fish were observed in some studies to have less asthma (OR 0.26) but in other studies to have a higher prevalence of asthma (OR of 1.117 and 2.03 for n-3 and n-6 fatty acids combined). A Cochrane review from 2003, which evaluated nine randomised controlled trials of oral LC n-3 PUFA administration for periods greater than four weeks in children with asthma and older than two years, did not identify a significant effect. In two studies a positive effect which was not significant was observed. Administration of 500 mg fish oil to 616 high-risk (for atopy) infants at six months or as soon as they were weaned resulted in a 9.8 % reduction in wheeze and a 7.8 % reduction of wheeze over more than one week at the age of 18 months (review by Prescott and Calder, 2004).

More controlled long-term studies in well-defined population groups are necessary to assess the preventive potential of LC n-3 PUFA for allergic and chronic inflammatory diseases.

Mental ability

Several observational and intervention studies have reported a positive effect of fish or fish oil consumption or administration of isolated LC n-3 PUFA on cognitive function in the elderly and on depressive disorders. While a potential positive effect on vascular function in the brain is possible, there are as yet no unbiased studies which control adequately for confounding factors.

6.3. Adverse effects

Adverse effects associated with fish oil consumption are dose dependent and consist predominantly of gastrointestinal disturbances. Both fish oil and LC n-3 PUFA supplements can cause excessive bleeding, increases of cholesterol, especially in subjects with familial combined hyperlipidaemia, can suppress certain cellular immune responses and can cause oxidative damage when they contain low vitamin E concentrations (Sidhu, 2003). In addition, a high supply of LC n-3 PUFA may inhibit desaturation and elongation of LA. Again, possible effects of contaminants must be considered when interpreting the evidence.

Blood coagulation

Adolescents with familial hypercholesterolaemia who were treated with 1.5 g of LC n-3 PUFA from fish oil showed a high prevalence of epistaxis after several months of treatment (Clarke *et al.*, 1990). Clinical bleeding occurred in the same frequency in the treatment and placebo arms of randomised controlled trials with LC n-3 PUFA and was often associated with concomitant anticoagulative medication (Wang *et al.*, 2004).

Increase in serum cholesterol

EPA can specifically increase LDL cholesterol if administered without DHA (Nelson *et al.*, 1997). LC n-3 PUFA can increase cholesterol levels in familial combined hyperlipidaemia (Stone, 1996; Nelson *et al.*, 1997a; Harris, 1997)).

Suppression of immune parameters

A fish consumption of 121 - 188 g/day over 24 weeks by eleven elderly subjects as part of a National Cholesterol Education Program step-2 diet resulted in the suppression of the immune response when compared with a low-fish diet. This alteration may be beneficial for the prevention and treatment of atherosclerotic and inflammatory diseases but may be detrimental with regard to host defence against pathogens (Meydani *et al.*, 1993). Suppression of various aspects of human immune function *in vitro* or *ex vivo* in isolated peripheral leukocytes has been observed in many studies with LC n-3 PUFA supplementation. The minimum dose to elicit such effects was 0.9 g EPA and 0.6 g DHA (e.g. Kelley *et al.*, 1999; Lee *et al.*, 1985); Endres *et al.*, 1989).

6.4. Summary and conclusions

Fish is an important source of proteins of high biological value, LC n-3 PUFA, essential minerals, especially iodine, selenium and calcium, and vitamins, especially vitamins A and D and B₁₂. LC n-3 PUFA are not essential in human nutrition beyond the foetal and neonatal period, but may be conditionally essential in immature and young infants.

There is an increased demand for LC n-3 PUFA of the foetus with advancing pregnancy. This has to be satisfied predominantly by the mother by enhanced synthesis from the precursor LNA, by mobilisation of tissue stores or by dietary intake. Fish consumption corresponding to at least 0.2 g DHA/day can satisfy both the demands of the fetes and maternal requirements. However, both the intake of high amounts of LC n-3 PUFA (> 2 g/day) and of LNA (> 3 g/day) can decrease the AA status of the infant, which is undesirable. Therefore, both the requirement of LC n-3 PUFA and the relationship between n-3 and n-6 fatty acids and AA content in the maternal diet are of concern and the optimal mixture needs to be identified. The Scientific Committee on Food (2003) considered the available evidence insufficient for setting a mandatory minimum content of LC PUFA for infant formula intended for healthy mature infants.

There is evidence that fish consumption, preferably of fatty fish - one to two servings of 130 g per week, which may correspond to an LC n-3 PUFA intake ranging from 1.86 g for one meal (0.26 g/day) up to 9.7 g LC n-3 PUFA for two meals/week (1.38 g/day), depending on the type of fish - and, alternatively, fish oil or isolated LC n-3 PUFA (about 1 g of LC n-3 PUFA/day) benefit the cardiovascular system and are suitable for secondary prevention in manifest coronary artery disease. Nevertheless, results from both epidemiologic and interventional studies suggest that health benefits are associated with the consumption of certain levels of EPA/DHA from fish and fish oils also in the healthy population. The

expected benefits include a decrease in the risk of cardiovascular disease and stroke and improved neurodevelopmental and perinatal growth in infants.

7. Evaluation overview on fish consumption

7.1. Toxicity

7.1.1. Toxicity of metals

Metals and other elements are present in water both from natural sources, such as the rocks of the sea bed, and as a result of human activities, such as emissions from industrial processes. These elements are taken up by marine organisms and many tend to accumulate in organisms such as predatory fish which are higher up the food chain. As a result, the concentrations in fish of many elements, including arsenic, cadmium, lead, and mercury can be relatively high compared with levels in other foods.

Arsenic

Arsenic levels are usually higher in the aquatic environment than on land. Therefore, fish and marine crustaceans and molluscs accumulate arsenic compounds. Most arsenic in fish (> 90 %) is in the form of arsenobetaine which is also the main form found in crustaceans and bivalve molluscs, the remainder is arsenocholine and a small amount of inorganic arsenic (usually < 1 %) (Kohlmeyer *et al.*, 2002). Fish is the main source of arsenic in the human diet; arsenobetaine is therefore the main form of arsenic present in food.

Inorganic arsenic is more toxic than organic forms and inorganic arsenic is classified by the International Agency for Research on Cancer (IARC) as “carcinogenic to humans” (Group 1) on the basis of “sufficient evidence” for an increased risk for cancer of the urinary bladder, lung and skin (IARC, 2002). Besides cancer induction, chronic human exposure to arsenic in drinking water (mainly inorganic arsenic) has also been associated with peripheral vascular diseases, cardiovascular diseases and possibly with diabetes and reproductive effects. In 1988, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) confirmed its previously established provisional tolerable weekly intake (PTWI) for inorganic arsenic of 15 µg/kg b.w./week (WHO, 1988) and noted that organic forms of arsenic present in sea foods need different consideration from the inorganic arsenic in water. There are many regional and ethnic populations who consume large quantities of fish that result in organoarsenic intakes of about 50 µg/kg b.w./day. There are no reports of toxicity in man or animals from the consumption of organoarsenicals in seafood. Organic arsenic compounds such as arsenobetaine and arsenocholine seem not to be converted to inorganic arsenic *in vivo* as they are eliminated unchanged from the body. Arsenobetaine and arsenocholine are also not genotoxic in mammalian cells *in vitro*. Therefore, arsenobetaine and arsenocholine from fish and sea food consumption is not considered to represent a significant health risk.

Cadmium

Cadmium exerts a variety of toxic effects including nephrotoxicity, osteoporosis, neurotoxicity, carcinogenicity and genotoxicity, teratogenicity, and endocrine and reproductive effects (EFSA, 2004d). Upon absorption, cadmium is bound with high affinity to metallothionein. The actual level of metallothionein, (which is induced by cadmium) in individual tissues, and, in turn the remaining free cadmium fraction determines the cellular toxicity (Ju and Nordberg, 1998). The cadmium-metallothionein complex represents the major transport form of cadmium in the organism, but cadmium may be released from metallothionein by lysosomal enzymes. Particularly in proximal tubule cells of the kidney, this mechanism results in an increase in free cadmium. Free cadmium readily binds to other

macromolecules including calmodulin. Interaction with calmodulin signalling pathways and disturbance of intracellular calcium homeostasis seems to account for many of the clinically observed toxicities of cadmium, including renal proximal tubule injury and tubular cell necrosis (Brzóska and Moniuszko-Jakoniuk, 1998). The kidney is, therefore, the most sensitive target organ of cadmium toxicity (e.g. renal tubular dysfunction). Exposure to cadmium may also induce oxidative stress, which occurs secondary to the immediate cadmium toxicity, and includes lipid peroxidation and DNA single strand breaks (Goering *et al.*, 1993).

Cadmium has a long biological half-life in mammals. In humans, steady-state concentrations in the renal cortex are reached only after about 40 years. A number of recent epidemiological studies have been published which evaluated the relationships of cadmium exposure to various health effects, particularly renal dysfunction, mortality, and calcium/bone metabolism. Specifically, studies conducted in Japan, Europe, China, and the United States have attempted to refine estimates of the dose-effect relationship between environmental exposure to cadmium and renal dysfunction (WHO, 2004). In aggregate, the new data are consistent with the hypothesis that low-level environmental exposure to cadmium is associated with an increased prevalence of proximal renal tubular dysfunction, using beta₂-microglobulinuria as a biomarker of effects. However, the long-term health implications of the changes in renal function observed at low urinary cadmium levels are uncertain. The JECFA concluded that the new data available did not provide a sufficient basis for revising the PTWI and therefore maintained the PTWI of 7 µg/kg b.w., a dose level at which a small proportion of the general population may already be at increased risk for tubular dysfunction.

Lead

In humans, blood lead levels exceeding 300 µg/L as a consequence of occupational exposure have been related to a number of toxic effects such as anaemia, renal toxicity and subsequent carcinogenicity, cardiovascular and neurological/behavioural effects, and impairment of the reproductive system. The most important and best-documented effect of lead at the exposures most commonly encountered outside occupational settings is retardation in the neurobehavioral development observed in children of mothers having been exposed to lead (Lidsky and Schneider, 2003). A number of cohort studies carried out during the last 20 years have demonstrated an inverse association between lead levels at birth (cord blood: 60 – 80 µg/L) and during the first years of life of children (blood levels: 60 - 400 µg/L), and intellectual performances at pre-school and primary school ages. The most recent research on developmental toxicity in children suggest that detectable deficits may occur even at exposure levels previously considered to be safe (Lanphear *et al.*, 2000, Canfield *et al.*, 2003, Selevan *et al.*, 2003).

The principal mechanisms involved in the neurotoxicity of lead, which readily crosses the blood brain barrier, include apoptosis, excitatory modification of neurotransmitter storage and release, as well as alteration of the mitochondrial and second messenger function affecting cerebrovascular endothelial cells, astrocytes, and oligodendrocytes (EFSA, 2004c; Lidsky and Schneider, 2003). Lead is not genotoxic but it seems to affect cellular defence mechanisms such as DNA repair and regulation of tumour suppressor and promoter genes. In rats, tumour formation has been shown to occur at dose levels below the maximum tolerated dose of 200 mg lead (as lead acetate) per litre of drinking water. Recent epidemiological data provide evidence for an association between lead intake and an increased risk for cancer (Silbergeld, 2003). The IARC has classified lead into group 2A (probably carcinogenic for humans) (IARC, 2004).

The JECFA has established a PTWI for lead in 1986. It is set to 25 µg/kg b.w./week for infants and children on the basis that lead is accumulating in the body and an increase of the body burden of lead should be avoided. In 1993 and 2000, the JECFA reconfirmed this PTWI and extended it to all age groups (WHO, 1993; WHO, 2000).

Mercury

Humans may be exposed to elemental, inorganic and organic forms of mercury. There is a common mechanism of mercury toxicity, binding of mercuric ions to thiol groups in proteins leading to alterations in cell function and cell death. However, the organs affected vary as a result of differences in the physicochemical properties between the three forms. Organic mercury is considered to be more toxic than other forms of mercury following ingestion. Methylmercury is the predominant form of mercury in fish. Studies have reported methylmercury percentages with respect to total mercury of 75 – 100 % in tuna (with an average of 91 % - Storelli *et al.*, 2002), greater than 85 % in muscle of sardines (Joiris *et al.*, 1999), between 67 % and 100 % in swordfish muscle and canned tuna (Kamps and Miller, 1972) and 81 – 100 % in shark (Storelli *et al.*, 2001).

The primary target of methylmercury toxicity is the nervous system. High exposure *in utero* has resulted in cerebral palsy or severe mental retardation in the neonate. Based on a number of poisoning incidents (Minamata, Niigata, Iraq), the JECFA concluded that a minimum level of toxicity would be associated with exposures resulting in 200 µg/L mercury in blood or 50 µg/g mercury in hair. This association was used to derive a PTWI for methylmercury of 3.3 µg/kg b.w./week, corresponding to a blood mercury level of 33 µg/L or hair mercury level of 8.25 µg/g in a 70 kg adult. This PTWI was maintained over several re-evaluations but the JECFA noted that pregnant women and nursing mothers may be at greater risk than the general population from the adverse effects of methylmercury (WHO, 2000). In its sixty-first meeting the JECFA (WHO, 2004) revised its PTWI to 1.6 µg/kg b.w./week, in order to be protective of the developing foetus. Neurodevelopment was considered to be the most sensitive health outcome and *in utero* exposure the most sensitive period of exposure. This evaluation took into account new data from the large epidemiological studies performed in the Seychelles and the Faroe Islands, as well as additional epidemiological data. In prospective studies in Finland, n-3 PUFA intake from fish appeared to prevent cardiovascular mortality, but this beneficial effect seemed to be counteracted or overwhelmed by concomitant exposure to methylmercury (Rissanen *et al.*, 2000; Virtanen *et al.*, 2005), see also chapter 6.2.1. The JECFA (WHO, 2004) already noted that an increasing body of data is now indicating that raised methylmercury exposure may augment the risk of cardiovascular morbidity and mortality but considered that the data available are not conclusive and therefore could not be used as a basis for estimating the PTWI.

An evaluation performed by the EFSA considered the reduction of the PTWI for methylmercury by the JECFA as justified because the new PTWI is based on the most susceptible lifestage, i.e. the developing foetus and intake during pregnancy, rather than on the general adult population (EFSA, 2004a). The EFSA evaluation noted that the JECFA and the U.S.-NRC considered several sources of uncertainties and that the health based guidance values differed by a factor of two, largely because different uncertainty factors were used.

7.1.2 Toxicity of selective organochlorine compounds

There are numerous organochlorine contaminants in the environment such as PCDD/F, PCBs, camphechlor, dichlorodiphenyltrichloroethane (DDT) and its metabolites (DDD, DDE), chlordane, dieldrin, aldrin, endrin, heptachlor, hexachlorbenzene. They belong to a class of

chemicals generally referred to as persistent organic pollutants (POPs). Most of these compounds are no longer in use and levels in the environment are generally decreasing. This section summarises the toxicological properties of the most relevant compounds, i.e. dioxin-like compounds, and examples of some of the other organochlorine contaminants, i.e. camphechlor and hexachlorocyclohexane.

Polychlorinated dioxins and furans

There are in total 210 different congeners of polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzo-*p*-furans (PCDFs) which are often referred to as ‘dioxins’. PCDD/Fs are not intentionally produced, but are formed as by-products or impurities in several industrial processes as well as from most combustion processes, such as chemical, pulp and metal industries, incineration of municipal and hazardous waste and other high-temperature processes including small scale burning. PCDD/Fs are also found in soil and sediment and these can act as secondary sources of dioxins to the environment.

PCDD/Fs are bioaccumulated and biomagnified in the environment and are therefore found at elevated concentrations in predatory animals at high trophic levels, especially in aquatic food chains. As PCDD/Fs are lipophilic, high levels are found in fatty fish such as salmon and herring.

Among the 210 congeners the 17 PCDD/Fs with chlorine substitution in positions 2, 3, 7 and 8 are the most toxic. All 17 congeners as well as 12 dioxin-like polychlorinated biphenyls (see below) have the same mode of action, elicited by binding to the same receptor, the Ah receptor and show comparable qualitative effects but with different potencies. These differences in potency are expressed in the toxic equivalency factors (TEFs). Consensus on the TEFs for PCDD/Fs and dioxin-like PCBs for human risk assessment (WHO-TEFs) was obtained at a WHO meeting in 1997 (van den Berg *et al.*, 1998). Long-term exposure leads to increased dioxin levels in fatty tissues and may result in developmental effects in children, as well as cancer and other diseases.

The SCF and JECFA established tolerable intake levels for ‘dioxins’ in 2001 (EC SCF, 2001; WHO, 2001). Both committees concluded that the risk assessment should be based on effects of TCDD on the developing male reproductive system resulting from the maternal body burden. A threshold approach was used to derive a tolerable intake of 2 pg TCDD/kg b.w. per day. This was extended to include other PCDDs, PCDFs and dioxin-like PCBs (see below), and expressed over a longer time period because of the long half-lives in the human body. SCF established a group Provisional Tolerable Weekly intake of 14 pg WHO-TEQ/kg (EC, 2001). JECFA established a group Provisional Tolerable Monthly Intake of 70 pg WHO-TEQ/kg b.w. (WHO, 2001). There are some differences in approaches used by other authorities for the assessment of dioxins and dioxin-like compounds to human health risk and the international approaches were recently discussed in an EFSA colloquium (EFSA, 2004e). While there is wide agreement in the scientific community on the principle in deriving health-based guidance values, the interpretation of the results of such assessments may vary greatly depending on the policy of national authorities.

Polychlorinated biphenyls

Polychlorinated biphenyls (PCBs) consist of the biphenyl structure with two linked benzene rings, in which some or all of the hydrogen atoms have been substituted by chlorine atoms. There are in total 209 different congeners. All PCB congeners are lipophilic and the lipophilicity increases with increasing degree of chlorination. PCBs are highly persistent and accumulate within food chains.

Due to their unique physical and chemical properties such as non-flammability, stability, high boiling point, low heat conductivity and high dielectric constants, PCBs have been widely used commercially since the 1920s as dielectric and heat exchange fluids and in a variety of other industrial applications. Since the 1970s there were strong restrictions for the use of PCBs in several EU countries and today PCBs are banned in most countries. However, entry into the environment due to improper disposal practices of PCB-containing materials or leakage from transformers and hydraulic systems still in use cannot be excluded.

For technical purposes, PCBs were never used as single compounds, but always as complex technical mixtures. Among the 209 individual PCB congeners that are theoretically possible, 12 have a chemical structure, which enable them to fit into the ligand-binding domain of the Ah receptor. These congeners, referred to as dioxin-like PCBs, exhibit toxicological effects (effects on liver, thyroid, immune function, reproduction and behaviour) similar to those caused by TCDD/Fs. WHO have assigned TEFs to them (see above). Typically, dioxin-like PCB congeners have chlorine substituents in para- and meta-positions and at the most, in one ortho-position. The dioxin-like PCBs are included in the tolerable intake levels established for PCDDs and PCDFs (see above).

The other group of PCBs, non-dioxin-like PCBs constitute a major part of the PCB congeners found in human tissues and food. They do not bind to the Ah receptor and do not show dioxin-like toxicity, but exhibit a different toxicological profile. This comprises in particular effects on the developing nervous system and neurotransmitter function. Mixtures used to study the toxicity of PCBs contain both non-dioxin-like and dioxin-like PCBs. It is therefore difficult, if not impossible, to differentiate between the toxic effects of dioxins and non-dioxin-like and dioxin-like PCBs

With the exception of specific cases of accidental or occupational exposure, for the majority of the human population dietary intake is considered the main pathway of exposure to non-dioxin as well as dioxin-like PCBs.

Currently for NDL-PCBs there is no reliable health based guidance value available for human risk assessment.

Camphechlor

Camphechlor is a technical mixture of a large number of chlorinated bornanes containing 67 - 78 % chlorine resulting from chlorination of camphene. The number of congeners in technical mixtures has been estimated as up to 670 but no individual congener exceeds 5 % by weight. Camphechlor is a non-systemic contact and stomach insecticide with some acaricidal action. Camphechlor has been the most heavily applied insecticide in the U.S. and in many parts of the world and replaced DDT as a major insecticide in the early 1970s. Environmental degradation and congener specific accumulation in the aquatic environment has led to relatively high levels of certain camphechlor congeners in fish, fish oil, marine mammals and sea birds, i.e. congener #26, #50 and #62 (Parlar coding system). Depending on species, origin, fat content and procedure of calculating total camphechlor, the sum of these three congeners normally amounts to approximately 8 – 50 % of total camphechlor.

Camphechlor was evaluated by WHO in 1984 (WHO, EHC, 1984) and by a Nordic expert group under the Nordic Committee of Senior Officers for Food Issues, the Nordic Council of Ministers in 1996 (Anonymous, 1997) and more recently by Brüscheiler *et al.* (2004).

The evaluation was based on repeated dosing in laboratory animals (mice, rats, guinea pigs, dogs and macaques) and effects in liver, thyroid and kidney as well as the immune system.

Also developmental and reproductive toxicity has been observed. The Canadian Authorities established in 1999 (Health Canada, personal communication) a TDI of 200 ng/kg b.w./day based on the NOAEL value (0.2 mg/kg/day) for liver toxicity in a 13 - week study in dogs (Chu *et al.*, 1986), using an uncertainty factor of 1000. Brüscheiler *et al.* (2004) derived a TDI of 100 ng/kg b.w./day based on the NOAEL of 0.1 mg/kg b.w./day for immunotoxicity in a 33 weeks study in macaques (Tryphonas *et al.*, 2001) by using also a factor of 1000 (an additional uncertainty factor of 10 to the normal 100 default uncertainty factor was applied, because humans are exposed to a different mixture of camphechlor through food than the technical mixture used in experiment).

Hexachlorocyclohexane

Technical hexachlorocyclohexane (HCH) is a mixture of various HCH isomers; alpha, beta, gamma and delta. Both technical HCH and gamma-HCH (lindane), which possesses the insecticidal activity, have been globally used as insecticides. Because of the lipophilic properties and persistence in the environment, beta-HCH followed by alpha-HCH and to a less extent gamma-HCH may give rise to bioaccumulation and biomagnification through the food chain (EFSA, 2005c).

HCHs are rapidly absorbed from gastrointestinal tract, pass the placenta and are transferred into milk. The toxicity of the isomers varies, gamma-HCH being the most acutely neurotoxic followed by alpha-HCH. Beta-HCH penetrates less readily into the central nervous system and is more persistent and tends to accumulate in the body over time. All isomers cause liver hyperplasia and/or liver tumours most likely by a non-genotoxic mode of action. Beta-HCH has weak estrogenic activity (EFSA, 2005c). HCHs were classified in group 2B, showing sufficient (alpha-HCH, and technical HCH) or limited evidence (beta-HCH and gamma-HCH) for carcinogenicity in animals, by IARC (1979 and 1987)

The FAO/WHO Joint Meeting on Pesticide Residues (JMPR) established an ADI of 0 – 0.005 mg/kg b.w./day on the basis of liver toxicity in rats (WHO, 2002). In 1992 Health Canada set a group TDI for all HCH isomers of 0.3 µg/kg b.w. (Feeley, 2005).

7.1.3. Toxicity of brominated flame retardants

There are more than 75 different brominated flame retardants (BFRs) recognised commercially. Approximately 500,000 metric tons of bromine are produced each year, and BFRs accounted for 38 % of this in 2000. There are five major classes of BFR: brominated bisphenols, diphenyl ethers, cyclododecanes, phenols and phthalic acid derivatives, with the first three classes representing the highest production volumes. Polybrominated biphenyls (PBBs) were removed from the market in the early 1970s following an incident involving contamination of animal feed with Firemaster FF-1. Currently, 5 major BFRs constitute the overwhelming majority, but the situation changes as new substances are introduced or discontinued. These five are: tetrabromobisphenol A (TBBPA), hexabromocyclododecane (HBCD) and three commercial mixtures of polybrominated diphenyl ethers (PBDEs), referred to as decabromodiphenyl ether (DBDE), octabromodiphenyl ether (OBDE), and pentabromodiphenyl ether (pentaBDE). As a result of their potential to bioaccumulate in the environment, the manufacture and use of OBDE and pentaBDE have been banned in the EU since August 2004, and industrial producers are engaged in a voluntary initiative to cease production and use worldwide. The Restriction on Hazardous Substances Directive bans PBDEs in new electrical goods from July 2006 which will tend to reduce DBPE use, which is not otherwise explicitly controlled.

Some of these substances are persistent organic contaminants in the environment, with the potential to contaminate the food chain long after production has ceased. Risk assessment is complicated by incomplete toxicological databases and lack of information on exposure to humans from different sources. European Union risk assessment reports and scientific opinions of the EC Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE) and the Scientific Committee on Health and Environmental Risks (SCHER) are available for pentabromodiphenyl ether, octabromodiphenyl ether, decabromodiphenyl ether, hexabromocyclododecane and tetrabromobisphenol (EC 2005, EC JRC 2004, EC JRC 2003 EC JRC 2002, EC 2002, and EC 2000).

Polybrominated diphenyl ethers (PBDEs)

There are 209 individual PBDE congeners, identified by congener numbers related to the number and positions of the bromine substituents. The three commercial PBDE flame-retardants, pentaBDE, OBDE and DBDE are not pure products but a mixture of various diphenyl ethers with varying degrees of bromination. Example compositions of PBDEs have been published (see Table 14).

Table 14. Relative congener distribution for penta- and octaBDE

Commercial product	% congeners in commercial product						
	Tetra	Penta	Hexa	Hepta	Octa	Nona	Deca
PentaBDE	24 - 38	50 - 62	4 - 12	-	-	-	-
OBDE	-	-	≤ 12	≤ 45	≤ 33	≤ 10	-
DBDE	-	-	-	-	-	≤ 3	≤ 97

The available regulatory toxicity studies have been conducted with the commercial PBDE mixtures. In contrast, the data on occurrence of PBDEs in food relate to a range of individual congeners, which are commonly summed as total PBDE. The range of congeners measured in different studies may vary, and thus the data for total PBDE are not necessarily comparable. Similarly, dietary exposure is to complex mixtures of PBDEs, which may vary in proportion depending on the type of food and region of sampling, but the most persistent congeners are expected to predominate.

Toxicological evaluations of PBDEs rely on the risk assessments conducted under the EU Existing Substances Regulations, because much of the data is unpublished. In general the toxicological databases are poor. The UK Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) noted that the majority of studies reviewed were relatively old and would not meet current requirements for study design and reporting (FSA, 2004b).

The liver is a target organ for the PBDEs, with pentaBDE being the most toxic, and DBDE the least. It is also notable that the duration of the longest studies undertaken with pentaBDE was similar to the reported half-life and the resulting tissue concentrations would only have reached half of their maximal value by the end of the study. OBDE has been found to exhibit reproductive toxicity, whereas pentaBDE and DBDE did not produce adverse effects in routine developmental studies. Non-routine studies suggest that the most sensitive endpoints are neurodevelopmental. Two of the congeners that are present in commercial pentaBDE (PBDE-47: 2,2',4,4'-tetraBDE; PBDE-99: 2,2',4,4',5-pentaBDE) have been shown to cause neurobehavioral effects in adult mice following administration of a single postnatal oral dose at the lowest doses tested (0.6 and 0.8 mg/kg body weight/day respectively). This type of effect may be linked to a decrease in circulating thyroid hormones observed in PBDE treated rodents. At the time of the COT evaluation, data from this type of study were not available for

OBDE and DBDE, and the congeners commonly found in them. However recently Viberg *et al.* (2003a,b) reported that DBDE and PBDE 153 can affect neonatal brain development in mice. The COT noted that neonatal mice would be at a developmental stage comparable to infants up to one month of age. Some of the higher brominated congeners of PBDEs, such as BDE-153, may be subject to biomagnification, and decabrominated BDE (BDE-209) will be more extensively used in the future. Evidence is therefore needed on the fate of these congeners in regard to contamination of farmed fish. There are no neurodevelopmental studies with exposure *in utero* or via lactation and a lack of information on concentrations in breast milk that could result from consumption of fish by the mother. Overall, the COT considered it was not possible to base a risk assessment on the neurodevelopmental effects.

In view of the inadequacies in the toxicological database and the absence of identifiable no-effect levels for some endpoints, the COT concluded that it was not possible to determine a tolerable daily intake (TDI). The COT therefore decided to take a Margin of Exposure (MoE) approach in which the estimated human exposures were compared with the relevant NOAEL or LOAEL identified from the animal studies. Based on the evidence that pentaBDE is the most toxic, the COT assumed that comparison of the estimated intakes of the sum of the measured PBDE congeners with the reported effect levels for pentaBDE provides a precautionary approach because some of the congeners are expected to be less toxic.

Had it been possible to establish a TDI from a NOAEL, uncertainty factors (UFs) would be required to allow for inter- and intraspecies differences in toxicokinetics and toxicodynamics (100) and limitations in the database such as study duration and gaps in the data (up to 10). Combining these uncertainty factors suggested a target MoE of 1000 for liver toxicity of penta-BDE, above which risks to health would not be expected. Comparison with the NOAEL of 0.45 mg/kg b.w./day indicates that exposures to total PBDE below 0.45 µg/kg b.w./day would not be a concern (FSA, 2004b).

Since the COT evaluation, information became available demonstrating that administration of a single postnatal oral dose of DBDE to neonatal mice also produced neurobehavioral effects in adult mice (at lowest test dose of 2.2 mg/kg b.w./day). This observation adds to uncertainty about the potential for neurodevelopmental effects, but is unlikely to alter the COT evaluation. The Scientific Committee on Health and Environmental Risks (SCHER) concluded that there is a need for further studies on the neurotoxicity of PBDEs (EC, 2005). SCHER also noted concern that following release to the environment, DBDE may be degraded to lower brominated, bioaccumulating substance.

The JECFA evaluated the toxicology of PBDEs in February 2005. JECFA concluded that for the more toxic PBDEs, adverse effects would be unlikely to occur in rodents at doses of less than approximately 100 µg/kg b.w./day (WHO, 2005). Dividing this dose by the target MoE of 1000 recommended by the COT indicates that exposure of 0.1 µg/kg b.w./day or less in humans would be of low concern.

Hexabromocyclododecane (HBCD)

HBCD is synthesised through bromination of cyclododecatriene. It is commercially available in the EU as a mixture of three stereoisomers α , β and γ . All toxicological studies with HBCD were conducted using the commercial mixture. The α -isomer has been found to accumulate more than the β - and γ -isomers, and therefore predominates in food. The extent of metabolism of the commercial HBCD is unknown.

The limitations of the toxicological databases on the PBDEs, similarly apply to HBCD. HBCD is also hepatotoxic (LOAEL of 100 mg/kg b.w./day). It has not shown evidence of

developmental toxicity in routine studies, but neurodevelopmental effects have been observed following administration to neonatal mice using a protocol similar to that with PBDEs (LOAEL of 0.9 mg/kg b.w./day, study available in abstract form only at the time of the COT evaluation).

Following a similar approach to that taken for the PBDEs, the COT considered that a target MoE of 3,000 - 10,000 was required for HBCD. Comparison with the LOAEL of 100 mg/kg b.w./day indicates that exposures below 10 µg/kg b.w./day would not be a concern (FSA, 2004b).

Additional data or evaluations, subsequent to the COT opinion, have not been identified.

Tetrabromobisphenol A (TBBPA)

Tetrabromobisphenol A (TBBPA) is a single compound and the database is relatively more complete than for the PBDEs and HBCD. A recent COT evaluation of TBBPA is available (FSA, 2004c).

Repeat dose studies revealed no toxicologically significant effects at doses up to 10,000 mg/kg b.w. per day, administered for 90 days. No long-term carcinogenicity study was available. However, the results of mutagenicity studies were negative and the subchronic studies provided no indication of a mechanism to suggest that TBBPA could lead to carcinogenesis of relevance to humans following life-time exposure.

TBBPA has been shown to be nephrotoxic at high doses in newborn rats (Fukuda *et al.*, 2004).

The lack of effects in a recent 2-generation reproductive toxicity study in rats suggests that TBBPA is not an endocrine disruptor *in vivo* at doses relevant to human exposure. Discussions focussed on results of two neurotoxicity studies in rats, showing conflicting results. A developmental study indicated that TBBPA administration to rats by gavage at 25 mg/kg b.w./day from gestational day 7 to postnatal day 17 resulted in altered performance in some neurobehavioral tests conducted in the offspring at 7 months of age. In contrast, a 2-generation study revealed no toxicologically significant effects on neurodevelopment at doses of up to 1000 mg/kg b.w./day by gavage. After detailed consideration, the COT concluded that the findings at 250 mg/kg in one of the studies were likely to have been due to chance.

The COT agreed that the highest dose tested of 1000 mg/kg b.w./day, which was without clear adverse effects in a 90-day study and in a two-generation reproductive toxicity study, could be used as the basis for deriving a Tolerable Daily Intake (TDI). A total uncertainty factor of 1000 was applied in order to set a TDI of 1 mg/kg b.w./day. This uncertainty factor comprised the default uncertainty factor of 100 to allow for inter- and intra-species variation and an additional factor of 10 for the absence of chronic toxicity studies. TBBPA has been shown to have weak estrogenic activity in some *in vitro* assays.

Polybrominated biphenyls (PBBs)

The toxicological profiles of the PBBs are expected to resemble that of the polychlorinated biphenyls (PCBs). However, Toxic Equivalency Factors (TEFs) have not been allocated for the co-planar congeners, and relevant toxicological evaluations have not been identified.

7.1.4. Toxicity of organotin compounds

Organotin compounds (OTC) are lipophilic contaminants which tend to bioaccumulate through the food chain (particularly in fish and seafood). OTC compounds have been recently

assessed by EFSA (EFSA, 2004b). The main source of OTC in fish is likely to be tri-substituted compounds, e.g. tributyltin (TBT), and triphenyltin, which have been used widely as biocides in wood preservatives, in antifouling paints for boats and as pesticides. Mono- and di-substituted OTC, e.g. dibutyltin (DBT), di-n-octyltin (DOT) are generally used in mixtures in various amounts as stabilisers in polyvinyl chlorides (PVC). TBT, DBT, TPT and DOT act by a similar mode of immunotoxic action and potency (lymphocyte depletion in thymus and peripheral lymphoid tissues). A NOAEL for immunotoxicity of 0.025 mg/kg b.w./day was identified for TBT oxide from chronic rodent feeding studies. By applying an uncertainty factor of 100, a group TDI of 0.25 µg/kg b.w. for TBT, DBT, TPT and DOT compounds was established (based on TBT oxide molecular mass, this group TDI is 0.1 µg/kg b.w. when expressed as Sn content or 0.27 µg/kg b.w. when expressed as TBT chloride).

7.2. Exposure scenarios

7.2.1. General considerations

Scenarios for exposure to chemicals from fish were created by combining data on fish consumption by consumers (Table 15) with concentrations of chemical in fish. For a vast range of organic contaminants in the environment, quantitative data on occurrence is virtually lacking for various fish species. Moreover, for a number of contaminants, fish does not contribute significantly to the human exposure; therefore, the present opinion focuses on those chemicals for which fish is a major contributor to total dietary exposure. When available, the contribution of individual fish species to the overall exposure to these contaminants was provided. It should be noted that in all national surveys, the consumption of fish by species is in general underestimated because consumers are often unaware of the type of fish they are eating. Therefore, scenarios for the total exposure from all fish was also calculated. This last calculation should be considered as a reasonable overestimation of the exposure, assuming that the consumer eats only a single fish species.

Food consumption data from 6 countries (France, Italy, Norway, Sweden, the Netherlands and the UK) were used. The consumption of salmon, herring, tuna, trout, carp, pilchard/sardines and mackerel was considered in those countries based on individual food consumption surveys. In addition, data on carp consumption were provided by the Czech Republic. The survey methodologies used were very different between countries and included individual records from 2 to 7 days and food frequency questionnaires.

In order to compare those data, all results were provided for consumers only (mean daily consumption over the total number of days of the survey for all individuals who consumed the specific food group at least once during the survey).

The overall average indicative fish consumption ranged from 34 to 77 g/day and the 99th percentile of indicative fish consumption ranged from 113 to 190 g/day (Table 15). The Norwegian data were taken from fish consumption in coastal communities. The consumption of carp was estimated in the Czech Republic (Ruprich, personal communication). In this country, anglers represent a population of high consumers of fresh water fish with a reported median consumption of 39 g/day and a 95th percentile consumption of 105 g/day. Those values are similar to those observed in other European countries for total fish.



Table 15. Indicative fish consumption figures for ‘consumers only’ from 6 countries in g/day

Consumption	Mean FR	Mean IT	Mean NL	Mean NO	Mean SE	Mean UK	99th FR	99th IT	99th NL	95th NO	99th SE	97.5th UK
Salmon	15	14	19	8	5	19	63	60	65	14	27	66
Herring	11	20	30	3	5	19	42	66	94	7	30	53
Tuna	11	15	14	8	3	16	31	61	55	31	27	52
Trout	32	30	NA	5	NA	22	55	87	NA	14	NA	53
Carp	NA	8	NA	NA	NA	NA	NA	8	NA	NA	NA	NA
Pilchards/sardines	10	22	NA	NA	NA	15	47	70	NA	NA	NA	62
Mackerel	13	25	16	4	NA	17	54	245	40	10	NA	47
Other fishes	31	43	33	NA	18	32	99	190	101	NA	75	87
Non specified fish	29	NA	39.0	NA	NA	NA	129	NA	107	NA	NA	NA
Total Fish	36	48	34	77	29	43	141	190	123	174	113	136

NA: Data not available

In addition, the average portion size for fish was estimated in 5 countries (Norway used food frequency questionnaires and typical portion size was not measured or estimated by the subjects of the survey). The average portion size represents individual mean daily consumption on an eating occasion. The average portion size ranged from 99 to 129 grams in The Netherlands and Italy, respectively. These figures are similar to the high percentiles of consumption, meaning that high level consumers of fish eat about 1 average portion of fish each day.

The percentage of consumers of fish (total) ranges from 15 to 78 % respectively for Netherlands and Sweden, meaning that 15 to 78 % of the population of the surveys, consumed a portion of fish at least once during the survey. It should be noted that short survey duration (2 to 7 days) underestimates the percentage of consumers over a longer period of time. This is particularly evident for Netherlands for which the survey is conducted on 2 consecutive days.

Average portion sizes for meat and eggs were also estimated in 4 countries. This assumption was used for the scenarios of substitution of fish by other foods of animal origin (meat, poultry and eggs). Results are summarised in Table 16.

Table 16. Average portion sizes (in g) for fish, meat and eggs in 4 countries

	Italy	France	Netherlands	Sweden
Fish	129	118	99	114
Meat	116	131	61	105
Egg	41	107	43	67

For this opinion a typical portion size of 130 g was used for both fish and meat.

It should be noted that the national differences are probably in part due to differences in the survey methodology. In certain countries (e.g. Italy), the consumption is split relative to the recipes which has the effect of decreasing the mean value for certain foods that can be included in recipes in small amounts (eggs in salad or fish on pizza for example).

The concentrations of dioxins (PCDD/F and DL-PCBs), non-dioxin-like PCBs, PBDEs, camphechlor, organotin compounds and mercury were combined with both the average and the high percentile of consumption. In order to reflect a long term exposure, the mean, or when available the median, contamination level were used.

7.2.2 Baltic herring

The Baltic Sea is one of the most thoroughly studied water bodies in the world. It is characterized by high levels of a number of pollutants in biota and sediments. The explanation of this is not fully elucidated. Industrial activities in the past together with the long retention time of the water might be important factors. Available data show that concentrations of hazardous substances such as dioxins and PCBs have declined in Baltic fish over the past three decades. This decreasing trend has however become less obvious in many areas and during the last decade no further decrease was seen.

There are many different strains of Baltic herring that migrate and feed in different areas of the Baltic Sea. There are also strains that migrate to and from the North Sea area. These differences in strain, behaviour and feeding area is part of the explanation for the great variation in concentration of persistent organic contaminants found in Baltic herring. In Sweden the National Food Administration has investigated the consumption of fatty fish from the Baltic (herring and salmon) by different age groups of the general population. The results

show that younger people eat less fish from the Baltic than do older people. Women above 40 in general consume 6 g/day, whereas women below 40 consume 2 g/day. The total fish consumption in these two groups was found to be 36 and 27 g/day respectively.

7.2.3. Methylmercury

Analytical results are expressed as total mercury but the most toxic substance from the diet for human health is the organic form, methylmercury (MeHg), which occurs primarily as a result of microbial activity on the mercury present in sea. MeHg is present in aquatic food with the highest level occurring in predatory fishes, particularly those at the top of the aquatic food chain (Claisse *et al.*, 2001; Cossa *et al.*, 1989). In order to be conservative, it is assumed that all the mercury present in fish is MeHg. The recent opinion of the CONTAM panel of the EFSA concluded that fish is the main contributor to the exposure to MeHg even if an additional contribution is from molluscs and crustaceans (EFSA, 2004a).

Among the fish species considered important for this opinion, tuna is the major contributor to human exposure to MeHg because of both its contamination levels (tuna is at the end of the food chain) and its level of consumption. Methylmercury levels are not related to the fat content. In the different tuna species, MeHg concentrations are very different and respective average concentrations were associated to 4 specific tuna species (French Authorities, DGAL, DGCCRF, IFREMER, FIAC). These data were combined with consumption data for all tuna, rather than for the consumption of the individual tuna species. Therefore estimates of exposure to methylmercury may be overestimated for the more contaminated species if they are not consumed frequently. In particular canned tuna seems to have lower levels of methylmercury than fresh tuna due to different species and/or size used. For other fish species, the mean levels of total mercury reported in the SCOOP task on heavy metals were used (see Table 9 of Annex 1).

In order to compare human exposure with health based guidance values (HBGV) exposure scenarios were expressed in $\mu\text{g}/\text{kg}$ b.w/week for adults, assuming 60 kg body weight (Table 17). In addition, because the consumption can be underestimated by misreporting of fish species from the survey, simulations were performed assuming that the total fish consumed is contaminated at 0.1 and 0.4 $\mu\text{g}/\text{g}$ total mercury. These 2 values represent reasonable overestimates of the exposure to MeHg respectively for a consumer of non predatory fish and for a regular eater of predatory fish. It should be noted that the case of a high consumer exclusively of top predatory fish contaminated at 0.4 $\mu\text{g}/\text{g}$ is unlikely in Europe. Because the highest intakes come from tuna, these data are used in the risk characterisation.

The exposure scenarios should not be considered as national exposure assessments. For example in the UK most of the tuna consumed is canned skipjack and therefore calculations based on albacore or blufin are considerable overestimates.

Limited data on methylmercury in Baltic herring indicate that although levels are slightly higher than in non-Baltic herring, they are lower than in tuna and therefore not a specific concern (see Table 10 of Annex 1).

Table 17. Exposure scenarios for methylmercury (in µg/kg b.w./wk) from selected fish species in 6 European countries (for more details on occurrence data, see Table 9 of Annex 1).

Assumed MeHg levels in µg/g fish	Mean scenario	99th scenario	99th scenario	99th scenario	95th scenario	99th scenario	97.5th scenario					
	A	B	C	D	E	F	A	B	C	D	E	F
Salmon (0.04)	0.07	0.06	0.09	0.04	0.02	0.09		0.27	0.23	0.06	0.12	0.30
Herring (0.03)	0.04	0.08	0.12	0.01	0.02	0.08	0.17	0.27	0.38	0.03	0.12	0.22
Tuna Skipjack (0.15)	0.19	0.26	0.24	0.14	0.05	0.28	0.54	1.07	0.96	0.54	0.47	0.91
Tuna Yellowfin (0.3)	0.38	0.52	0.49	0.28	0.10	0.56	1.08	2.13	1.92	1.08	0.94	1.82
Tuna Albacore (0.49)	0.63	0.86	0.80	0.46	0.17	0.91	1.77	3.49	3.14	1.77	1.54	2.97
Tuna Bluefin (0.49)	0.63	0.86	0.80	0.46	0.17	0.91	1.77	3.49	3.14	1.77	1.54	2.97
Trout (0.04)	0.15	0.14	NA	0.02	NA	0.10	0.26	0.42	NA	0.07	NA	NA
Carp (0.03)	NA	0.03	NA	NA	NA	NA	NA	0.03	NA	NA	NA	NA
Pilchards/sardines (0.04)	0.04	0.09	NA	NA	NA	0.06	0.20	0.29	NA	NA	NA	0.26
Mackerel (0.04)	0.06	0.11	0.07	0.02	NA	0.08	0.25	1.11	0.18	0.05	NA	0.21
Total (0.4)	1.68	2.24	1.59	3.60	1.35	2.01	6.58	8.87	5.74	8.12	5.27	6.35
Total (0.1)	0.42	0.56	0.40	0.90	0.34	0.50	1.64	2.22	1.43	2.03	1.32	1.59

Note: MeHg concentrations for fishes other than tuna were compiled from the European SCOOP task on heavy metals (EC, 2004) and from Norwegian data submitted to the panel (Jan Alexander, personal communication). Contamination data of tuna by species were provided by the French authorities (DGAL, DGCCRF, IFREMER, FIAC) and correspond to a survey from 1997 to 2002 (174 samples).

NA: Data not available

The principal tuna species used for canning are skipjack and yellowfin (FAO 2003b).

7.2.4. Organochlorine compounds

PCDD/F and DL-PCBs

The average concentration of PCDD/Fs and DL-PCBs in herring from the Baltic Sea, expressed as total TEQ, was reported to be 11.5 ng WHO-TEQ/kg. The concentration of PCDD/F and DL-PCBs in herring NOT from the Baltic Sea was reported to be 3.2 ng WHO-TEQ/kg fish (Gallani *et al.*, 2004, see Table 5 of main text). More recent data provided by Sweden on PCDD/Fs and DL-PCBs values in Baltic herring confirm this level, see table 13 of Annex 1

From the call for data by DG SANCO (Gallani *et al.*, 2004), the reported concentrations of PCDD/F and DL-PCBs in salmon ranged from 0.6 to 6.8 (non-Baltic salmon, mainly farmed) and 6.0 to 32 pg (Baltic salmon) WHO-TEQ/g fresh weight. Based on the most frequent levels of contamination, simulations can be performed in order to assess the impact of salmon intake on exposure to dioxins. Two levels of 1 and 4 pg WHO-TEQ/g fresh weight were used as representative levels for low and high contamination of non-Baltic salmon.

Because the exposure estimate can be underestimated by misreporting of fish species from the survey, additional simulations were performed assuming that the total fish consumed is contaminated at 1 (median reported contamination level for oily fish) vs. 4 pg/g total TEQ (90th %-ile of the reported contamination level), see Table 18. The exposure to PCDD/F and DL-PCBs for consumers eating only oily fish ranges from 0.03 and 0.76 pg WHO-TEQ/day corresponding to respectively 0.5 and 12 pg/kg b.w./day assuming 60 kg body weight.



Table 18. Exposure scenarios for PCDD/F and DL-PCBs (in pg total TEQ/g b.w./wk) from selected fish species in 6 European countries

Assumed total TEQ levels in pg/g fish	Mean scenario A	Mean scenario B	Mean scenario C	Mean scenario D	Mean scenario E	Mean scenario F	99th scenario A	99th scenario B	99th scenario C	95th scenario D	99th scenario E	97.5th scenario F
Salmon (1)	1.75	1.63	2.22	0.93	0.58	2.22	7.35	7.00	7.58	1.63	3.15	7.70
Salmon (4)	7.00	6.53	8.87	3.73	2.33	8.87	29.40	28.00	30.33	6.53	12.60	30.80
Herring (3.2)	4.11	7.47	11.20	1.12	1.87	7.09	15.68	24.64	35.09	2.61	11.20	19.79
Baltic herring (11.5)	NS	NS	NS	4.0	6.7	NS	NS	NS	NS	9.4	40.1	NS
Baltic salmon (16.47)	NS	NS	NS	15.37	9.60	NS	NS	NS	NS	26.90	51.89	NS
Tuna (1.76)	2.26	3.08	2.87	1.64	0.62	3.29	6.37	12.53	11.29	6.37	5.54	10.68
Trout (1)	3.73	3.50	NA	0.58	NA	2.57	6.42	10.15	NA	1.63	NA	6.18
Pilchards/sardines (1.3)	1.52	3.34	NA	NA	NA	2.28	7.13	10.62	NA	NA	NA	9.40
Mackerel (0.9)	1.47	2.83	1.81	0.45	NA	1.92	6.11	27.73	4.53	1.13	NA	5.32
Total fish (1)	4.20	5.60	3.97	8.98	3.38	5.02	16.45	22.17	14.35	20.30	13.18	15.87
Total fish (4)	16.8	22.4	15.9	35.9	13.5	20.1	65.8	88.7	57.4	81.2	52.7	63.5

NS: No simulations performed as assumed not to be consumed in significant amounts in these countries, or no data available

NA: Data not available

The average concentration of PCDD/F and DL-PCBs in meat (ruminants and poultry) is 1.1 ng WHO-TEQ/kg fat. Assuming 12 % of lipids (beef rib steak, ref Repertoire Général de Aliments, TEC et DOC ed. 1991), a 130 g portion of meat would correspond to 0.017 ng TEQ of dioxins and PCBs or 0.3 pg/kg b.w.

Non-dioxin like PCBs

For NDL-PCBs, contamination data are limited for individual fish species. Therefore exposure scenarios were based on total fish consumption.

The average concentration of NDL-PCBs in fish was estimated to be 12.5 ng/g of fish (Gallani *et al.*, 2004). Therefore the scenarios for average and the 99th percentile of intake for NDL-PCBs from fish range from 0.36 to 0.96 µg/day/person and from 1.41 to 2.38 µg/day/person (42 to 112 and 165 to 277 ng/kg b.w./week), respectively (Table 19).

The average concentration of total NDL-PCBs in meat is 12.7 ng/g fat (in ruminants). Assuming 12 % of lipids (beef rib steak, ref Repertoire Général de Aliments, TEC et DOC ed. 1991), a portion of meat of 130 g would correspond to 0.16 µg of NDL-PCBs.

From occurrence data provided by Sweden for NDL-PCBs in Baltic herring, the median concentration was estimated to be 20.9 ng/g fish (range: 3.7-125 ng/g fish; average: 38 ng/g fish), see table 13 of Annex 1. Data are supported by the report of Gallani *et al.*, (2004), see also table 7 of main text.

Table 19. Exposure scenarios for **NDL-PCBs** in (ng/kg b.w./wk) from fish in 6 European countries

Assumed levels in ng/g fish	Mean scenario A	Mean scenario B	Mean scenario C	Mean scenario D	Mean scenario E	Mean scenario F	99th scenario A	99th scenario B	99th scenario C	95th scenario D	99th scenario E	97.5th scenario F
Total fish (12.5)	53	70	50	112	42	63	206	277	179	254	165	198
Baltic herring (20.9)	NS	NS	NS	7.3	12.2	NS	NS	NS	NS	17.1	73.1	NS

NS: No simulations performed as assumed not to be consumed in significant amounts in these countries

Table 20. Exposure scenarios for **camphechlor** (in µg/kg b.w./wk) from selected fish species in 6 European countries

Assumed levels in ng/g fish	Mean scenario A	Mean scenario B	Mean scenario C	Mean scenario D	Mean scenario E	Mean scenario F	99th scenario A	99th scenario B	99th scenario C	95th scenario D	99th scenario E	97.5th scenario F
Salmon (56) n=13	0.10	0.09	0.12	0.05	0.03	0.12	0.41	0.39	0.42	0.09	0.18	0.43
Herring (32) n=292	0.04	0.07	0.11	0.01	0.02	0.07	0.16	0.25	0.35	0.03	0.11	0.20
Trout (4 µg) n=115	0.01	0.01	0.00	0.00	NA	0.01	0.03	0.04	0.00	0.01	NA	0.02
Mackerel (44) n=19	0.07	0.13	0.08	0.02	NA	0.09	0.28	1.26	0.21	0.05	NA	0.24
Total Fish (25) n=1314	0.11	0.14	0.10	0.22	0.08	0.13	0.41	0.55	0.36	0.51	0.33	0.40

NA: Data not available

Camphechlor

For camphechlor, contamination data were submitted by Germany (Germany food monitoring) for 4 fish species (salmon, herring, rainbow trout, mackerel).

The average concentration of camphechlor was estimated to be 25 µg/kg of fish (EFSA 2005b) resulting from the sum of 3 indicator congeners measured (#26, #50, #62, Parlar nomenclature) and multiplication by a factor of 4, which is a conservative assumption to account for all congeners. Therefore the scenarios for average and the 99th percentile of intake for camphechlor from total fish range from 0.7 to 1.9 µg/day/person and from 2.8 to 4.8 µg/day/person (0.08 to 0.22 and 0.33 to 0.55 µg/kg b.w./week), respectively (Table 20).

The Panel was not aware of any data on camphechlor in Baltic herring.

Fish, in particular fatty species are the main source of human exposure to camphechlor. Other sources, e.g. meat are less important (EFSA, 2005b).

7.2.5. Brominated flame retardants

PBDEs

For PBDEs, contamination data are not sufficient to allow differentiation between individual fish species. Therefore the exposure scenario was based on total fish consumption.

The median concentration of total PBDE in fish and shellfish was estimated to be 1.78 ng/g fresh weight (WHO, 2005). Therefore the scenarios for average and the 99th percentile of intake for PBDE from fish range from 51 to 137 ng/day/person and from 201 to 338 ng/day/person (6 to 16 and 23 to 39 ng/kg b.w./week), respectively (Table 21).

Limited data indicate that median levels of PBDEs in Baltic herring range from 0.8 - 5.5 ng/g fish depending on the location (see Table 11 and 12 of Annex 1).

The median concentration of total PBDE in meat and poultry was estimated to be 0.06 ng/g fresh weight (WHO, 2005). A portion of meat of 130 g would correspond to 7.8 ng/day or 0.1 ng/kg b.w.

Other brominated flame retardants

The few data available for other brominated flame retardants are not sufficient to perform an exposure assessment.



Table 21. Exposure scenarios for **PBDE** (in ng/kg b.w./wk) from fish in 6 European countries

Assumed levels in ng/g fish	Mean	Mean	Mean	Mean	Mean	Mean	99th	99th	99th	95th	99th	97.5th
	scenario A	scenario B	scenario C	scenario D	scenario E	scenario F	scenario A	scenario B	scenario C	scenario D	scenario E	scenario F
Total fish (1.78)	7.5	10.0	7.1	16.0	6.0	8.9	29.3	39.5	25.5	36.1	23.5	28.2

Table 22. Exposure scenarios for **organotin** compounds (in ng/kg b.w./wk) from fish in 6 European countries

Assumed levels in ng/g fish	Mean	Mean	Mean	Mean	Mean	Mean	99th	99th	99th	95th	99th	97.5th
	scenario A	scenario B	scenario C	scenario D	scenario E	scenario F	scenario A	scenario B	scenario C	scenario D	scenario E	scenario F
Total Fish (13.5)	8.1	10.8	7.7	17.3	6.5	9.7	31.7	42.8	27.7	39.2	25.4	30.6

7.2.5. Organotin compounds

For organotin compounds (OTC), contamination data do not exist or are not available for individual fish species. Therefore the exposure scenarios were based on total fish consumption.

The median concentration of OTC in fish (expressed as sum of tributyltin, dibutyltin, triphenyltin) was estimated to be 13.5 µg/kg of fish (EFSA, 2004b). Therefore the scenarios for average and the 99th percentile of intake for OTC from fish range from 0.4 to 1 µg/day/person and from 1.5 to 2.6 µg/day/person (8 to 17 and 25 to 43 ng/kg b.w./week), respectively (Table 22).

The Panel was not aware of any data on OTC in Baltic herring.

Fish and seafood are the major contributors to total dietary exposure (EFSA, 2004b), and intake from meat is therefore not considered in this opinion.

7.2.6. Nutrient intake

Fish is an important source of proteins of high biological value, LC n-3 PUFAs and certain vitamins and minerals. The intake of LC n-3 PUFA was estimated by multiplying the FA content by the amount of fish consumed (Table 23).

Table 23. Intake scenarios for LC n-3 PUFA (in g/day) from selected fish species in 6 European countries

Assumed levels in g/100 g fish	Mean scenario A	Mean scenario B	Mean scenario C	Mean scenario D	Mean scenario E	Mean scenario F	99th scenario A	99th scenario B	99th scenario C	95th scenario D	99th scenario E	97.5th scenario F
Wild salmon (1.5)	0.23	0.21	0.29	0.12	0.08	0.29	0.95	0.90	0.98	0.21	0.41	0.99
Farmed salmon (1.5)	0.23	0.21	0.29	0.12	0.08	0.29	0.95	0.90	0.98	0.21	0.41	0.99
Herring (2)	0.22	0.40	0.60	0.06	0.10	0.38	0.84	1.32	1.88	0.14	0.60	1.06
Canned tuna (0.25)	0.03	0.04	0.04	0.02	0.01	0.04	0.08	0.15	0.14	0.08	0.07	0.13
Fresh tuna (1.3)	0.14	0.20	0.18	0.10	0.04	0.21	0.40	0.79	0.72	0.40	0.35	0.68
Trout (0.8)	0.26	0.24	NA	0.04	NA	0.18	0.44	0.70	NA	0.11	NA	0.42
Carp (0.3)	NA	0.02	NA	NA	NA	NA	NA	0.02	NA	NA	NA	NA
Fresh pilchards/sardines (1.4)	0.14	0.31	NA	NA	NA	0.21	0.66	0.98	NA	NA	NA	0.87
Canned sardines (3.3)	0.33	0.73	NA	NA	NA	0.50	1.55	2.31	NA	NA	NA	2.05
Mackerel (2.51)	0.33	0.63	0.40	0.10	NA	0.43	1.36	6.15	1.00	0.25	NA	1.18
Mackerel (0.34)	0.04	0.09	0.05	0.01	NA	0.06	0.18	0.83	0.14	0.03	NA	0.16
Total fish low (0.3)	0.11	0.14	0.10	0.23	0.09	0.13	0.42	0.57	0.37	0.52	0.34	0.41
Total fish high (3)	1.08	1.44	1.02	2.31	0.87	1.29	4.23	5.70	3.69	5.22	3.39	4.08

NA: Data not available

Several assumptions were made regarding the published data on nutritional composition. Because it is difficult to differentiate between wild and farmed salmon an average value was chosen (1.5 g/100g). For both sardines and mackerel, data from the literature are considerably different; therefore calculations were performed with the lowest and the highest reported mean value.

An additional calculation was made to see globally the contribution of fish to the intake of LC n-3 PUFA assuming for total fish consumed the lowest and the highest reported concentrations (0.3 vs. 3 g/100g).

Normally the levels of LC n-3 PUFA in meat is very low compared to fish (MAFF, 1998; Souci-Fachman-Kraut, 2000), however it can be increased to a limited extent by modifying the composition of the feed.

A comparison between the amounts of fat, saturated fatty acids, LC n-3 PUFA, preformed vitamin A, vitamin D, vitamin B₁₂, iodine and selenium provided by an equal fresh weight of fish and meat demonstrates the difference between those two foods. A portion size of 130 g and salmon and beef (sheer muscle) were chosen see table 24:

Table 24. Comparison of nutrient content in fish and meat in portions of 130 g

	Salmon		Beef (sheer muscle)	
	Mean	Range	Mean	Range
Fat	17.7 g	16.3-21.5 g	2.5 g	2.0-3.9 g
Saturated fatty acids	4.4	2.5-6.5 g	1 g	0.9-1.2 g
LC n-3 PUFA	3.9 g	1.1-5.9 g	0.006 g	NA
Vitamin A	53.3 µg	11.7-84.5 µg	26.0 µg	NA
Vitamin D	20.8 µg	6.5-26.0 µg	NA	NA
Vitamin B ₁₂	3.8 µg	NA	6.5 µg	1.3-10.4 µg
Selenium	37.7 µg	26.0-44.2 µg	7 µg	3.9-13 µg
Iodine	44.2 µg	NA	7 µg	2.2-8.8 µg

NA: data not available

7.3. Risk Characterisation

7.3.1. General considerations

Concentrations of environmental contaminants in fish vary with the nature of the contaminant and the type of fish. Lipophilic organic contaminants are localised, and often accumulated, in adipose tissue. Therefore the amount present in fish as consumed depends both on the fat content and the anatomical region of the fish in which the fat is stored. Although data are not available for all lipophilic contaminants of interest, it can be concluded that higher levels may be found in fatty fish such as salmon and herring. In contrast, methylmercury is not lipophilic and does not accumulate in fatty fish. Methylmercury concentrations are determined by the trophic level and size (age) of the fish. Of the fish selected as important for this evaluation, the highest levels are found in tuna, particularly in the larger species such as albacore and bluefin.

Table 25 summarises the toxicological information on a range of metals and organic contaminants in fish. Exposure to contaminants from fish needs to be considered in the context of total dietary exposure. Arsenic is considered unimportant for the present evaluation, because the toxicological concerns relate mainly to inorganic arsenic, whereas the

predominant forms in fish are the organic arsenobetaine and arsenocholine. Cadmium and lead in finfish are not significant contributors to total dietary exposure and therefore are not considered further. In contrast, the form of mercury that is of most toxicological concern is methylmercury, which is the predominant form in fish. Other dietary sources of methylmercury are negligible by comparison. Of the brominated flame retardants, the data relating to HBCD, TBBPA and PBBs are inadequate to perform an exposure assessment, and these are not considered further. Therefore the lipophilic organic contaminants important for this risk characterisation are the PCDD/Fs, PCBs, PBDEs and the organotins.

PTWIs have been established for methylmercury, and for the PCDD/Fs and DL-PCBs. A TDI has been established for the organotins. So far the toxicological data are not adequate to establish tolerable intakes for the PBDEs or non-dioxin-like PCBs. The PTWI and the TDI are health based guidance values for an amount of contaminant that can be consumed weekly or daily over an entire lifetime without appreciable risk to health. They are derived from doses that have been investigated in studies of human populations or experimental animals, incorporating uncertainty factors to allow for variability between and within species and uncertainties in the toxicological data. These tolerable intakes are not thresholds for toxicity. The health impact of exceeding the tolerable intake by a small amount is uncertain, but it can be concluded that as the amount and frequency of exceedance increase, the likelihood of adverse effects occurring in at least some of the exposed population will also increase. Generally, these health-based guidance values are set for individual compounds. When information is available that individual compounds may interact with each other (e.g. eliciting their effects through a common mode of action) health-based guidance values may be set for groups of compounds. As an example, the PTWI for chlorinated dioxins, furans and DL-PCBs is a group PTWI derived from data of the most toxic congener.

The scenarios for estimated exposures to contaminants in fish, described in section 7.2, have been compared with the PTWI or other toxicological information in table 26. For methylmercury and PCDD/Fs and the DL-PCBs, the comparisons are presented for the species of fish with the highest level of contaminants, and also for total fish. The exposure estimates for the other contaminants are based on fewer data, and verified data for individual fish species are not available. The estimates are based on raw fish and certain cooking procedures such as grilling fish may lead to a partial loss of fat and thus of fat soluble contaminants. Nevertheless, the available data clearly demonstrate that methylmercury and the PCDD/Fs and DL-PCBs are the contaminants for which fish consumption could result in exceedance of the PTWIs. Intakes from fish of the other contaminants considered in this evaluation are not a health concern because they are below the available toxicological comparator (e.g. TDI) or contribute only minimal to overall human dietary exposure.

Because exposure to contaminants from fish needs to be considered in the context of total dietary exposure, Table 26 also summarises the health impact of eating meat instead of fish.

7.3.2. Methylmercury

Table 26 indicates that some high level consumers of certain species of tuna may exceed the PTWI of 1.6 µg/kg b.w./wk and a greater proportion may exceed the US EPA reference dose of 0.7 µg/kg b.w./week. The highest levels of methylmercury occur in bluefin and albacore tuna. In practice it is unlikely that a high level consumer of tuna would eat exclusively these types of tuna, and actual exposure may be lower. Dietary intake calculations for total mercury in Norwegian women conducted by the Norwegian Food Safety Authority showed that even high fish consumers (95thile) are below the PTWI of 1.6 µg/kg b.w./week (EFSA, 2004a).

Nevertheless, it is likely that small numbers of consumers may be at risk from methylmercury in some types of fish. It is important to note that the health based guidance values are set to protect the most susceptible lifestage, i.e. the developing foetus exposed as a result of the mercury in the mother's body. Therefore the subgroups of special consideration are women who are pregnant or may become pregnant. People at other life stages are likely to be less susceptible, although cardiovascular risk may include other sections of the population.

The Panel noted that EFSA already recommends that women of childbearing age (in particular, those intending to become pregnant), pregnant and breastfeeding women as well as young children select fish from a wide range of species without giving undue preference to top predatory fish such as swordfish and tuna.

http://www.efsa.eu.int/press_room/press_release/258_en.html

Although some progress has been made with respect to farming of tuna especially bluefin tuna, the vast majority available in the EU is caught from the wild. The Panel has received no data comparing methylmercury levels of farmed and wild tuna.

7.3.3. PCDD/Fs and DL-PCBs

The PCDD/F and DL-PCB content of some fatty fish may lead some high level consumers to exceed the PTWI, before taking into account exposure from the rest of the diet. A complete risk characterisation needs to be based on total dietary exposure for different groups of consumer. The PTWI is set to protect against effects on the foetus, which is considered to be the most vulnerable lifestage. Therefore pregnant women could be considered to be particularly at risk. However, because PCDD/F and DL-PCB have very long half-lives in the human body, the body burden during pregnancy is not determined by the PCDD/F or PCB intake at that time, but by the total previous intake over many years until that time. Women past reproductive age and males are likely to be at lesser risk from PCDD/F and DL-PCBs.

In comparing the safety of farmed and wild fish, it is necessary to consider both the types of fish that are available to consumers as well as whether there are differences resulting from farming practices. The fish with highest levels of contamination are salmon and herring. Available data do not allow distinction between levels of contamination in farmed and wild salmon of the same species, with the exception of salmon from the Baltic. Herring are caught from the wild; the majority of salmon available to EU consumers are farmed. There is no meaningful difference in exposure, and hence in risk, to consumers of these fish.

7.3.4. Baltic herring and salmon

On average, the total TEQ from PCDD/F and DL-PCBs in Baltic herring is about 3.5 times that in herring that are not from the Baltic Sea. In Baltic salmon, on average, the total TEQ from PCDD/F and DL-PCBs in wild salmon is about 5 times that of farmed salmon. As a result there is a greater potential for consumers to exceed the PTWI if they eat herring or wild salmon from the Baltic more than once a week (see Table 18 and 26). The Panel noted that the available data supports the Finnish and Swedish recommendations on Baltic herring consumption as outlined in the background.

Limited data on methylmercury in Baltic herring indicate that although levels are slightly higher than in non-Baltic herring, they are lower than in tuna and therefore not a specific concern.

Consumption of Baltic herring is 6 % of total fish consumption in Finland (National Food Agency of Finland). In addition the few data available indicate that about half of the herring consumed in Finland and Sweden is from the Baltic.

7.3.5. Possible impact of not eating fish

Substantial dietary intake of LC n-3 PUFA can be obtained readily by eating fatty fish or larger amounts of lean fish (Table 26). Therefore individuals who eat no fish will have difficulties in meeting the daily intakes of LC n-3 PUFA recommended with regard to cardiovascular health and foetal development. The benefits of fish consumption also relate to the general nutritional content, particularly protein of high biological value, vitamins D, A and B12, iodine and selenium. In particular fish is an important source of iodine and selenium.

Lipophilic contaminants such as PCDD/Fs are widespread in food, especially in fatty foods. Some high level consumers of meat may also exceed the PTWI for PCDD/F, regardless of their fish intake. Therefore reducing fish consumption for example by eating meat will not inevitably lead to decreased dietary exposure to PCDD/F and dioxin-like compounds.

Table 25. Summary of toxicological information of contaminants in fish

Compound	NOAEL / Effect	TDI/PTWI (if exists)	Source and Date of evaluation	Estimated exposure level from fish	Remarks
Arsenic	Not established. Narrow margin between PTWI and effect level	15 µg/kg b.w./week	WHO, 1988	Not relevant	PTWI for inorganic arsenic. Not relevant for fish which contains mainly organic arsenic compounds
Cadmium	Not established. Renal tubular dysfunction may be seen in a small proportion of the general population at PTWI	7 µg/kg b.w./week	WHO, 2004	Not relevant	PTWI based on biomarker of effect in humans, dose level at which may already be at increased risk for tubular dysfunction. Fish is only a minor contributor to overall exposure to cadmium.
Lead	PTWI at NOEL of biomarker, neurobehavioral development in children	25 µg/kg b.w./week	WHO, 1986, 2000	Not relevant	PTWI based on biomarker of exposure (blood levels) in infants and children. Intake not leading to an increase in blood lead levels. Fish is only a minor contributor to overall exposure to lead.
Mercury	1.5 µg/kg b.w./day, averaged from NOAEL in one study and BMDL in 2nd study for neurodevelopmental effects	1.6 µg/kg b.w./week	WHO, 2004 EFSA, 2004a	See Table 26	PTWI based on biomarker of exposure (hair levels) in mother-infant pairs.
PCDD/F and DL-PCBs	Lowest LOAEL is for effects of TCDD on developing male reproductive system resulting from maternal body burden	14 pg WHO-TEQ/kg b.w./week	EC, 2001	See Table 26	TWI established using body burden approach to allow for species differences in toxicokinetics
NDL-PCBs				See Table 26	
Camphchlor	NOAELs of 0.2 mg/kg b.w./day for liver toxicity in dogs and 0.1 mg kg/b.w./day for immunotoxicity in macaques.	Proposed TDI 100 or 200 ng/kg b.w./day	Brüschweiler <i>et al.</i> , 2004 M. Feeley, personal communication	See Table 26	No official TDI established by EFSA or other international body.

Compound	NOAEL / Effect	TDI/PTWI (if exists)	Source and Date of evaluation	Estimated exposure level from fish	Remarks
Hexachloro-cyclohexanes	NOAEL is 0.47 mg/kg b.w. for liver toxicity in rats for gamma-HCH.	TDI 0.005 mg/kg b.w./day for gamma-HCH	JMPR, 2002	Not relevant	TDI for alpha- and beta-HCH not established by EFSA or other international body.
PBDEs	Lowest NOAEL is 0.45 mg/kg b.w./day for liver toxicity of pentaBDE. Neurodevelopmental effects seen with single neonatal oral dose of ≥ 0.6 mg/kg b.w./day for some PBDE congeners. JECFA concluded effects in animals unlikely below 100 μ g/kg b.w./day.	No TDI Guideline of 0.1 μ g/kg b.w./day	FSA, 2004b; WHO, 2005	See table 26	Lack of long term and reproductive studies. Multiple congeners with no established method of risk assessment for the mixtures present in fish. COT proposed MoE of at least 1000 is needed because of uncertainties in the data.
HBDCD	LOAEL is 100 mg/kg b.w./day for liver toxicity. Neurodevelopmental effects seen with single neonatal oral dose of ≥ 0.9 mg/kg b.w./day.	No TDI Target MoE of 3000-10000	FSA, 2004b	Data not adequate	Lack of chronic and reproductive studies
TBBPA	No toxicologically significant effects up to 1000 mg/kg b.w./day	1 mg/kg b.w./day	FSA, 2004c	Data not adequate	Lack of chronic studies
PBBs				Data not adequate	No risk assessment available
Organotin compounds (TBT, DBT, TPT, DOT)	NOAEL: 0.025 mg/kg b.w./day for immunotoxicity for TBT oxide in chronic rat study	Group TDI of 0.25 μ g/kg b.w./day	EFSA, 2004b	See Table 26	Group TDI has been established as TBT, TPT, DBT and DOT have similar mode of immunotoxic action (lymphocyte depletion in thymus and peripheral lymphoid tissues)

Table 26. Comparison of estimated exposures to contaminants in fish with the PTWI or other toxicological information and health impact of eating meat instead of fish.

Substance	Recommended intake or PTWI ^a	Critical fish	Dietary exposure scenario				% recommended intake or PTWI ^b			
			From fish ^c		From equivalent amount of meat ^d		From fish		From equivalent amount of meat	
			Mean consumption	High consumption	Mean	High	Mean	High	Mean	High
LC n-3 PUFAs	0.5 g/person/day	Lean fish (3 g/kg)	0.1 – 0.2 g/person/day	0.3 – 0.6 g/person/day	~ 0	~ 0	20 – 40	60 – 120	0	0
		Fatty fish (30 g/kg)	0.9 – 2.3 g/person/day	3.4 – 5.7 g/person/day	~ 0	~ 0	180 – 260	680 – 1140	0	0
Mercury	1.6 µg/kg b.w./week	Tuna – skipjack (0.15 mg/kg)	0.05 – 0.3 µg/kg b.w./wk	0.5 – 1.1 µg/kg b.w./wk	~ 0	~ 0	3 – 19	31 – 69	0	0
		Tuna – albacore, bluefin (0.49 mg/kg)	0.2 – 0.9 µg/kg b.w./wk	1.5 – 3.5 µg/kg b.w./wk	~ 0	~ 0	13 – 56	94 – 219	0	0
		Total non-predatory fish (0.1 mg/kg)	0.3 – 0.9 µg/kg b.w./wk	1.3 – 2.2 µg/kg b.w./wk	~ 0	~ 0	19 – 56	81 – 138	0	0
PCDD/F and DL-PCBs	14 pg TEQ/kg b.w./week	Salmon (1 ng/kg)	0.6 – 2.2 pg TEQ/kg b.w./wk	1.6 – 7.7 pg TEQ/kg b.w./wk	0.1 – 0.3 pg TEQ/kg b.w./wk	0.2 – 0.9 pg TEQ/kg b.w./wk	4 – 16	11 – 55	0.7 – 2	1.4 – 6
		Salmon (4 ng/kg)	2.3 – 8.9 pg TEQ/kg b.w./wk	6.5 – 31 pg TEQ/kg b.w./wk	0.4 – 1.6 pg TEQ/kg b.w./wk	1.2 – 5.5 pg TEQ/kg b.w./wk	16 – 64	46 – 214	3 – 11	9 – 39
		Herring (3.2 ng/kg)	1 – 11 pg TEQ/kg b.w./wk	2.6 – 35 pg TEQ/kg b.w./wk	NR	NR	7 – 79	19 – 250	NR	NR
		Baltic herring (11.5 ng/kg)	4 – 40 pg TEQ/kg b.w./wk	9 – 126 pg TEQ/kg b.w./wk	NR	NR	29 – 286	64 – 900	NR	NR

Substance	Recommended intake or PTWI ^a	Critical fish	Dietary exposure scenario				% recommended intake or PTWI ^b			
			From fish ^c		From equivalent amount of meat ^d		From fish		From equivalent amount of meat	
			Mean consumption	High consumption	Mean	High	Mean	High	Mean	High
NDL-PCBs ^e	NA	Total fish (12.5 µg/kg)	42 – 112 ng/kg b.w./wk	165 - 277 ng/kg b.w./wk	5 - 13 ng/kg b.w./wk	22 – 33 ng/kg b.w./wk	NA	NA	NA	NA
Camphechlor	0.7 or 1.4 µg/kg b.w./wk	Salmon (56µg/kg)	0.03-0.12 µg/kg b.w./wk	0.09 – 0.43 µg/kg b.w./wk	~ 0	~ 0	4 - 17 or 2 - 9	13 - 6 or 6 – 31		
		Total fish (25µg/kg)	0.08 - 0.22 µg/kg b.w./wk	0.33 - 0.55 µg/kg b.w./wk	~ 0	~ 0	11 – 31 or 6 - 16	47 – 79 or 24 - 39		
PBDEs	0.7 µg/kg b.w./wk	Total fish (1.78 µg/kg)	6 - 16 ng/kg b.w./wk	23 - 39 ng/kg b.w./wk	1.7-4.6	6.8-11	1 - 2	3 – 6	0.2-0.7	1-1.6
Organotin ^f compounds (TBT,DBT, TPT,DOT)	1.75 µg/kg b.w./wk ^g	Total fish (13.5 µg/kg)	8 – 17 ng/kg b.w./wk	25 - 43 ng/kg b.w./wk	~ 0	~ 0	0.5 - 1	1.4 - 2.5	0	0

^a Recommended intake for LC n-3 PUFAs, PTWI (or equivalent) for the contaminants

^b Overall risk assessment needs to consider other sources of dietary exposure for the lipophilic organic contaminants (negligible for methylmercury, camphechlor and organotins)

^c Based on fish consumption figures for ‘consumer only’ from 6 countries, see table 15

^d Intake from meat at the same portion sizes as the specified fish. Total intake from meat is likely to be higher. Based on meat (ruminants and poultry) assuming 12 % of lipids average and 99th percentile concentration of PCDD/F and DL-PCBS, and average concentration of non-dioxin like PCBs.

^e Data in italics are derived from limited data and are considered less robust.

^f shellfish is a major contributor to total dietary intake of OTC

^g For the purposes of this comparison the TDI for organotin compounds has been multiplied by 7 to provide a PTWI.

NA: Data not available

NR: Not relevant to the comparison of Baltic and non-Baltic herring.

CONCLUSIONS AND RECOMMENDATIONS

- Fish is an important source of proteins of high biological value, LC n-3 PUFAs, certain vitamins and minerals.
- There is evidence that fish consumption, especially fatty fish, benefits the cardiovascular system and may also benefit foetal development.
- One to two portions (of about 130 g per portion) of fatty fish per week are sufficient to reach the daily intake for LC n-3 PUFA recommended for potential benefits to health.
- Fish can make a major contribution to dietary exposure to environmental contaminants, particularly PCDD/F, DL-PCBs and methylmercury.
- Species, season, location, diet, life stage and age have a major impact on the levels of both the nutrients and contaminants in fish. These levels vary broadly within species and between species.
- The available data do not allow a robust comparison of nutrient and contaminant levels of wild and farmed fish. The limited data available indicate that there are no consistent differences between wild and farmed fish. However, regional differences exist, e.g. with increased contaminant concentrations in wild fish from the Baltic sea.
- High level consumers of top predatory fish such as tuna may exceed the PTWI for methylmercury. High level consumers of fatty fish may exceed the PTWI for PCDD/Fs and DL-PCBs, even without taking into account other sources of dietary exposure. Exposure to these contaminants may counteract some of the beneficial effects. Advice on fish consumption needs to take into account total dietary exposure of relevant contaminants, based on national consumption patterns including relevant fish species.
- The greatest susceptibility to the critical contaminants, (e.g. methylmercury and the dioxin-like compounds) occurs during early development. Exposure during this life stage results from the total amount in the mother's body. For methylmercury there is a possibility of decreasing the mother's body burden by decreasing intake in the months preceding and during pregnancy whereas this is not possible for the PCDD/Fs and DL-PCBs because of their much longer half lives.
- Pregnant women eating up to two portions/week of the fish considered in this opinion are unlikely to exceed the PTWI for methylmercury, provided that one of these portions is not bluefin or albacore tuna. Other top predatory fish, such as marlin, pike, swordfish, and shark frequently contain high levels of methylmercury.
- Consumption of up to two portions per week of fatty fish such as non-Baltic herring or salmon will not lead to the exceedance of the PTWI for PCDD/Fs and DL-PCBs, although other sources of dietary exposure need to be taken into account.
- Contaminant levels in wild fish can only be reduced by long-term control of emissions of pollutants to the environment.
- Fish farming offers the possibility of managing the contaminant levels in fish in order to minimize the risks while maintaining the benefits.
- Fish oil and fish meal are the most important sources of contamination of farmed fish feed with dioxin-like compounds. EU regulations on PCDD/F in fish feed were introduced in 2002 and DL-PCB will be included in the near future. A possibility for reducing

contaminant levels in farmed fish is the selective use of feed ingredients with low levels of contaminants.

- When fish oil is replaced by vegetable oils in fish feed because of shortage of fish oil or to reduce contaminant levels it has to be ensured that the nutritional composition of the fish is not adversely affected.
- Further research into the practicality of different decontamination procedures for fish oil for use in farmed fish feed is needed.
- Special consideration is needed for fish from the Baltic sea. The main concern is the higher contamination with PCDD/F and DL-PCBs. Specific advice is recommended for consumers of Baltic herring and wild Baltic salmon, particularly for girls, in order to protect against accumulation of PCDD/Fs and DL-PCBs to a body burden in adulthood that could be harmful to the foetus during pregnancy.
- At present there is no agreed methodology for taking into account risk and benefit in a quantitative way. The Panel recommends that for future purposes a framework should be developed allowing a quantitative comparison of human health risks and benefits of food based on a common scale of measurement.

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SCIENTIFIC PANEL MEMBERS

Jan Alexander, Herman Autrup, Denis Bard, Angelo Carere, Lucio Guido Costa; Jean-Pierre Cravedi, Alessandro Di Domenico, Roberto Fanelli, Johanna Fink-Gremmels, John Gilbert, Philippe Grandjean, Niklas Johansson, Agneta Oskarsson, Jirí Ruprich, Josef Schlatter, Greet Schoeters, Dieter Schrenk, Rolaf van Leeuwen, Philippe Verger.

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DOCUMENTATION PROVIDED TO EFSA

Czech Republic National Institute of Public Health

- Carp production and consumption

Estonia Ministry of Agriculture, Food and Veterinary Department

- Occurrence of PCBs and heavy metals in Baltic herring and other fish species 2000-2004
- Consumption of fish by household's 200-2003

Food Safety Authority of Ireland (FSAI)

- Marine Institute a small survey on PBDE and HBCP occurrence in farmed Atlantic salmon from Ireland (analysed by RIVO, Netherlands)
- BIM - Irish Sea Fisheries Board - Survey on PCDD/F & 12 WHO PCB occurrence in farmed cooked Atlantic salmon (analysed by Eurofins GfA, Germany)
- FSAI - PCDD/F, PCB, PBDE, HBCD, FPA and cooking study in several fish species available on the Irish market (landed, farmed and retail (canned samples)) (analysed by Eurofins GFA, Germany)

National Public Health Institute of Finland

- Occurrence data for PCDD/F and PCBs, PBDE, PCN and DDT in Baltic herring and wild salmon (catchment date 2002)
- Finnish results of monitoring of dioxin levels in Baltic Sea fish 2004 and actions to limit human exposure to dioxin through fish
- Heavy metals in sea and fresh water fish
- Catches of herring and Baltic herring 2002.
- Export of Baltic herring, 2003.

State Food and Veterinary Service of Lithuania

- Occurrence data on POPs and PCBs in fish from 2003 and 2004, PCDD/PCDF and PCB in Baltic herring, and heavy metals in farmed fish in 2003 and 2004
- National Nutrition Centre. Fish consumption in Lithuania 2001-2002

Norwegian Food and Control Authority and Institute of Public Health

- Fish consumption data
- Estimated daily intake of total mercury from fish, crustaceans and bivalves

Spain

- Occurrence data on heavy metals in different fish species

Swedish National Food Administration

- Occurrence data on heavy metals in different fish species 2002 and 2003, and data on PCDD/PCDF, PCBs, HBCD, PBDE, DDT, HCB, HCH, chlordan and nonchlordan in herring and salmon from the Baltic, Bothnian and Eastersea regions from 2000-2002.
- Fish consumption in Sweden, 1997-98
- Results from Interim report 4 – study of dioxin levels in fatty fish from Sweden 2000-2003

FEFAC

- Occurrence data on heavy metals, POPs, PBDE, dioxins, furans and PCBs in fish feed.

ABBREVIATIONS AND GLOSSARY FOR SOME TERMS

AA	Arachidonic acid (C20:4 n-6)
ADI	Acceptable daily intake: Estimated maximum amount of an agent, expressed on a body mass basis, to which individuals in a (sub)population may be exposed daily over their lifetimes without appreciable health risk*
BFRs	Brominated flame retardants
Consumers only	Daily or weekly consumption or intake averaged over the total number of days of the survey for all individuals who consumed the specific food group at least once during the survey
CI	Confidence interval
COT	The UK Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment
DBDE	Decabromodiphenyl ether
DBT	Dibutyltin
DDE	Dichlorodiphenyldichloroethylene, metabolite of DDT
DDD	Dichlorodiphenyldichloroethane, metabolite of DDT
DDT	Dichlorodiphenyltrichloroethane
DHA	Docosahexaenoic acid (C22:6 n-3)
DL-PCBs	Dioxin-like PCBs, dioxin-like compounds
DPA	Docosapentaenoic acid (C22:5 n-3)
EPA	Eicosapentaenoic acid (C20:5 n-3)
TBBPA	Tetrabromobisphenol A
HBCD	Hexabromocyclododecane
HCH	Hexachlorocyclohexane
HDL	High-density lipoprotein
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
LA	Linoleic acid (C18:2 n-6)
LC n-3 PUFA	Long-chain n-3 polyunsaturated fatty acid: includes the cis fatty acids eicosapentaenoic acid (C20:5 n-3; EPA), docosapentaenoic acid (C22:5 n-3; DPA) and docosahexaenoic acid (C22:6 n-3; DHA). These fatty acids have in common that they have a double bond at the third C atom from the methyl-end of the fatty acid. They can be formed from the essential fatty acid α -linolenic acid (C18:3 n-3; LNA) through the actions of various desaturases and elongases
LDL	Low-density lipoprotein
LNA	α -linolenic acid (C18:3 n-3)
LOAEL	Lowest observed adverse effect level: Lowest dose of an agent, found by experiment or observation, that causes an adverse alteration of morphology, functional capacity, growth, development or life span in an organism, system or (sub) population
MeHg	Methylmercury
MoE	Margin of Exposure: Ratio of the point of comparison on the dose-response curve (e.g. NOAEL or benchmark dose) to the estimated intake in humans

MUFA	Monounsaturated fatty acid
n-3 fatty acid	Omega-3 fatty acid
n-6 PUFA	n-6 polyunsaturated fatty acid
NDL-PCBs	Non dioxin-like PCBs
NOAEL	No-observed adverse effect level: Greatest dose of an agent, found by experiment or observation, that caused no detectable adverse alteration of morphology, functional capacity, growth development or life span in an organism, system or (sub)population
n-3 fatty acid	Omega-3 fatty acid
OBDE	Octabromodiphenyl ether
OTC	Organotin compounds
PBBs	Polybrominated biphenyls
PBDE	Polybrominated diphenyl ethers
PCBs	Polychlorinated biphenyls
PIH	Pregnancy-induced hypertension
PCDD	Polychlorinated dibenzo-p-dioxins
PCDF	Polychlorinated dibenzo-p-furans
PCDD/F	Often referred to as dioxins
PentaBDE	Pentabromodiphenyl ether
POPs	Persistent organic pollutants
PTWI	Provisionally tolerable weekly intake: Analogous to TDI, term used for contaminants that accumulate in the body.
PUFA	Polyunsaturated fatty acid
PVC	Polyvinyl chlorides
RR	Relative risk
SACN	UK Scientific Advisory Committee on Nutrition
SCAN	Scientific Committee on Animal Nutrition of the European Commission
SCF	Scientific Committee on Food of the European Commission
SCHER	Scientific Committee on Health and Environmental Risks of the European Commission
SFA	Saturated fatty acid
TBBPA	Tetrabromobisphenol A
Tributyltin	Tributyltin
TDI	Tolerable daily intake: analogous to the ADI and PTWI. The term 'tolerable' is used for agents that are not deliberately added, such as contaminants in food
TEFs	Toxic equivalency factors
TEQ	Toxic equivalents
TPT	Triphenyltin
UFs	Uncertainty factors (sometimes referred to as safety factors)
VLDL	Very low-density lipoprotein
WHO	World Health Organization

APPENDIX

ANNEX 1: Tables

Table 1. Common and Latin names of some fish species

Amberjack		<i>Seriola dumerili</i> (Temminck & Schlegel)
Anchovy (Striped)		<i>Anchoa hepsetus</i> (Linnaeus)
(Silver)		<i>Engraulis eurystole</i> (Swain & Meek)
Barramundi		<i>Lates calcarifer</i> (Bloch)
Bass (Sea)		<i>Dicentrarchus labrax</i> (Linnaeus)
Bream (Gilthead Sea)		<i>Sparus aurata</i> (Linnaeus)
Carp		<i>Cyprinus carpio</i> (Linnaeus)
Cod		<i>Gadus morhua</i> (Linnaeus)
Eel		<i>Anguilla anguilla</i> (Linnaeus)
Haddock		<i>Melanogrammus aeglefinus</i> (Linnaeus)
Halibut		<i>Hippoglossus hippoglossus</i> (Linnaeus)
Herring		<i>Clupea harengus</i> (Linnaeus)
Mackerel		<i>Scomber scombrus</i> (Linnaeus)
Perch		<i>Perca fluviatilis</i> (Linnaeus)
Pike		<i>Esox lucius</i> (Linnaeus)
Salmon (Atlantic)		<i>Salmo salar</i> (Linnaeus)
(Chinook)		<i>Oncorhynchus tshawytscha</i> (Walbaum)
(Chum)		<i>Oncorhynchus keta</i> (Walbaum)
(Coho)		<i>Oncorhynchus kisutch</i> (Walbaum)
(Pink)		<i>Oncorhynchus gorbuscha</i> (Walbaum)
(Sockeye)		<i>Oncorhynchus nerka</i> (Walbaum)
Pilchard		<i>Harengula clupeola</i> (Cuvier)
Trout (Rainbow)		<i>Oncorhynchus mykiss</i> (Walbaum)
Tuna (Bluefin)		<i>Thunnus thynnus</i> (Linnaeus)
(Yellowfin)		<i>Thunnus albacares</i> (Bonaterre)
(Blackfin)		<i>Thunnus atlanticus</i> (Lesson)
Turbot		<i>Scophthalmus maximus</i> (Linnaeus)

Table 2. Results on dioxin levels in fatty fish from Sweden, Interim Report . WHO-TEQ levels of 17 dioxins and furans (pg/g fresh weight) in fish muscle, fish muscle + skin and fish roe from 52 pooled fish samples caught along the Swedish east and south coast and lakes Vänern, Vättern and Rebnisjaure year 2000-2003. Values below LOD were set to 1/1 LOD in all calculations. Please note that there are large differences in age within the species reported

No.	Species	Gender	Mean age years (range)	Mean weight (g)	Fat content (%)	Location caught	Year caught	No. of indiv. in pool	PCDD-TEQ (pg/g fw)	PCDF-TEQ (pg/g fw)	Σ PCDD/F-TEQ (pg/g fw)
94	Salmon	F	2.0	6907	6.1	Luleå archipelago	2002	9	1.4	3.5	4.9
95	Salmon	M	1.9 (1-3)	5796	7.2	Luleå archipelago	2002	11	1.6	3.6	5.2
96	Salmon	F	2.8 (2-3)	9558	7.3	Luleå archipelago	2002	10	1.8	4.1	5.9
97	Salmon	M	2.6 (2-3)	10450	7.6	Luleå archipelago	2002	10	1.6	3.9	5.6
98	Salmon	Mixed	1.9 (1-2)	2500	4.8	Baltic proper	2002	8	0.7	1.6	2.3
99	Salmon	Mixed	1.9 (1-3)	4000	6.1	Baltic proper	2002	10	0.9	2.1	3.0
100	Salmon	Mixed	2.0	3900	6.0	Baltic proper	2002	10	0.9	2.1	3.0
101	Salmon	Mixed	2.0	6400	9.0	Baltic proper	2002	9	1.5	3.3	4.8
102	Salmon	Mixed	2.0	6500	9.6	Baltic proper	2002	9	1.5	3.6	5.1
103	Salmon	Mixed	2.5 (2-3)	10100	9.3	Baltic proper	2002	6	1.7	3.7	5.4
104	Salmon	Mixed	2.0 (1-3)	5300	8.1	Gävle bay	2002	9	1.3	3.5	4.9
105	Salmon	Mixed	1.4 (1-2)	5200	8.2	Gävle bay	2002	10	1.6	4.1	5.7
106	Salmon	Mixed	2.9 (2-4)	10800	7.0	Gävle bay	2002	7	1.5	4.3	5.8
107	Salmon	Mixed	2.8 (2-4)	10600	7.3	Gävle bay	2002	6	1.6	4.1	5.7
108	Salmon	Mixed	NA	3279	6.1	S. Vänern, Dalbosjön	2002	9	0.8	0.8	1.5
109	Salmon	Mixed	NA	4539	6.9	S. Vänern, Dalbosjön	2002	10	0.9	0.9	1.8
110	Salmon	Mixed	NA	3254	6.0	N. Vänern	2003	10	1.4	1.2	2.6
111	Salmon	Mixed	NA	4614	8.0	N. Vänern	2003	7	2.2	1.8	4.0
112	Salmon	Mixed	NA	4863	9.4	N. Vättern	2002-3	7	0.8	1.3	2.0
113	Salmon	Mixed	NA	7629	10.5	N. Vättern	2002-3	7	1.2	1.9	3.1
114	Salmon	Mixed	NA	6175	4.3	S. Vättern	2003	8	1.2	1.8	3.0
115	Salmon	Mixed	5.6	4536	2.4	Ormön Stockholm (farmed)	2002	10	0.3	0.4	0.7
116	Arctic char	Mixed	4.6	3790	1.4	Lake Rebnisjaure	2002	11	0.07	0.12	0.19
117	Arctic char	Mixed	3.3	1640	1.0	Lake Rebnisjaure	2002	22	0.05	0.08	0.13
118	Rainbow trout	Mixed	2.0	1401	4.9	Ormön Stockholm (farmed)	2002	10	0.2	0.3	0.44

No.	Species	Gender	Mean age years (range)	Mean weight (g)	Fat content (%)	Location caught	Year caught	No. of indiv. in pool	PCDD-TEQ (pg/g fw)	PCDF-TEQ (pg/g fw)	Σ PCDD/F-TEQ (pg/g fw)
119	Herring	Mixed	3.7 (2-5)	90.3	10.4	Rugen	2002	15	1.5	3.7	5.1
120	Herring	Mixed	3.1 (2-5)	74.2	10.1	Rugen	2002	15	1.3	3.3	4.6
121	Herring	Mixed	3.6 (2-7)	62.7	3.9	Rugen	2002	17	0.9	2.0	3.0
122	Herring	Mixed	2.4 (1-5)	59.2	7.0	Rugen	2002	18	0.5	1.2	1.6
123	Herring	Mixed	2.4 (1-4)	54.2	2.4	Rugen	2002	20	0.5	1.0	1.5
124	Herring	Mixed	2.8 (2-4)	69.9	2.2	Rugen	2002	15	0.5	1.0	1.6
125	Herring	Mixed	2.9 (1-5)	49.6	7.2	Rugen	2002	36	0.5	1.3	1.8
126	Herring	Mixed	2.7 (2-5)	48.7	7.4	Rugen	2002	30	0.5	1.2	1.6
127	Sprat	Mixed	4.1 (1-8)	9.3	13.0	Baltic sea	2002	106	0.9	2.6	3.5
128	Sprat	Mixed	4.2 (2-9)	9.6	10.6	Baltic sea	2002	105	0.9	2.5	3.4
129	Sprat	Mixed	4.2 (2-9)	9.7	11.2	Baltic sea	2002	96	1.0	2.8	3.8
130	Sprat	Mixed	3.9 (2-8)	9.4	11.2	Baltic sea	2002	104	0.8	2.4	3.3
131	Sprat	Mixed	4.9 (2-8)	9.1	7.1	Baltic sea	2002	100	0.9	2.3	3.1
132	Sprat	Mixed	4.1 (2-9)	8.9	7.9	Baltic sea	2002	102	0.7	2.1	2.8
133	Sprat	Mixed	2.5 (2-3)	18	10.6	W. Bornholm	2002	16	0.7	1.6	2.4
134	Sprat	Mixed	5 (2-8)	9	9.1	W. Gotland	2002	22	1.0	2.7	3.6
135	Turbot	F	7.5 (5-11)	704	0.9	NE of Gotland	2002	10	0.2	0.6	0.8
136	Turbot	F	8.5 (5-14)	1116	0.9	NE of Gotland	2002	10	0.3	0.7	1.0
137	Turbot	F	5.4 (4-8)	649	0.8	Gotska Sandön	2002	10	0.1	0.4	0.5
138	Turbot	F	9.0 (5-13)	903	0.7	NE of Gotland	2002	10	0.2	0.7	0.9
139	Turbot	F	6.7 (5-11)	720	0.6	S. Marsö	2002	9	0.1	0.4	0.5
140	Turbot	F	10.5 (5-13)	1012	0.6	Öland Kårehamn	2002	10	0.2	0.7	0.8
141	Cod	Mixed	3.4 (3-5)	1224	0.6	Baltic proper	2002	11	0.1	0.1	0.2
142	Vendace roe ^a	F	-	-	6.10	Lake Vänern N. Djurö	2002	-	1.9	1.8	3.7
143	Vendace roe	F	-	27.7 ^b	13.2	Lake Vänern	2002	88	2.9	3.2	6.1
144	Vendace roe	F	-	16.6 ^b	11.9	Luleå archipelago	2000	69	0.6	1.3	1.9

Fish = for all fish species in this report, testing was carried out on muscle tissue (fish meat), or the muscle including fish skin (herring and sprat). All analyses in this report have been done on pooled samples (pooled sample = an equivalent amount of material from several fish is mixed and used for analysis).

a: Final product. b: Mean weight of the Vendace

Table 3. PBDEs (total BDE, sum of various congeners, for example 17, 28, 47, 66, 77, 99, 100, 153, 154, 183, 209) in fish feed (ng/g); Jacobs *et al.* (2002)

Sample	Origin	Collection	Mean values	Range	n
Salmon feed	different Scottish sources	1999	16.7	8.1 – 23.9	8
Fish oil	different Scottish sources	1999	2.54	ND – 12.7	5

Table 4. BFR values in fish

BFR	Type of fish	N	Farmed or Wild	Location	Concentration	Citation
HBCD	Herring	Unk1	Wild	Swedish Coast (6 sites)	10.4 ng/g lw	Asplund <i>et al.</i> , 2004
HBCD	Salmon	Unk2	Wild	Baltic	51.0 ng/g lw	Darnerud <i>et al.</i> , 2000
HBCD	Trout	Unk2	Wild	Lake Ontario	0.5 - 4.6 ng/g ww	Tomy <i>et al.</i> , 2004
HBCD	Trout	1-26	Wild	Ricknall Grange, Skerne-Tees, UK	21 - 119 ng/g fw	CEFAS, 2003
HBCD	Trout	1-26	Wild	Haughton Road, Skerne-Tees, UK	159 - 6758 ng/g fw	CEFAS, 2003
HBCD	Trout	1-26	Wild	Oxenfield Bridge, Skerne-Tees, UK	106 - 414 ng/g fw	CEFAS, 2003
HBCD	Trout	1-26	Wild	Middleton in Teesdale, Skerne-Tees, UK	3.6 - 51.0 ng/g fw	CEFAS, 2003
HBCD	Trout	1-26	Wild	Low Coniscliffe, Skerne-Tees, UK	3.6 - 26.0 ng/g fw	CEFAS, 2003
HBCD	Trout	1-26	Wild	Croft-on-Tees, Skerne-Tees, UK	27 - 198 ng/g fw	CEFAS, 2003
HBCD	Trout, Rainbow	Unk2	Farmed	Sweden	6.7 ng/g lw	Darnerud <i>et al.</i> , 2000
PBB	Trout, Lake	10	Wild	Lake Superior	0.25 +/- 0.13 ng/g ww	Luross <i>et al.</i> , 2002
PBB	Trout, Lake	10	Wild	Lake Huron	3.1 +/- 1.7 ng/g ww	Luross <i>et al.</i> , 2002
PBB	Trout, Lake	10	Wild	Lake Eerie	0.33 +/- ng/g ww	Luross <i>et al.</i> , 2002
PBB	Trout, Lake	10	Wild	Lake Ontario	1.1 +/- 0.57 ng/g ww	Luross <i>et al.</i> , 2002
PBDE	Carp	1-83	Wild	Kahramanmaras, Turkey	<1.6 ng/g ww	Erdogrul <i>et al.</i> , 2004
PBDE	Carp	Unk2	Wild	Boezinge, Flanders, Belgium	1.56 ng/g ww	Covaci <i>et al.</i> , 2004
PBDE	Carp	Unk2	Wild	Antwerp, Flanders, Belgium	0.6 ng/g ww	Covaci <i>et al.</i> , 2004
PBDE	Carp	Unk2	Wild	Hamme, Flanders, Belgium	6.0 ng/g ww	Covaci <i>et al.</i> , 2004
PBDE	Carp	1	Wild	Hadley Lake, USA (suspected source)	6.2 ng/g ww	Dodder <i>et al.</i> , 2002
PBDE	Carp	1	Wild	Hadley Lake, USA (suspected source)	20 ng/g ww	Dodder <i>et al.</i> , 2002
PBDE	Carp	Unk2	Wild	US	13 - 22 ng/g lw	Loganathan <i>et al.</i> , 1995
PBDE	Carp, Gibel	Unk2	Wild	Sint-Pieters-Leeuw, Flanders, Belgium	0.62 ng/g ww	Covaci <i>et al.</i> , 2004
PBDE	Carp, Gibel	Unk2	Wild	Willebroek, Flanders, Belgium	3.75 ng/g ww	Covaci <i>et al.</i> , 2004
PBDE	Carp, Gibel	Unk2	Wild	Balen, Flanders, Belgium	0.97 ng/g ww	Covaci <i>et al.</i> , 2004
PBDE	Carp, Nose-carp	1-83	Wild	Kahramanmaras, Turkey	<1.5 ng/g ww	Erdogrul <i>et al.</i> , 2004
PBDE	Herring	Unk1	Wild	North Sea	13.9 ng/g lw	Paepke <i>et al.</i> , 2004
PBDE	Herring	12-20	Wild	Baltic Sea (7 sites)	17.0 ng/g lw	Nylund <i>et al.</i> , 2001
PBDE	Herring	Unk1	Wild	Swedish Coast (6 sites)	10.4 ng/g lw	Asplund <i>et al.</i> , 2004
PBDE	Herring	Unk2	Wild	Baltic Sea	30 - 61 ng/g lw	Sellstrom <i>et al.</i> , 1993, 1996

BFR	Type of fish	N	Farmed or Wild	Location	Concentration	Citation
PBDE	Herring	Unk2	Wild	Kattegat	17 ng/g lw	Sellstrom <i>et al.</i> , 1993, 1996
PBDE	Herring	Unk2	Wild	Baltic Sea	12 ng/g lw	Burreau <i>et al.</i> , 1999
PBDE	Herring	Unk2	Wild	Baltic Sea	12.9 - 28.3 ng/g lw	Strandman <i>et al.</i> , 1999
PBDE	Herring, Atlantic	3	Wild	St Lawrence Estuary, Canada	13 ng/g ww	Law <i>et al.</i> , 2003
PBDE	Mackerel	16	Wild	Japan	1.44ng/g fw	Ohta <i>et al.</i> , 2002
PBDE	Salmon	Unk1	Wild	California, USA	0.6 ng/g ww	Luksemburg <i>et al.</i> , 2004
PBDE	Salmon	Unk1	Farmed	California, USA	3.0 ng/g ww	Luksemburg <i>et al.</i> , 2004
PBDE	Salmon	Unk1	Wild	Chile	1.76 ng/g lw	Paepke <i>et al.</i> , 2004
PBDE	Salmon	8	Wild	Scotland	56.3 ng/g lw	Jacobs <i>et al.</i> , 2002
PBDE	Salmon	5	Wild	Belgium	85.2 ng/g lw	Jacobs <i>et al.</i> , 2002
PBDE	Salmon	16	Farmed	Japan	0.97 ng/g fw	Ohta <i>et al.</i> , 2002
PBDE	Salmon	16	Wild	Japan	0.70 ng/g fw	Ohta <i>et al.</i> , 2002
PBDE	Salmon	Unk2	Wild	Baltic Sea	220 ng/g lw	Hagland <i>et al.</i> , 1997
PBDE	Salmon	Unk2	Wild	Baltic Sea	290 ng/g lw	Asplund <i>et al.</i> , 1999
PBDE	Salmon	1-144	Farmed	EU	3.0 ng/g ww	Hites <i>et al.</i> , 2004b
PBDE	Salmon	1-144	Farmed	N. America	1.0 ng/g ww	Hites <i>et al.</i> , 2004b
PBDE	Salmon, Atlantic	Unk1	Farmed	California, USA	1.6 ng/g ww	Luksemburg <i>et al.</i> , 2004
PBDE	Salmon, Atlantic	Unk1	Farmed	California, USA	1.7 ng/g ww	Luksemburg <i>et al.</i> , 2004
PBDE	Salmon, Atlantic	1-459	Farmed	EU	3.0 ng/g ww	Hites <i>et al.</i> , 2004b
PBDE	Salmon, Atlantic	1-459	Farmed	N. America	3.0 ng/g ww	Hites <i>et al.</i> , 2004b
PBDE	Salmon, Atlantic	1-459	Farmed	Chile	0.8 ng/g ww	Hites <i>et al.</i> , 2004b
PBDE	Salmon, Chinook	1	Farmed	Pacific Coast, USA	1.2 ng/g ww	Easton <i>et al.</i> , 2002
PBDE	Salmon, Chinook	1	Farmed	Pacific Coast, USA	4.5 ng/g ww	Easton <i>et al.</i> , 2002
PBDE	Salmon, Chinook	1	Wild	Pacific Coast, USA	0.49 ng/g ww	Easton <i>et al.</i> , 2002
PBDE	Salmon, Chum	1	Wild	Pacific Coast, USA	0.04 ng/g ww	Easton <i>et al.</i> , 2002
PBDE	Salmon, Coho	Unk1	Wild	California, USA	0.3 ng/g ww	Luksemburg <i>et al.</i> , 2004
PBDE	Salmon, Pacific	135	Wild	Pacific	0.2 ng/g ww	Hites <i>et al.</i> , 2004b
PBDE	Salmon, Sockeye	Unk1	Wild	California, USA	0.2 ng/g ww	Luksemburg <i>et al.</i> , 2004
PBDE	Salmon, Sockeye	1	Wild	Pacific Coast, USA	0.10 ng/g ww	Easton <i>et al.</i> , 2002
PBDE	Salmon, Sockeye	1	Wild	Pacific Coast, USA	0.09 ng/g ww	Easton <i>et al.</i> , 2002
PBDE	Trout	Unk1	Wild	NE Atlantic	9.74 ng/g lw	Paepke <i>et al.</i> , 2004
PBDE	Trout	1/lake	Wild	Norway (13 lakes)	43.2 ng/g lw	Schlabach <i>et al.</i> , 2001



BFR	Type of fish	N	Farmed or Wild	Location	Concentration	Citation
PBDE	Trout	1-26	Wild	Ricknall Grange, Skerne-Tees, UK	12.0 - 14.0 ng/g fw	CEFAS, 2003
PBDE	Trout	1-26	Wild	Haughton Road, Skerne-Tees, UK	59.0 - 197.0 ng/g fw	CEFAS, 2003
PBDE	Trout	1-26	Wild	Oxenfield Bridge, Skerne-Tees, UK	27.0- 123.0 ng/g fw	CEFAS, 2003
PBDE	Trout	1-26	Wild	Middleton in Teesdale, Skerne-Tees, UK	1.3 - 18.0 ng/g fw	CEFAS, 2003
PBDE	Trout	1-26	Wild	Low Coniscliffe, Skerne-Tees, UK	3.0 - 7.6 ng/g fw	CEFAS, 2003
PBDE	Trout	1-26	Wild	Croft-on-Tees, Skerne-Tees, UK	1.0 - 57.7 ng/g fw	CEFAS, 2003
PBDE	Trout	Unk2	Wild	Dalslands Canal, Sweden	280 - 120ng/g lw	Sellstrom <i>et al.</i> , 1993, 1996
PBDE	Trout, Lake	10	Wild	Lake Superior	56 +/-19 ng/g ww	Luross <i>et al.</i> , 2002
PBDE	Trout, Lake	10	Wild	Lake Huron	50 +/-19ng/g ww	Luross <i>et al.</i> , 2002
PBDE	Trout, Lake	10	Wild	Lake Erie	27 +/- ng/g ww	Luross <i>et al.</i> , 2002
PBDE	Trout, Lake	10	Wild	Lake Ontario	95 +/-22 ng/g ww	Luross <i>et al.</i> , 2002
PBDE	Trout, Lake	10	Wild	Lake Erie	117 ng/g lw	Luross <i>et al.</i> , 2000
PBDE	Trout, Lake	Unk1	Wild	Lake Ontario	545 ng/g lw	Alaee <i>et al.</i> , 1999
PBDE	Trout, Lake	Unk1	Wild	Lake Huron	237 ng/g lw	Alaee <i>et al.</i> , 1999
PBDE	Trout, Lake	Unk1	Wild	Lake Superior	135 ng/g lw	Alaee <i>et al.</i> , 1999
PBDE	Trout, Rainbow	3	Farmed	Farm, Switzerland	0.74 ng/g ww	Zennegg <i>et al.</i> , 2003
PBDE	Trout, Rainbow	3	Farmed	Farm, Switzerland	1.0 ng/g ww	Zennegg <i>et al.</i> , 2003
PBDE	Trout, Rainbow	3	Farmed	Farm, Switzerland	1.3 ng/g ww	Zennegg <i>et al.</i> , 2003
PBDE	Trout, Rainbow	3	Farmed	Farm, Switzerland	0.97 ng/g ww	Zennegg <i>et al.</i> , 2003
PBDE	Tuna	Unk1	Wild	California, USA	0.2 ng/g ww	Luksemburg <i>et al.</i> , 2004
PBDE	Tuna	Unk1	Wild	California, USA	0.01 ng/g ww	Luksemburg <i>et al.</i> , 2004
PBDE	Tuna, Yellow	16	Wild	Japan	0.02 ng/g fw	Ohta <i>et al.</i> , 2002
PBDE	Tuna, Yellowfin	Unk1	Wild	California, USA	0.2 ng/g ww	Luksemburg <i>et al.</i> , 2004

N: Sample size (where values A - B are given, exact sample size for that particular species is not known. B = the sample size for the whole study)

fw: Fresh weight

ww: Wet weight

lw: Lipid weight

Unk1: Unknown sample size (data not contained within publication)

Unk2: Unknown sample size (publication unavailable)

Table 5. Camphechlor (Parlar 26, Parlar 50, Parlar 62) in fish ($\mu\text{g}/\text{kg}$ wet weight) and fish feed samples, transfer rates from feed into rainbow trout (%) according to Karl *et al.* (2002)

Sample	Origin	Collection	Parlar	Mean values	Range	n
Fish feed	Norway	1997	26	5.2	2.0 – 9.7	3
			50	7.4	4.1 – 13.0	3
			62	4.8	2.1 – 8.8	3
Farmed rainbow trout		1997 - 1999	26	1.2	0.1 – 2.7	7
			50	2.2	0.3 – 4.8	7
			62	1.1	0.2 – 2.2	7
Transfer rates (%)			26	24.8	17.3 – 34.4	6
			50	28.7	15.3 – 45.0	6
			62	22.4	15.0 – 30.3	6

Table 6. Composition of different wild salmon species [average values, unit/100g raw weight] (<http://www.nal.usda.gov/fnic/foodcomp/search>)

	Atlantic	Chinook	sockeye	coho
Latin name	<i>Salmo salar L.</i>	<i>Oncorhynchus tshawytscha</i> (Walbaum)	<i>Oncorhynchus nerka</i> (Walbaum)	<i>Oncorhynchus kisutch</i> (Walbaum)
Energy [kcal]	142	179	168	146
Protein [g]	19.8 ± 0.66	19.9 ± 0.4	21.3 ± 0.2	21.6 ± 0.04
Total lipid/[g]	6.3 ± 1.8	10.4 ± 1.5	8.6 ± 0.4	5.9 ± .2-
SFA [g]	0.98	3.1	1.5	1.3
MUFA [g]	2.1	4.4	4.1	2.1
PUFA [g]	2.5	2.8	1.9	2.0
20:5 n-3 [g]	0.32	1.01	0.52	0.43
22:5 n-3 [g]	0.29	0.30	0.04	0.23
22:6 n-3 [g]	1.12	0.94	0.65	0.66
Sum LC n-3 PUFA [g]	1.73	2.25	1.21	1.32
Retinol [µg]	12	136	58	30
Selenium [µg]	36.5	36.5	33.7	36.5

SFA: Total saturated fatty acids

MUFA: Total mono-unsaturated fatty acids

PUFA: Total poly-unsaturated fatty acids

Table 7. Composition of different tuna species [average values, unit/100g raw weight] (<http://www.nal.usda.gov/fnic/foodcomp/search>)

	bluefin	skipjack	yellowfin
Latin name	<i>Thunnus thynnus L.</i>	<i>Euthynnus pelamis L.</i>	<i>Thunnus albacares (Bonnaterre)</i>
Energy [kcal]	144	103	108
Protein [g]	23.3 ± 0.4	22.0 ± 1.2	23.4
Total lipid [g]	4.9 ± 1.0	1.0 ± 0.1	1.0 ± .03
SFA [g]	1.3	0.33	0.24
MUFA [g]	1.6	0.19	0.15
PUFA [g]	1.4	0.32	0.28
20:5 n-3 [g]	0.28	0.07	0.04
22:5 n-3 [g]	0.13	0.01	0.01
22:6 n-3 [g]	0.89	0.19	0.18
Sum LC n-3 PUFA [g]	1.30	0.27	0.23
Retinol [µg]	655	16	18
Selenium [µg]	36.5	36.5	36.5

SFA: Total saturated fatty acids

MUFA: Total mono-unsaturated fatty acids

PUFA: Total poly-unsaturated fatty acids

Table 8. Composition of different mackerel species [average values, unit/100g raw weight] (<http://www.nal.usda.gov/fnic/foodcomp/search>)

	Atlantic	king	spanish
Latin name	<i>Scomber scombrus L.</i>	<i>Scombermorus cavalla</i> (Cuvier)	<i>Scombermorus maculatus</i> (Mitchill)
Energy [kcal]	205	105	139
Protein [g]	18.6 ± 0.2	20.3 ± 0.8	19.3 ± 1.2
Total lipid [g]	13.9 ± 0.4	2.0 ± 0.2	6.3 ± 3.8
SFA [g]	3.3	0.36	1.8
MUFA [g]	5.5	0.76	1.5
PUFA [g]	3.4	0.46	1.7
20:5 n-3 [g]	0.90	0.14	0.33
22:5 n-3 [g]	0.21	0.02	0.10
22:6 n-3 [g]	1.40	0.18	1.01
Sum LC n-3 PUFA [g]	2.51	0.34	1.44
Retinol [µg]	50	218	30
Selenium [µg]	44.1	36.5	36.5

SFA: Total saturated fatty acids

MUFA: Total mono-unsaturated fatty acids

PUFA: Total poly-unsaturated fatty acids

Table 9. Mercury levels in various fish species. Data are from the EC SCOOP Task 3.2.11 (EC, 2004) and from Norway (Jan Alexander, personal communication) and are expressed in $\mu\text{g/g}$ fish. Levels in carp are provided by Jiri Ruprich (personal communication)

SPECIES	N° SAMPLES	MEAN	WEIGHTED MEAN
Salmon	15	0.07	1.02
Salmon	321	0.04	12.23
Salmon	2	0.04	0.07
Salmon	5	0.03	0.16
Salmon	5	0.05	0.23
Salmon	3	0.02	0.07
Salmon	5	0.04	0.18
Salmon	1	0.02	0.02
Salmon	3	0.04	0.13
Salmon	4	0.03	0.12
MEAN	364		0.04
Trout	18	0.03	0.56
Trout	1	0.05	0.05
Trout	51	0.05	2.34
Trout	2	0.02	0.03
MEAN	72		0.04
Herring	32	0.04	1.12
Herring	1	0.04	0.04
Herring	2	0.03	0.06
Herring	8	0.03	0.26
Herring	50	0.04	1.80
MEAN	93		0.04
Mackerel	129	0.04	5.29
Mackerel	9	0.06	0.56
Mackerel	1	0.04	0.04
Mackerel	3	0.03	0.10
Mackerel	1	0.02	0.02
Mackerel	5	0.04	0.20
Mackerel	44	0.03	1.36
MEAN	192		0.04
Sardine	5	0.04	0.22
Sardine	3	0.04	0.11
Sardine	4	0.07	0.28
Sardine	26	0.03	0.78
MEAN	38		0.04
Carp	12	0.03	0.03
Carp	12	0.05	0.05
Carp	12	0.03	0.03
Carp	12	0.04	0.04
Carp	12	0.02	0.02
Carp	12	0.04	0.04
Carp	4	0.03	0.03
Carp	4	0.02	0.02
Carp	4	0.02	0.02
Mean	84	0.03	0.03



Table 10. Mercury levels in Baltic herring ($\mu\text{g/g}$ fish)

Location	N	Year	Mean (mg/kg fw)	Range	Source
Galö	1*	2001	0.057		Swedish National Food Administration
East Gräsö	1*	2001	0.12		Swedish National Food Administration
Öregrundsgrepen	1*	2001	0.14		Swedish National Food Administration
Östra Gräsö	8 (f)	2002	0.083		Swedish National Food Administration
	1 (m)		0.048		
	1		0.037		
Öregrundsgrepen	2 (f)	2002	0.08		Swedish National Food Administration
	7 (m)		0.066		
	1		0.04		
Not specified	5		0.03	0.005-0.11	Finish Ministry of Trade and Industry, 2004
	9	2003	0.018		Estonia Ministry of Agriculture, Food and Veterinary Department

* 10 pooled samples



Table 11. Occurrence data for PBDE and DDT in Baltic Herring provided by Finland to EFSA in March 2005

Location	n	Year		Sum PBDE (ng/g f.w.)	Sum DDT (ng/g f.w.)
Bothnian Sea	90	2002	Mean	1.94	26.7
			Median	1.4	18.6
			Min	0.36	2.56
			Max	8.125	141.3
Bothnian Bay	9	2002	Mean	2.1	10.5
			Median	1.8	12.0
			Min	0.44	1.89
			Max	4.48	20.56
Archipelago Sea	10	2002	Mean	1.3	14.9
			Median	0.8	9.4
			Min	0.37	4.37
			Max	3.97	44.24
Southern Baltic Sea	5	2002	Mean	2.4	28.6
			Median	2.8	20.2
			Min	0.79	12.15
			Max	4.12	50.99
Total	113	2002	Mean	1.8	23.2
			Median	1.4	16.1
			Min	0.3	1.2
			Max	8.1	141.3

n: Number of individual samples .

Table 12. Occurrence data HBCD, PBDE, DDT, HCB and HCH in Baltic Herring provided by Sweden to EFSA in March 2005

Location	N	Year		HBCD (ng/g f.w.)	Sum PBDE (ng/g f.w.)	Sum DDT (ng/g f.w.)	HCB (ng/g f.w.)	Sum HCH (ng/g f.w.)
Bothnian Bay	91	2001	Mean	0.5	1.0	7.9	1.4	1.35
			Median	0.5	1.0	7.9	1.3	1.3
			Min:	0.23	0.64	4.0	0.66	0.91
			Max:	1.05	1.76	11.7	2.55	2.08
Baltic proper	103		Mean	1.3	2.8	59.9	2.9	4.8
			Median	1.3	2.4	48.9	3.1	5.0
			Min	0.48	1.14	20.9	0.94	1.82
			Max	2.85	7.27	178.5	4.62	8.74
Bothnian Sea	66	2001 and 2003	Mean	2.1	5.7	92.3	5.1	4.5
			Median	1.9	5.5	91.7	5	4.5
			Min:	1.49	2.87	52.8	4.09	3.64
			Max:	3.22	8.15	130.6	6.76	5.34
W Bornholm	186		Mean:			39.4	1.9	2.7
			Median			29.3	1.8	2.6
			Min:			25.1	0.85	0.67
			Max:			83.6	3.39	4.74
Total	446	2001-2003	Mean:	1.4	3.1	50.8	2.8	3.5
			Median	1.36	2.67	39.6	2.43	3.9
			Min:	0.2	0.64	4	0.6	0.67
			Max:	3.2	8.15	178.5	6.76	8.74

N: Number of fish in pooled analysis of 5-11 fish in each pool.



Table 13. Occurrence data for PCDD/F, DL PCB and NDL-PCB in Baltic Herring provided by Sweden to EFSA in March 2005

Location	N	Year		Sum PCDD (pgTEQ/g f.w.)	Sum PCDF (pgTEQ/g f.w.)	DL-PCB (pgTEQ/g f.w.)	SUM PCDD/F + DL-PCB (pgTEQ/g f.w.)	Sum 6 PCB (ngTEQ/g f.w.)
Bothnian Bay	91	2001	Mean	0.62	1.76			11.01
			Median	0.7	1.6			11.2
			Min	0.20	0.67			3.70
			Max	1.12	2.98			19.79
Bothnian Sea	106	2001-2003	Mean	4.21	11.78			75.88
			Median	4.59	12.35			77.02
			Min	2.30	7.33			9.11
			Max	5.83	17.12			117.7
Baltic proper	103	2000	Mean	1.5	4.2			36.4
			Median	1.44	3.64			26.91
			Min	0.5	1.2			12.4
			Max	2.9	8.4			124.7
W Bornholm	186	2002	Mean	0.8	1.7			21.0
			Median	0.6	1.2			18.9
			Min	0.5	1.0			13.9
			Max	1.5	3.7			37.1
Total	486	2001-2003	Mean	1.8	5	4.5	11.2	38.0
			Median	1.2	3.26	3.42	8.0	20.9
			Min:	0.2	0.67	0.82	1.7	3.7
			Max:	5.8	17.1	14.19	33.5	125

N: Number of fish in pooled analysis of 5-11 fish in each pool.

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ANNEX 2. Details of metabolism, function and physiological requirement of LC n-3 PUFA

Metabolism and function

Adult humans are able to desaturate and elongate LNA to EPA and DPA, but further desaturation to DHA is limited. Conversion is higher in women than in men (Burdge *et al.*, 2002; Burdge and Wootton, 2002). Both $\Delta 6$ and $\Delta 5$ desaturases have been demonstrated in human fetal tissue from as early as 17 to 18 weeks of gestation (Chambaz *et al.*, 1985; Rodriguez *et al.*, 1998) and both term and preterm infants have been shown to convert LNA to DHA (Carnielli *et al.*, 1996; Salem *et al.*, 1996; Sauerwald *et al.*, 1997; Uauy *et al.*, 2000).

The affinity of the $\Delta 6$ desaturase to its substrates is LNA > LA > oleic acid. High dietary intakes of LNA, LA and of LC n-3 PUFA and arachidonic acid (AA) reduce the efficiency of the conversion of LNA to DHA and of LA to AA. Both a high intake of LNA and of EPA/DHA result in reduced tissue concentrations of AA, whereas a high DHA intake leads to increased levels of EPA, probably by retroconversion (Emken *et al.*, 1999).

Both AA and DHA from the diet can cross the blood brain barrier, but DHA can also be formed from precursors in astrocytes. In mice, an increased intake of EPA, DPA and DHA from fish oil resulted in incorporation into whole brain, hippocampal, and hepatic phospholipids (Berger *et al.*, 2002). n-3 Fatty acids from the diet are almost completely absorbed and either oxidised, incorporated into tissue lipids or converted to eicosanoids.

LC n-3 PUFA are incorporated in cellular membrane lipids particularly in nerve tissue and in the retina and they are, like AA, precursors for eicosanoids which antagonise AA-derived prostaglandins, thromboxanes and leukotrienes in having anti-inflammatory and anti-thrombotic effects (Kelley, 2001). EPA and DHA have been shown in numerous studies to suppress human immune function *in vitro* or *ex vivo* in peripheral blood mononuclear cells or in isolated neutrophils or monocytes in a minimum dose of 0.9 g EPA plus 0.6 g DHA as fish oil during 6 to 8 weeks in healthy subjects (Cooper *et al.*, 1993). EPA and DHA doses which caused some type of immunosuppression in intervention studies with either fish or fish oil or single-cell (algal) oils ranged from 0.9 to 9.4 g/day and from 0.6 to 6.0 g/day, respectively. Effects included a diminished potential of the immune system to attack pathogens (Kelley *et al.*, 1998; 1999; Lee *et al.*, 1985; Schmidt *et al.*, 1989). This was associated with a suppression of inflammatory responses such as occur in chronic degenerative autoimmune disease. The reported effects on proliferation of peripheral blood mononuclear cell proliferation and neutrophil function, and on cytokine production were dependent on amounts and type of LC n-3 PUFA, basal diet, age and gender and were inconsistent. This may also be due to the individual genetic background of the study populations (Markovic *et al.*, 2004).

LC n-3 PUFA alter the postprandial lipoprotein production and clearance and lower circulating triglyceride-rich lipoproteins (Roche and Gibney, 2000). LC n-3 PUFA activate the peroxisome proliferator-activated receptor- α (PPAR- α) and thereby decrease triglyceride synthesis (Wong *et al.*, 1985), increase mitochondrial β -oxidation and fatty acid oxidation in peroxisomes (Gronn *et al.*, 1992; Jump and Clarke, 1999; Willumsen *et al.*, 1993) and decrease the formation of very-low density (VLDL) lipoproteins (Nossen *et al.*, 1986). Fish oil may also lower lipids indirectly by suppressing inflammation. Inflammation results in mobilisation of non-esterified fatty acids from adipose tissue and increased hepatic synthesis and secretion of VLDL-triglycerides in response to cytokines, cortisol and catecholamines (Gallin *et al.*, 1969; Samra *et al.*, 1996).

The effects are mediated by actions of LC n-3 PUFA on gene transcription, either directly by activating PPARs or indirectly through additional transcription factors (Berger *et al.*, 2002; Jump, 2002).

Physiological background for the requirement for LC n-3 PUFA

Clinical signs (skin changes) that have been attributed to an insufficient supply of LNA have been reported in humans at LNA intakes of < 0.02 % of energy intake during enteral nutrition (Bjerve *et al.*, 1987). The estimated minimum requirement of LC n-3 PUFA to cure deficiency symptoms in these patients was 0.1 - 0.2 g/day, corresponding to 0.1 - 0.2 % of the energy intake. The endogenous formation of LC n-3 PUFA from their precursor LNA is rather inefficient and, in addition negatively influenced by the LA intake, which is high in some European countries. There is no consensus on the need for an intake of preformed DHA in adults, but there are data to indicate that DHA is conditionally indispensable for preterm infants. A functional shortage of DHA is characterised by an increased synthesis of n-6 docosapentaenoic acid (C22:5 n-6) or by a lowered DHA/n-6 docosapentaenoic acid ratio (Hornstra, 1992).

An increase in n-6 docosapentaenoic (Osbond) acid and n-9 eicosatrienoic (Mead) acid (C20:3 n-9), another surrogate marker for LC-PUFA deficiency, by 348 and 244 % in blood lipids is regularly seen early in pregnancy (Al *et al.*, 1995a; Otto *et al.*, 2001a), while the concentrations of DHA and AA in plasma and red blood cell phospholipids increase and their relative concentrations (percent of total fatty acids) decrease. Accretion of LC-PUFA in fetal and placental tissues is especially high in the last trimester of pregnancy when the fetus accumulates about 60 - 70 mg LC n-3 PUFA (predominantly DHA) per day (Clandinin *et al.*, 1980; 1981). It is estimated that a total amount of at least 10 g DHA is needed by the fetus during pregnancy. This amount has for the greatest part to be provided by the pregnant woman either by increasing her intake, by mobilisation of body stores or by an increase in synthesis. To this amount must be added the DHA secreted into milk by the breastfeeding mother, i.e. another 12 g over a lactation period of six months (Makrides and Gibson, 2000). After delivery the LC-PUFA in maternal plasma phospholipid normalises slowly after an initial decrease in the relative DHA concentration in both breastfeeding and non-lactating mothers. However, in breastfeeding mothers this decrease is more pronounced. The concomitant increase of other LC n-3 PUFA suggests that DHA is selectively transferred into milk (Otto *et al.*, 2001b). It is, therefore, recommended that a pregnant woman should increase her intake of DHA to about 0.2 g/day and that a breastfeeding woman should increase her intake to approximately 0.16 - 0.17 g/day during the lactation period (FSA, 2004a). This is of special importance in multiparous women. The absolute (mg/L) and relative (% of total fatty acids) amounts of DHA in maternal plasma phospholipids were significantly lower in multiparous than primigravid women and consequently the ratio DHA/n-6 docosapentaenoic acid was significantly lower in umbilical plasma and the DHA content of umbilical artery and vein vessel walls was significantly lower in infants born to multiparous women (Al *et al.*, 1997).

DHA is the major LC-PUFA in the central nervous system (Innis, 2003; Uauy *et al.*, 2001) which shows in the human infant a spurt in growth during the third trimester of pregnancy and in the neonatal period. No significant associations have been observed between either DHA or AA concentration in cord blood phospholipids and cognitive performance at 7 years of age (Bakker *et al.*, 2003) or between LC-PUFA status of neonatal red blood cells and cognitive performance at 3.5 years of age (Ghys *et al.*, 2002). But DHA status at birth was significantly and positively related to movement quality and visual acuity at 7-8 years of age and to

behaviour at 7 years (Hornstra, 2005). This underlines the importance of an adequate maternal DHA intake during pregnancy as a condition for an ample supply of the fetus and its importance for cognitive, motor, visual and behavioural development of the infant and child. This adequate maternal intake during pregnancy and lactation can be reached by a higher intake of fatty fish (or of fish oil). Supplementation of pregnant women with 2.8 g LNA plus 9 g LA, however, did not increase maternal and neonatal DHA concentrations in blood phospholipids, but increased EPA and DPA concentrations. It increased the functional DHA status of the infant (lower n-6 docosapentaenoic acid in umbilical arterial vessel wall), but decreased LC n-6 PUFA concentrations in the infant's phospholipids (de Groot *et al.*, 2004). Because AA is the second abundant LC-PUFA in neural tissues, a decrease by either LNA or fish oil supplementation in high amounts (Helland *et al.*, 2003; van Houwelingen *et al.*, 1995) may, however, not be desirable.

Consumption of fish oils

An alternative to eating fish as a source of LC n-3 PUFA is the use of fish oils which can be produced from the livers of lean fish, e. g. cod liver oil, or from the bodies of fatty fish. These oils can either be native, like cod liver oil with a high content of vitamins A and D, or winterised oils, from which saturated fatty acids, vitamins A and D and lipophilic contaminants have virtually been removed (heavy metals have for the greater part been removed with the aqueous phase during pressing of the heated fish material). With special processing the LC n-3 PUFA can be further concentrated. The resulting product rich in LC n-3 PUFA should be stabilised against oxidation.

Table 1 contains compositional data on fish oils from different species. If farmed fish is the source of the oil, the lipid source of the fish feed will be reflected in the fatty acid pattern of the oil.

The total LC n-3 PUFA content can contribute one third of the total lipid content but can be as low as one tenth. The relative amounts of DHA vary independent of the total LC n-3 PUFA content. With the exception of menhaden and herring oil DHA predominates over EPA

Table 1: Fatty acid composition of oils from different fish species [units/100g] (<http://www.nal.usda.gov/fnic/foodcomp/search>)

	Cod liver oil	Menhaden oil	Herring oil	Salmon oil	Sardine oil
Energy/kcal	902	902	902	902	902
Total lipid/g	100	100	100	100	100
Vitamin A/ μ g	30 000	0	0	0	0
Vitamin D/ μ g	250	0	0	0	8
SFA/g	22.6	30.4	21.3	19.9	30.0
MUFA/g	46.7	26.7	56.6	29.0	33.8
PUFA/g	22.5	34.2	15.6	40.3	31.9
20:5 n-3/g	6.9	13.2	6.3	13.0	10.1
22:5 n-3/g	0.9	4.9	0.6	3.0	2.0
22:6 n-3/g	11.0	8.6	4.2	18.2	10.7
Sum LC n-3 PUFA/g	18.8	26.7	11.1	34.2	22.8
DHA/EPA	1.6	0.7	0.7	1.4	1.1
Cholesterol/mg	570	521	766	485	710

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