The Nutritional-Toxicological Conflict related to Seafood Consumption

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The Nutritional-Toxicological Conflict related to Seafood Consumption

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Thesis submitted in fulfilment of the requirements for the degree of Doctor in Medical Sciences

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“You shall offer something good to your body,
so that the soul feels well to live in it.”

Anonymous

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Abbreviations

%E	percentage of total energy intake
AA	arachidonic acid
AHA	American Heart Association
AMDR	acceptable macronutrient distribution range
bw	body weight
CEC	Central Economic Council
CHD	coronary heart disease
COT	Committee on Toxicity (United Kingdom)
Cons.	consumers-only
CVD	cardiovascular disease
DHA	docosahexaenoic acid
dl PCBs	dioxin-like PCBs
DPA	docosapentaenoic acid
DRI	dietary reference intake
EDR	estimated diet record
EFSA	European Food Safety Authority
EPA	eicosapentaenoic acid
FA	fatty acid
FAO	Food and Agriculture Organization
FCDB	food composition database
FCS	food consumption survey
FFQ	food frequency questionnaire
Hg	mercury
HIS	health interview survey
ICES	International Council for the Exploration of the Sea
IOM	Institute of Medicine (United States of America)
iPCBs	indicator PCBs
ISSFAL	International Society for the Study of Fatty Acids ans Lipids
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LA	linoleic acid
LNA	α -linolenic acid
LC	long chain
LC n-3 PUFAs	long chain omega-3 poly-unsaturated fatty acids
LOD	limit of detection
LOQ	limit of quantification
MeHg	methyl mercury
n-3	omega-3
n-6	omega-6
PBDEs	polybrominated diphenyl ethers
ndl PCBs	non-dioxin like PCBs
PCBs	polychlorinated biphenyls
PCDDs	polychlorinated dibenzo-*p*-dioxins
PCDFs	polychlorinated dibenzofurans
PUFAs	poly-unsaturated fatty acids
SACN	Scientific Advisory Committee on Nutrition (United Kingdom)
SCO	single cell oil
SFA	saturated fatty acid

TDI	tolerable daily intake
TEFs	toxic equivalency factors
TEQ	toxic equivalent
totTEQ	total TEQ
TWI	tolerable weekly intake
UL	tolerable upper intake level
WHO	World Health Organization

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Chapter I.

General introduction

Pioneer research in the nineteen sixties and seventies by Danish scientists indicated that the consumption of fish was associated with a reduced risk for heart disease in Greenland Eskimos (Bang & Dyerberg, 1980; Dyerberg *et al*, 1975). This Eskimo population experienced a low mortality from coronary heart disease despite a diet rich in fat and cholesterol. The investigators proposed that this could be related to the high content of omega-3 fatty acids, typically present in marine foods.

These Eskimo studies have triggered a much broader and intensified research on the importance of omega-3 fatty acids and seafood in the human diet. This PhD-thesis is embedded in this research area and examines in the first place the intake of omega-3 and other fatty acids by the Flemish and Belgian population. In a next step, the question is raised whether seafood is a safe dietary source of these fatty acids and whether the consumption conform with physiological needs induces any toxicological concern. The latter is of importance since the favourable health perception is troubled by information regarding the potential adverse health impact of chemical contaminants in marine foods, occuring naturally or resulting from man-made processes. Persistent organochlorine compounds, e.g. dioxin-like substances, and organochlorine pesticides, as well as heavy metals, e.g. mercury, accumulate in the marine food chain. As a result, people consuming seafood are potentially at increased risk to ingest simultaneously compounds that can have toxicological effects. These conflicting facts form a potential base for an important public health conflict between dietary recommendations on the one hand and toxicological safety assurance on the other hand.

The introduction of this PhD-thesis starts with the position of omega-3 fatty acids in the human diet, their role in the human body, and the current recommendations for omega-3 fatty acids. Next, an introduction to the nutritional-toxicological conflict related to seafood is given, followed by a detailed description on how the evaluation of the intakes of nutrients and contaminants will be performed in this PhD-thesis.

1. Omega-3 fatty acids and the human diet

Scientific evidence exists that long chain (LC) omega-3 poly-unsaturated fatty acids (PUFAs) play an essential role in human health (Din *et al*, 2004; Kris-Etherton *et al*, 2002; Kris-Etherton *et al*, 2003; Ruxton, 2004; Ruxton *et al*, 2004; Ruxton *et al*, 2005; Sidhu, 2003; Simopoulos *et al*, 2000). To situate these omega-3 PUFAs in the overall group of fatty acids, the table below indicates the different types of fatty acids and gives some commonly occurring examples (Table I.1).

Table I.1: The different types of fatty acids and some examples commonly occurring in food

I. Saturated fatty acids		**II. Unsaturated fatty acids**	
Butyric acid	C4:0	II.a. Mono-unsaturated fatty acids	
Caproic acid	C6:0	Oleic acid	C18:1n-9
Lauric acid	C12:0	II.b. Poly-unsaturated fatty acids	
Myristic acid	C14:0	*II.b.1. Omega-6 fatty acids*	
Palmitic acid	C16:0	Linoleic acid	C18:2n-6
Stearic acid	C18:0	γ-linolenic acid	C18:3n-6
		Arachidonic acid	C20:4n-6
		II.b.2. Omega-3 fatty acids	
		α-linolenic acid	C18:3n-3
		Eicosapentaenoic acid	C20:5n-3
		Docosahexaenoic acid	C22:6n-3

The group of PUFAs is divided into two groups: omega-3 (n-3) and omega-6 (n-6) PUFAs, differing in the position where the first double C-bound is located. Two PUFAs are called 'essential fatty acids' since they cannot be synthesized in the human body and are vital for physiological integrity. Therefore, they must be obtained from the diet. One is linoleic acid (LA, C18:2n-6) and belongs to the n-6 family. The other one is α-linolenic acid (LNA, C18:3n-3) belonging to the n-3 family. These essential parent compounds can be converted in the human body to LC fatty acids, but humans can not interconvert n-3 and n-6 fatty acids (Ruxton *et al*, 2005). LA can be converted to arachidonic acid (AA, C20:4n-6) and further on to longer chain derivates, and LNA to eicosapentaenoic acid (EPA, C20:5n-3) in a first step and docosahexaenoic acid (C22:6n-3) in a next step. This conversion is summarized in Fig. I.1.

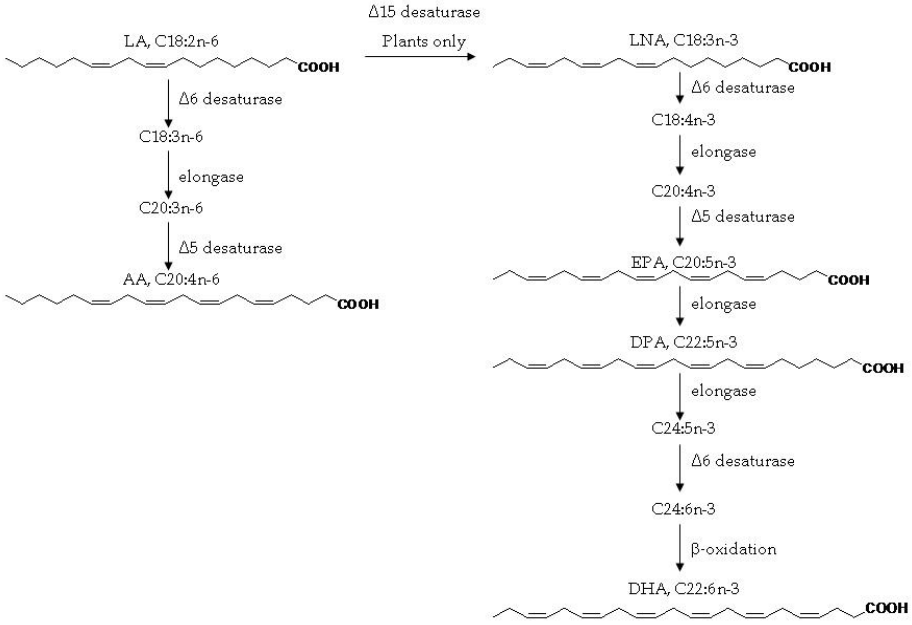


Fig. I.1 Desaturation and elongation of n-6 and n-3 fatty acids (based on Din et al (2004))

Different enzymatic steps are needed to fulfil these conversions which include desaturation and elongation. A crucial point is that the first enzymatic step in the desaturation of both LA and LNA involves the enzyme Δ6 desaturase. This results in a competition of both substrates for Δ6 desaturase. High intakes of LA have been suggested to decrease the desaturation of LNA to EPA and DHA and favour higher levels of AA.

In developed countries dietary intakes of LA have increased over the last century from ~3% to 5-7% of total energy intake due largely to an increased consumption of LA-rich vegetable oils (Cunnane & Griffin, 2002; Liou *et al*, 2007; Ruxton *et al*, 2005). As a result, the conversion of LNA to LC n-3 PUFAs in the human body is even more limited. In adult men conversion to EPA is limited to approximately 8% and conversion to DHA is extremely low (< 0.1%). In women conversion to DHA appears to be greater and affected by the physiological state (e.g. higher conversion during pregnancy to meet the provision of foetal DHA) (Otto *et al*, 2001; Williams & Burdge, 2006). It is questioned whether all human requirements for LC n-3 PUFAs can be met from the endogenous supply (Burdge & Wootton, 2002; Cunnane & Griffin, 2002; Ruxton *et al*, 2005; Williams & Burdge, 2006). Therefore, most nutritional guidelines now include recommendations for increased intakes of these fatty acids.

As already stated, over the past decades, the balance of fatty acids in the diet has shifted away from n-3 PUFAs to n-6 PUFAs. Explanations for this shift include changing farming

practices resulting in meat being less rich in n-3 PUFAs, low consumption of seafood, and developments in food technology leading to increased supply and high consumption of n-6 rich food products (e.g. margarines) (Ruxton, 2004; Simopoulos, 1999). As a result, the ratio of n-6 to n-3 PUFAs increased in developed countries. Simopoulos (1999; 2002) indicates that human beings evolved on a diet with a ratio of n-6 to n-3 fatty acids of around 1 to 4 whereas in Western diets the ratio is now 15/1–16.7/1. Considering the dietary sources of n-3 PUFAs, LNA can be found in plant sources, including green leafy vegetables, some seed oils (linseed oil, rapeseed oil, flaxseed oil, and soybean oil) and nuts (e.g. walnuts). Synthesis of EPA and DHA occurs in phytoplankton and animals, but not in plants. It is by planktivorous fishes that LC n-3 PUFAs enter in the marine food chain and accumulate in seafood (mainly in oily fish) (Cunnane & Griffin, 2002; Innis, 2007). Therefore, seafood is the food group with the highest natural LC n-3 PUFA concentration, followed by poultry and eggs since poultry also synthesise a considerable amount of LC n-3 PUFAs. Moreover, the fatty acids content of their feed will influence their LC n-3 PUFA content.

2. The role of omega-3 fatty acids in the human body

Because of the changes in dietary and lifestyle patterns, chronic non-communicable diseases - including obesity, diabetes mellitus, cardiovascular disease (CVD), hypertension and stroke, and some types of cancer – have globally become increasingly significant causes of disability and premature death (World Health Organization, 2003). In this context, the role of seafood consumption and the relation of n-3 PUFAs with several diseases are summarized below.

2.1. During development

Two major areas where n-3 PUFAs, particularly DHA, play a role are brain and eye function (Ruxton, 2004). Therefore, the maintenance of an adequate level of DHA in both the brain and the retina is important for proper nervous system and visual functions (Lands, 2005; Salem & Pawlosky, 1992; Simopoulos, 1989). DHA accounts for 25% of the phospholipids in cerebral grey matter, where it supports neural and brain activity (Ruxton *et al*, 2004; Ruxton *et al*, 2005). A supply of n-3 PUFAs from seafood and other food items may have led to large

brain expansion during the long evolution of hominids to *Homo sapiens*. Studies suggest that it is unlikely that the foetus can make sufficient DHA to support brain development. Given that maternal DHA stores compensate for the limited ability of the foetus to synthesise DHA, it is likely that an adequate intake of LC n-3 PUFA could impact foetal development (Otto *et al*, 2001; Ruxton *et al*, 2005). However, the question on how important dietary DHA is during human brain development remains unresolved (Innis, 2007).

2.2. Cardiovascular diseases

As mentioned before, the pioneering research of Bang and Dyerberg (1980) provided evidence that the consumption of fish reduced the risk on heart disease in Greenland Eskimos. The investigators proposed that this could be related to the high content of n-3 PUFAs in their diet. These findings initiated a lot of research to evaluate the effects of either fish or n-3 PUFA consumption on human health. Several prospective epidemiological studies have investigated the relationship between intake of n-3 PUFA and fatal coronary heart disease (CHD). Nowadays a large body of evidence exists to suggest beneficial effects of fish, oily fish and LC n-3 PUFAs on the molecular, cellular, and whole-body pathogenic processes of atherosclerosis and thrombosis (Kris-Etherton *et al*, 2002; Kris-Etherton *et al*, 2003; Ruxton *et al*, 2004; Williams & Burdge, 2006). Large-scale epidemiologic studies suggest that people at risk for CHD benefit from consuming n-3 PUFAs from plant and marine sources (Kris-Etherton *et al*, 2002). The ways in which n-3 PUFAs reduce CVD risk are still being studied. Possible mechanisms of action of n-3 PUFAs in the prevention of cardiovascular disease are (Din *et al*, 2004; Institute of Medicine, 2005; Kinsella *et al*, 1990; Kris-Etherton *et al*, 2002; Kris-Etherton *et al*, 2003; Ruxton *et al*, 2005; Sidhu, 2003):

- antiarrhythmic mechanism: n-3 PUFAs decrease risk of arrhythmias that might be implicated in sudden death;
- antithrombotic mechanism: n-3 PUFAs decrease risk of thrombosis, that might be implicated in heart attack and stroke;
- antiatherosclerotic mechanism: n-3 PUFAs decrease the rate of growth of atherosclerotic plaque;
- n-3 PUFAs lower triglyceride and remnant lipoprotein levels in a dose dependent manner;
- n-3 PUFAs lower blood pressure;

- antiinflammatory mechanism: n-3 PUFAs reduce inflammatory responses, which is important since inflammation has a central role in the development and progression of CHD.

However, evidence from trials with n-3 PUFAs is less clear. Moreover, a recent meta-analysis has revealed a lack of consistency between the different trials studying the influence of n-3 PUFAs on the risk of CHD (Hooper *et al*, 2006). The antiarrhythmic effect of fish oil remains unproven although the idea is still viable and is being actively tested in further trials (Brouwer *et al*, 2006). As a consequence, there is a need for further large-scale, well-executed, randomised controlled trials to reach a consensus with regard to the role of these fatty-acids in prevention of CHD and CVD in general (ISSFAL, 2007).

2.3. Other diseases

2.3.1. Inflammatory diseases

Lands (2005) stated that man must better balance n-3 and n-6 fatty acid intake to temper an overactive eicosanoid system that leads to development of chronic inflammatory diseases. Eicosanoids are produced from LC PUFAs and are biologically active substances which act locally to influence a wide range of functions in cells and tissues, including inflammation. There are different families of eicosanoids: prostaglandins, thromboxanes, leukotrienes, and other metabolites. For each, there are separated series, derived either from a LC n-3 or LC n-6 PUFA. At sufficiently high intakes, LC n-3 PUFAs decrease the production of inflammatory eicosanoids. Moreover, LC n-3 PUFAs give rise to a family of antiinflammatory mediators. Modulation of LC PUFA intake, mostly by increasing the relative proportion of n-3 versus n-6 PUFAs, may as such play a role in the prevention of diseases as asthma, rheumatoid arthritis, and bowel disease (Calder, 2006; De Caterina & Basta, 2001). Supplementation with large doses of fish oil has been shown to relieve symptoms in patients with rheumatoid arthritis but findings have been far less consistent for other inflammatory diseases (de Deckere *et al*, 1998). In the future, more, better designed and larger clinical trials are needed to assess the therapeutic potential of LC n-3 PUFAs in inflammatory diseases (Calder, 2006).

2.3.2. Cancer

Increasing evidence from animal and in vitro studies indicates that n-3 PUFAs present in fatty fish and fish oil inhibit carcinogenesis (Larsson *et al*, 2004; Terry *et al*, 2003). Ecologic studies have shown that high per capita fish consumption is correlated with a lower incidence of cancer in the populations (Caygill *et al*, 1996; Hursting *et al*, 1990; Kaizer *et al*, 1989; Sasaki *et al*, 1993). Several mechanisms whereby n-3 PUFAs may modify the carcinogenic process have been proposed. These include suppression of AA-derived eicosanoid biosynthesis, influences on transcription pathways, alteration of oestrogen metabolism, increased or decreased production of free radicals and reactive oxygen species, and mechanisms involving insulin sensitivity and membrane fluidity. On the basis of these multiple mechanisms, n-3 PUFAs may have an important influence on carcinogenesis. Further studies are needed to identify new mechanisms and to evaluate and verify the mechanisms in humans to gain more understanding of the effects of marine n-3 PUFA intake on cancer risk for humans (Larsson *et al*, 2004; Terry *et al*, 2003).

2.3.3. Mental function and mood

Research related lower intakes of LC n-3 PUFAs with mental illness. Epidemiological studies have found that countries with high seafood consumption tend to have a low prevalence of major depression (Hibbeln, 1998). However, Appleton *et al* (2006) reported in a recent review that trial evidence that examines the effects of n-3 PUFAs on depressed mood is limited and is difficult to evaluate because of considerable heterogeneity. The evidence available provides little support for the use of n-3 PUFAs to improve depressed mood. Furthermore, indications exist that imbalances in PUFA status could be linked to behavioural and learning disorders such as attention deficit hyperactivity disorder, dyslexia, dyspraxia, and autism (Richardson, 2004a; Richardson, 2004b; Richardson & Montgomery, 2005). The influence of higher intakes of LC n-3 PUFA is not clear yet. Also for dementia it is claimed that increasing prevalence is linked with low oily fish consumption (Ruxton, 2004; Ruxton *et al*, 2005). Nevertheless, there is a need for more evidence from intervention studies, particularly relating to postnatal depression, child intelligence, and ageing where LC n-3 PUFAs could have far-reaching benefits (Ruxton *et al*, 2005).

3. Recommendations for omega-3 fatty acids

Due to the positive health effects of n-3 PUFAs, recommendations for dietary intake have been formulated. In the past, dietary allowances have been developed with the aim of preventing signs or symptoms of deficiency. However, in the new guidelines the American Institute of Medicine (IOM) requires that 'reduction in the risk of chronic degenerative disease must be included in the formulation of dietary recommendations' (Gebauer *et al*, 2006; Institute of Medicine, 2005), being very relevant for the recommendations related to fatty acids. It is worthwhile to review some of the existing, international n-3 and other PUFA recommendations.

(1) The International Society for the Study of Fatty Acids and Lipids (**ISSFAL**) gives the following specific recommendations about the intake of PUFAs for healthy adults:

1. An adequate intake of LA equal to 2% of the total energy intake (%E);
2. A healthy intake of LNA equal to 0.7 %E;
3. For cardiovascular health, a minimum intake of EPA and DHA combined: 500 mg/d.

Moreover, the ISSFAL recognises that there may be a healthy upper limit to the intake of LA but insufficient data exist at present to set a precise value on such an upper limit (ISSFAL, 2007).

(2) The American **IOM** used the median PUFA intakes of the American population since n-6 and n-3 deficiency is nonexistent in healthy individuals. In summary, they determined an acceptable macronutrient distribution range (AMDR) of 5 to 10 %E for n-6 PUFA and 0.6 to 1.2 %E for n-3 PUFA. In both cases they stated that approximately 10% of the AMDR can be consumed as LC PUFAs (Institute of Medicine, 2005).

(3) In the **UK** a recommendation of 0.2 g LC n-3 PUFA per day was used until recently. However, new advice to increase fatty fish consumption led to new recommendations, suggesting that one to four portions of fatty fish per week should be consumed by the general UK population. This should be equal to an intake of 450 mg LC n-3 PUFA per day (SACN/COT, 2004).

(4) The World Health Organization (**WHO**) recommended an intake of n-6 PUFAs and n-3 PUFAs equal to 5 to 8%E and 1 to 2%E, respectively (World Health Organization, 2003).

This illustrates that the international recommendations on dietary PUFA intake are quite diverse in quantity as well as mode of expression. In this PhD-thesis, the recommendations formulated by the **Belgian Health Council** are applied. In a nutshell, for adults a dietary reference intake (DRI) of 4 to 8 %E is recommended for n-6 PUFA, with a minimum of 2 %E coming from LA and a DRI of 1.3 to 2.0 %E is recommended for n-3 PUFA with a minimum recommended intake of 1 and 0.3 %E for LNA and EPA+DHA, respectively (Belgian Health Council, 2007).

4. Seafood, the nutritional-toxicological conflict

To avoid confusion about terminology the generic term '**seafood**' will be used in this PhD-thesis to describe the group of fish and shellfish, the first being the sum of marine fishes and fresh water fishes and the latter being the sum of molluscs, crustacean, and cephalopods. Sometimes, the term 'marine foods' is used as an alternative for seafood. However, in literature the term 'fish' is mostly used when describing the relation of seafood with health and/or diseases. Reason for this is that the beneficial effects are related to EPA and DHA which are most abundant in fatty fish species. Seafood species other than fish (e.g. crustacean and molluscs) have in general a lower fat and n-3 PUFA content and are in general also less consumed.

There is no doubt that seafood is the most important source of LC n-3 PUFAs. Together with the fact that there is an imbalance between the current intake of LC n-3 PUFA in developed countries and the recommendations, and that an increase of the n-6/n3 PUFA ratio in most Western countries is seen, a further stimulation in seafood consumption should be an evident solution. In addition, seafood is a good source of high-quality proteins and micronutrients, e.g. iodine, vitamin D, selenium, and zinc.

However, during the last decades an increasing number of data on high levels of contaminants in seafood are published in scientific literature as well as in public media (Ashizuka *et al*, 2005; Corsolini *et al*, 2005; Foran *et al*, 2004; Hites *et al*, 2004a; Hites *et al*, 2004b; Isosaari *et al*, 2004; Karl *et al*, 2002; Sidhu, 2003; Simm *et al*, 2006). Due to the industrial revolution, oceans, seas, lakes, and rivers are contaminated with many persistent, chemical contaminants. Due to biomagnification and bioaccumulation, these contaminants are

concentrated in the aquatic food chain to concentrations that could form a health risk for consumers (Burreau *et al*, 2006). As a result, increased seafood consumption to achieve an adequate LC n-3 PUFA intake may simultaneously increase the contaminant intake to levels of toxicological concern. On the other hand, consumers decreasing their seafood intake in order to avoid contaminant exposure may be incurring an inadequate intake of LC n-3 PUFAs (Cohen *et al*, 2005). This nutritional-toxicological conflict forms the base of the study elaborated in this PhD-thesis and is summarized in the scheme below (Fig. I.2).

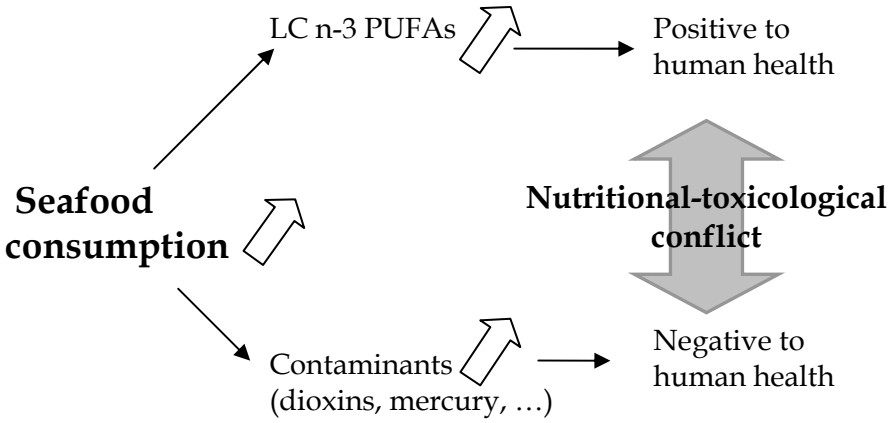


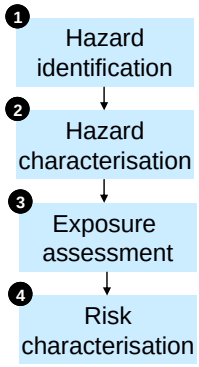
Fig. I.2 Nutritional-toxicological conflict linked to increased seafood consumption

Due to this nutritional-toxicological conflict, it was of interest to evaluate the risks and benefits related to the consumption of seafood in order to formulate safe recommendations about seafood consumption for the Belgian population. Also on international level, the FAO and WHO have demanded consultation of an expert group to study the risks and benefits related to the consumption of seafood. The latter is already discussed at the Commission of the Codex on Contaminants in Food (Commission of the Codex Alimentarius). Planning of the work is ongoing and some internal working groups at the level of the Food and Agriculture Organization (FAO) and WHO are now established, showing the pertinence of this subject.

5. Evaluation of risks and benefits

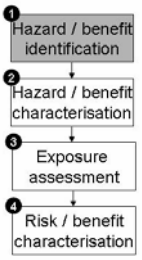
In this PhD-thesis the focus lies on the assessment of exposure to compounds ingested by food. Other ways of exposure are not considered. Food contains a wide range of substances which are either desired (nutrients, additives, and other components) or undesired, such as natural toxins, pesticide residues, mycotoxins, or contaminants. For all these materials, excessively high but in some cases also insufficiently low amounts can create a risk (Kroes *et al*, 2002). In the EU Regulation (EC) No 178/2002, risk is defined as 'a function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard' (European Commission, 2002). Regulatory measures taken solely from a risk point of view could restrict the consumption of a given food, whereas the health consequences of not eating that food might be more serious than the risk. Therefore, it is important to include also the benefits in such an evaluation.

Risk assessment is defined as a four-step process:



The latter step, risk characterisation, integrates the information collected in the preceding three steps (European Commission, 2002). It interprets the qualitative and quantitative information on the toxicological properties of a chemical in combination with the assessed amount to which individuals (parts of the population or the population at large) are exposed to this chemical (Kroes *et al*, 2002). On a colloquium of the European Food Safety Authority (EFSA) held in July 2006, experts and scientists came to a general consensus that a risk-benefit evaluation should mirror the paradigm already well established for risk analysis. This means that the benefit assessment part of the risk-benefit assessment should include benefit identification, benefit characterisation (dose-response assessment), exposure assessment, and (probability for) benefit characterisation. An introduction to the four different steps of the risk-benefit evaluation applied to the theme 'risks and benefits related to seafood consumption' is given below.

5.1. Hazard and benefit identification



The hazard and benefit identification concerns the determination of the different compounds to include in the analysis of the risks and benefits related to seafood.

5.1.1. Nutrients – benefit identification

Several nutritional benefits are related to seafood. Seafood contains very high quality proteins, being second only to egg protein in digestibility and supporting growth. With respect to vitamin content, seafood is an excellent source of the B vitamins niacin and B12, and is in general a better source of vitamins D and A compared to other protein sources as beef, pork, or chicken. Seafood can also contribute appreciable amounts of heme iron and zinc, nutrients that tend to be low in people's diets and it is among the best sources of dietary selenium.

In this PhD-study three nutritional compounds were studied in detail, chosen since they are present in seafood in a relatively high concentration compared to other food items. The first is the sum of **EPA and DHA**, the LC n-3 PUFAs most abundantly present in seafood. Furthermore, **vitamin D** and **iodine** were taken into account as well. The benefit identification of EPA and DHA is already described in previous parts of this introduction. Therefore, only vitamin D and iodine are considered below.

5.1.1.1. Vitamin D

Seafood - especially oily fish - is frequently regarded as the most important food source of the fat soluble vitamin D. The letter D origins from the German word 'Dörschleberöl', which means cod liver oil, hereby showing the historical link between vitamin D and seafood. Vitamin D is not an essential vitamin sensu stricto, since ultraviolet (UV) induced skin production constitutes the main contributor to vitamin D in humans who are sufficiently exposed to sunlight (Brustad *et al*, 2004). Apart from this endogen synthesis, food also provides vitamin D. Nevertheless, next to oily fish only a relatively small number of food items such as eggs, liver, and butter contain nutritionally significant quantities of vitamin D

(Bender, 2002; Lamberg-Allardt, 2006; Suzuki *et al*, 1988). To avoid vitamin D deficiency in Belgium all margarines and minarines are currently fortified with vitamin D (6,25 - 7,5 µg vitamin D per 100 g margarine) (Warenwetgeving, 1998).

The principal physiologic function of vitamin D is to maintain calcium homeostasis. Vitamin D functions as a hormone, synthesized far from the sites of biological action and reaching these distant sites through the blood stream. Vitamin D acts by binding to vitamin D receptors principally located in the nuclei of target cells. Due to that binding, the receptor will act as a transcription factor that modulates the gene expression of transport proteins, e.g. calbindin (Wolpowitz & Gilchrest, 2006). Severe deficiency of vitamin D leads to rickets in children and osteomalacia in adults (Bender, 2002). Yet, an adequate dietary intake of vitamin D is of great importance for young children, pregnant women, and elderly people.

Besides its role in the calcium homeostasis, more extensive roles for vitamin D were suggested by the discovery of the vitamin D receptor in tissues that are not involved in the calcium metabolism. As such, the role of vitamin D in regulation of the immune system and its possible role in the prevention and treatment of cancer and immune-mediated diseases was discovered (Mullin & Dobs, 2007).

5.1.1.2. Iodine

Oceans are considered as a huge natural reservoir of iodine. Iodine is distributed from the ocean in the atmosphere by evaporation and by rain it returns to earth. The biological function of iodine in the human body relates to its incorporation in the thyroid hormones (Institute of Medicine, 2005). The iodine content in most foods is low and can be affected by the content of the soil, irrigation, and fertilizers. Foods of marine origin have higher concentrations of iodine because marine species concentrate iodine from the seawater (Dahl *et al*, 2004; Karl *et al*, 2001). Another important dietary source is dairy food, due to the secretion of iodine in cow's milk. Lack of sufficient iodine results in inadequate thyroid production leading to the so-called iodine deficiency disorders, including mental retardation, hypothyroidism, goitre, cretinism, and varying degrees of other growth and developmental abnormalities (Institute of Medicine, 2005).

5.1.2. Contaminants – hazard identification

At the contaminant side, two different categories of persistent, chemical contaminants were considered: non-carcinogenic (e.g. mercury) and carcinogenic (e.g. dioxins) compounds. Although a very wide range of contaminants may accumulate in the marine food chain, it was decided to limit this study to the relevant ones for which enough data were available in the international scientific literature and other important publicly available data sources.

5.1.2.1. Mercury

The rationale to include mercury (Hg) is that seafood is the most important source of Hg in the human food chain. Moreover, in the marine environment, inorganic Hg is to high extent transformed to methyl mercury (MeHg), which further accumulates in the marine food chain (European Commission, 2006). This organic MeHg is very toxic for humans (Clarkson & Magos, 2006; EFSA, 2004). The primary target of MeHg is the central nervous system and the developing brain is thought to be the most sensitive target organ for MeHg toxicity (EFSA, 2004). In addition, there are indications that Hg can inhibit the preventive role of LC n-3 PUFAs on cardiovascular diseases (Chan & Egeland, 2004; Salonen *et al*, 2000).

5.1.2.2. PCDFs, PCDDs, PCBs

Next to Hg, two groups of persistent organic pollutants are considered in this work, being the polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) on the one hand and the polychlorinated biphenyls (PCBs) on the other hand. PCDDs, PCDFs and PCBs are ubiquitous in the environment and occur at picomolar levels in foods. PCDDs and PCDFs are by-products of combustion and of various industrial processes and thus unintentionally present in the environment. In contrast, PCBs were manufactured in the past for a variety of industrial uses, notably as electrical insulators or dielectric fluids and specialized hydraulic fluids. Most countries banned manufacture and use of PCBs in the 1970s. However, past improper handling of PCBs constitutes a continuing source of these compounds in the environment. Exposure of a typical person to these persistent organic pollutants occurs primarily through foods (> 90%), particularly animal fats because of the lipophilic characteristics of these substances. Bioaccumulation of dioxin-like compounds through the food chain begins at the point of consumption of contaminated plants, soil, or sediment by animals, which then are used to produce food for humans (EFSA, 2005c; World Health Organization, 2007; Yaktine *et al*, 2006).

The reason to consider these substances in this case study is that seafood has a higher concentration of these compounds per gram fat compared to other food items. Last decennia, a decreasing trend of the levels of PCBs, PCDDs, and PCDFs in food items is found, mainly due to strict rules and regulations to reduce human intake of these compounds. However, the decreasing trend seems to be quite limited in the aquatic environment since these compounds seem rather stable in large reservoirs like seas and rivers (AFSSA, 2005). As a result, seafood is currently one of the most important contributors to the total dietary intake of PCBs, PCDDs, and PCDFs (AFSSA, 2005; Bilau *et al*, 2007; Bocio & Domingo, 2005; Darnerud *et al*, 2006; Fattore *et al*, 2006; Kiviranta *et al*, 2004).

There exist 75, 135, and 209 different congeners of PCDDs, PCDFs, and PCBs, respectively, depending on the number and position of the chlorine atoms (Fig. I.3).

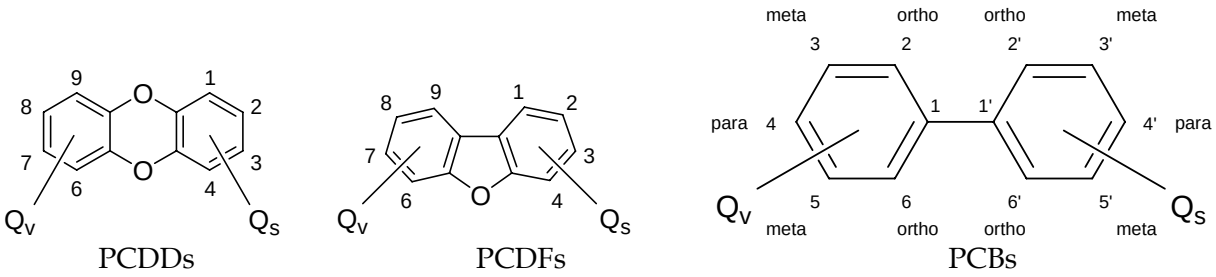


Fig. I.3 Chemical structures of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs); Q_s and Q_v represent chlorine substitution

Only 7, 10, and 12 of the existing PCDD, PCDF, and PCB congeners, respectively, exhibit relevant dioxin-like activity. The subset of 7 PCDDs and 10 PCDFs correspond to chlorine substitution at the 2, 3, 7, and 8 position (hereafter referred to as PCDD/Fs). The 12 dioxin-like PCBs (dl PCBs) included have either one or no chlorine substitution in the ortho position (non-ortho or mono-ortho PCBs) (World Health Organization, 2007).

For these 29 **dioxin-like compounds**, toxic equivalency factors (TEFs) for mammals have been derived (Van den Berg *et al*, 1998; Van den Berg *et al*, 2000; Van den Berg *et al*, 2006). The TEF approach relates the toxicity of these chemicals to that of 2,3,7,8-tetrachlorinated dibenzo-*p*-dioxin. In the TEF concept, the assumption is made that PCDD/Fs and dl PCBs have a common mechanism of action, which involves binding on the aryl hydrocarbon receptor, an intracellular receptor protein. This binding is considered to be the necessary first step in expressing the toxicity of these compounds as well as for the assumption that the

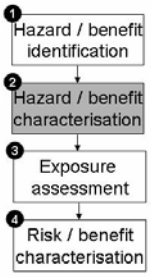
toxic effect of the different congeners is additive. Many uncertainties exist in the use of the TEF approach for human risk assessment, but pragmatically it is the most feasible approach (World Health Organization, 2007). TEFs allow determination of the toxic equivalent (TEQ) concentration of the residue. For a particular residue containing a mixture of *i* PCDDs, *j* PCDFs, and *k* PCBs, the TEQ is calculated according to the following equation (World Health Organization, 2007):

$$TEQ = \sum_i (PCDD_i * TEF_i) + \sum_j (PCDF_j * TEF_j) + \sum_k (PCB_k * TEF_k)$$

with *TEQ* the toxic equivalent (pg/g) of a mixture of *i* PCDDs (pg/g), *j* PCDFs (pg/g), and *k* PCBs (pg/g) all with their respective TEF value (dimensionless). Exposure to a mixture of PCDD/Fs and dl PCBs causes dermal toxicity, immunotoxicity, carcinogenicity, reproductive and developmental toxicity, and disruption of endocrine functions (World Health Organization, 2007).

Besides the twelve dl PCB congeners, the **non dioxin-like (ndl) PCBs** were considered as well. Ndl PCBs also cause multiorgan toxicity, the mechanism of which is not known. Much higher doses are needed than for the dl PCBs, but only very few data on a few individual congeners are available. Much more work has to be done before a similar quantitative risk assessment as for dioxin-like substances can be performed. Data on the occurrence of ndl PCBs in food and feed are often referred to as the sum of six or the sum of seven indicator PCBs (Σ6 iPCBs = congeners 28, 52, 101, 138, 153, and 180 or Σ7 iPCBs= congener 118 + Σ6 iPCBs, with congener 118 being a dl PCB). In this PhD-thesis, the Σ7 iPCBs were considered. In general, Σ6 iPCBs represent at least about 50% of the total amount of ndl PCBs in food (EFSA, 2005c).

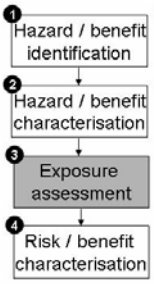
5.2. Hazard and benefit characterisation



Hazard characterisation involves the quantitative description of the level at which a compound has potential to cause adverse effects (based on dose-response relationships). Likewise, benefit characterisation can be defined as the quantitative description of the level at which a nutrient intake is sufficient to meet the requirement of all healthy individuals to stay in good health and to avoid deficiency. Details about dose-response relationships are beyond the

scope of this thesis and are therefore not described. However, two types of quantitative levels are applied in this PhD-thesis to evaluate nutrient and contaminant intakes related to seafood consumption; first, the DRIs for nutrients and second, the tolerable daily or weekly intakes (TDI or TWI) for contaminants. These reference levels are described in more detail in chapter II and IV.

5.3. Exposure assessment



The largest part of the work executed in this PhD-thesis is related to the exposure assessment. The latter has been defined by the WHO as the qualitative and/or quantitative evaluation of the likely intake of biological, chemical or physical agents via food as well as exposure from other sources if relevant (World Health Organization, 1997). In this PhD-thesis, only the exposure via food – and for the risk-benefit analysis only via seafood – is taken into consideration. The term 'exposure assessment' is often replaced by 'intake assessment' when considering only food as exposure route.

Dietary intake of nutrients and contaminants can be estimated in different ways. The most direct estimate is by measuring concentrations in duplicate diets collected over a certain time. This is a technique in which equivalent portions of all foods and beverages consumed by an individual are collected for direct analysis in order to estimate the individual's intake of energy, nutrients, and/or other food components (Cameron & Van Staveren, 1988). However, dietary intake is usually estimated by models combining data on food consumption with concentration data measured in foods and food groups (World Health Organization, 2007). The latter method was applied in this thesis and involved three important steps:

1. the collection of food **consumption** data;
2. the collection of **nutrient and contaminant** data; and
3. an appropriate **method** for combining both sources of data and assessing the intake.

5.3.1. Consumption data

In principle, to assess food consumption four different types of data can be used:

1. food supply data;
2. data from household consumption surveys;
3. data from dietary surveys of individuals;
4. the collection of duplicate diets (Kroes *et al*, 2002).

For the case study investigated in this PhD-thesis, data from **dietary surveys** were most appropriate. Different methods can be applied to assess such data, to be divided in two categories: **record** and **recall** methods.

- Record data collect information on current consumption over one or more days.
- Recall methods reflect past consumption, varying from intake over the previous day (24-hour recall) to usual food intake (food frequency questionnaire or dietary history) (Kroes *et al*, 2002; Willett, 1998).

Four different methods within the category of dietary surveys are distinguished:

(1) **Food records** (also called dietary records or food diaries) are kept for a specified time period, usually 1 to 7 days. If total daily intake is required, the food records should include all foods and beverages consumed at meals and in between, in quantified amount (Kroes *et al*, 2002; Willett, 1998).

(2) In the **24-hour recall** method the subject is asked by a trained interviewer to recall and describe the kinds and amounts of all foods and beverages ingested during the immediate past, mostly a 24- or 48-hour period. Food quantities are usually assessed by using household measures, food models, or photographs (Kroes *et al*, 2002; Willett, 1998).

(3) A **food frequency questionnaire** (FFQ) consists of a structured list of individual foods or food groups. The aim of the FFQ is to assess the frequency with which these items are consumed during a specified period (e.g. daily, weekly, monthly, yearly). FFQs may be qualitative, semi-quantitative or completely quantitative. Qualitative FFQs only obtain the usual number of times each food is eaten during a specified period. Semi-quantitative methods allow estimation of a standard portion or ask respondents to indicate how often they consume a specified common amount. A quantitative FFQ allows the respondent to indicate any amount of food typically consumed. The FFQ is often used to rank individuals

by food or nutrient intakes and also by food group intakes so that high and low intakes may be studied (Kroes *et al*, 2002; Willett, 1998).
(4) With the aid of a **dietary history method**, a trained interviewer assesses an individual's total usual food intake and meal pattern. The respondent is asked to provide information about his/her pattern of eating over an extended period of time (often a typical week) and also to recall the actual foods eaten during the preceding 24 hours. In addition, the interviewer completes a checklist of foods usually consumed. Finally as a cross-check, the respondent is often asked to complete a three-day estimated record (Kroes *et al*, 2002; Willett, 1998).

It should be noted that there is no single ideal method to assess food consumption. The choice depends on the objectives of the study, the foods of primary interest, the need for group versus individual data, the characteristics of the population, the time frame of interest, the level of specificity needed for describing foods, and available resources. Currently, most methods to assess food consumption are not developed explicitly from the perspective of risk assessment and the available data are used for other purposes than the original ones as well (Kroes *et al*, 2002).

5.3.2. Nutrient and contaminant concentration data

A key component in nutrient and contaminant intake assessment is the collection of quantitative data being accurate (i.e. agreeing with the actual concentration) and representative (i.e. reflecting the concentration of the whole group) whether it be nutrients or contaminants. In order to collect data, two different strategies can be applied. **First**, nutrient and contaminant concentrations can be measured in representative samples. Such an approach is scientifically preferable, but very expensive and laborious. Samples can be collected on the base of a representative sampling plan or by the application of a duplicate diet approach or a total diet study. The latter methodology is recommended by the FAO and WHO for estimating dietary exposure of the population. In total diet studies, representative samples of widely consumed foods are collected and analyzed for the constituents of interest (Kroes *et al*, 2002). **Alternatively**, data found in secondary sources like food composition databases (in the case of nutrients) and scientific literature, existing databases, and published reports (in the case of contaminants) can be used.

For the intake assessment executed in this thesis, the second strategy of using secondary data was adopted. This is in accordance with the strategy recently proposed by Brüders *et al* (2005), stating that existing data should be used in the most effective way as collecting samples and analyzing them is expensive. Nevertheless, the comparison of data from different sources is always difficult especially if one only has access to aggregated data without having sufficient information about the collecting scheme, location data, sampling procedure, or laboratory procedures (Brüders *et al*, 2005). Detailed information about the data collection and data handling as applied in this PhD-thesis is reported in chapter III.

5.5.3. Methodologies for intake assessment

In its simplest form, the model to represent dietary intake/exposure can be considered as:

Consumption x (Concentration or Residue) = Dietary exposure.

There are, however, three different approaches for combining the consumption data with nutrient/contaminant concentrations: deterministic modelling, simple distribution, and probabilistic modelling. Both the simple distribution and probabilistic approach are applied in this PhD-thesis.

5.5.3.1. Deterministic modelling

Deterministic modelling involves using a point estimate of each variable within the model (Vose, 1996). In the context of intake assessment, the term 'deterministic modelling' refers to a method whereby a fixed value for food consumption (such as the average or high level consumption value) is multiplied by a fixed value for the concentration and the intakes of all sources are then summed. In a schematic way, it can be summarized as follows:

μ(X)	x	μ(C)	=	μ(Y)	*Mean scenario*
Max(X)	x	Max(C)	=	Max(Y)	*Worst case scenario*

With X= consumption of a certain food item or food group, C= concentration of the considered compound in that food item, Y= exposure to the considered compound via the considered food item or food group.

Deterministic modelling is commonly used as a first step in exposure assessment because it is relatively simple and inexpensive to carry out. However, this approach does not provide insight into the range of possible exposures that may occur in a population (Kroes *et al*, 2002; Lambe, 2002) and it also obscures the ability to determine which scenarios present a risk that is likely to occur (Petersen, 2000).

5.5.3.2. Simple distribution

'Simple distribution' is a term used to describe a method that employs distributions of food intake but uses a fixed value for the nutrient and contaminant concentration variables (Fig. I.4).

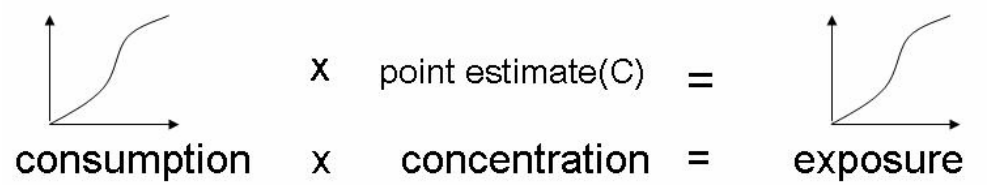


Fig. I.4 Scheme of the simple distribution approach

The results are more informative than those of the deterministic approach because they take into account the variability that exists in food consumption patterns (Kroes *et al*, 2002; Lambe, 2002). This approach is widely used for the intake assessment of macro- and micronutrients, where one value is used to represent the content of each nutrient in each food. This methodology was applied in this thesis to assess the intake of PUFAs via the total diet for different subgroups of the Flemish population and to assess the vitamin D intake of Flemish adolescents (chapter II).

5.5.3.3. Probabilistic modelling

In contrast to the deterministic approach, probabilistic modelling involves incorporation of the variability and/or uncertainty for the different parameters. As such, it takes into account all the possible values that each variable could take and weights each possible model outcome by the probability of its occurrence (Vose, 1996) (Fig. I.5).

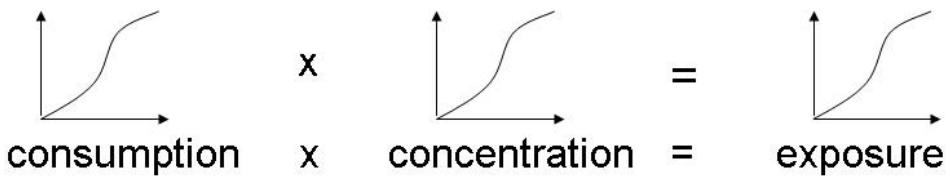


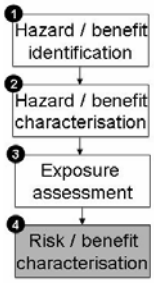
Fig. I.5 Scheme of the probabilistic approach

Food consumption and nutrient/contaminant concentration data can be entered in probabilistic models by two different approaches: non-parametric or parametric. The choice of the approach depends largely on the data resources available.

- In the **non-parametric** approach all the individual data are used as such, without assuming any underlying probability model.
- In the **parametric** approach probability distributions are fitted to the available data. This process involves making assumptions about the underlying mathematics of the distribution. In a next step, random values are drawn from these distributions and used as input for the mathematical model which describes the intake assessment process (Lambe, 2002).

The probabilistic analysis for intake assessment, requiring appropriate modelling software, permits the exposure assessor to consider the whole distribution of exposure, from minimum to maximum, with all modes and percentiles (Kroes *et al*, 2002). A probabilistic approach was applied in this thesis to assess the intake of multiple compounds of interest via seafood consumption (chapter IV). In this PhD-thesis, only variability (not uncertainty) was taken into account in the probabilistic intake assessment.

5.4. Characterisation of risks and benefits



Risk characterisation is the process of comparing the modelled estimates to toxicological endpoints and establishing the relevance for human health. In this PhD-thesis, a benefit characterisation was elaborated by comparing the assessed nutrient intakes with nutritional recommended endpoints. The results of this evaluation are described in chapter IV.

6. The public health relevance of this work

In very general terms, public health is the science that deals with optimisation of the health and wellbeing in the community at large, operating through organised societal efforts and diverse structural measures, including regulatory initiatives, health promotion campaigns, educational efforts, etc. Within this broad scientific area, both nutritional and environmental issues are important determinants and therefore deserve continued attention from scientific and from policy perspective.

A very important aspect within the broad instrumentarium of public health research is the assessment of 'exposure' in the general population and the development of algorithms that allow for the interpretation of such exposures in a broader context of potential health risk. This PhD-thesis is situated at the crossroads of nutritional and environmental epidemiology as it merges know-how from both areas and combines them in an attempt to optimise the understanding of their impact on public health. For example, some important topics that were handled within this PhD-thesis are:

- data management work leading to extensive databases relevant in exposure assessment (see chapter III),
- set up of a methodology and software module for probabilistic exposure assessment (chapter IV), and
- scenario analyses to evaluate the impact of certain dietary interventions (chapter IV).

The 'public health policy cycle' is an important concept that has been designed as a helpful tool when developing a strategy to tackle a public health problem. This cycle can – somewhat artificially - be broken down into seven different steps (Margetts, 2004):

1. Identify key nutrition-related problem
2. Set goal
3. Define objectives for goal
4. Create quantitative targets
5. Develop program
6. Implement program
7. Evaluate program

The work in this thesis is predominantly focusing on steps 1 and 7 and can also be of help in the development of interventional strategies.

Caraher & Coveney (2004) argue moreover that public health nutrition, through the medium of health promotion, needs to address the wider issues of who controls the food supply and

thus the influences on the food chain and the food choices of the individual and communities. Therefore, food policy should seek to make the social infrastructure conductive to health decisions about food (Caraher & Coveney, 2004). In the general discussion of this PhD-thesis (chapter V) some of these wider issues are discussed.

7. Outline of the thesis

Chapter II describes the intake of PUFAs via the total diet assessed for different subgroups of the Flemish population: Flemish preschool children, adolescents from the Ghent region, and young women from the Ghent region. Some thoughts relevant to the whole Belgian population are added in the discussion part of this chapter. Chapter II gives an indication on the contribution of seafood to the total intake of LC n-3 PUFAs and the overall adequacy of the PUFA intake compared to the recommendations. For the adolescent population, the importance of seafood as vitamin D source is described as well.

It is important to mention that chapter II of the thesis differs from the other parts in the way that for the analyses described in this chapter the total diet was taken into account (so no focus on seafood only). Furthermore, the intake was measured with the simple distribution approach whereas further on in the thesis a probabilistic approach was used to calculate nutrient and contaminant intakes via seafood consumption only.

Chapter III focuses on different important methodological aspects encountered during the setup of the different databases needed for the probabilistic intake assessment of nutrients and contaminants via seafood only. It describes the process of collecting data on the origin, the nutrient concentration, and the contaminant concentration of seafood available on the Belgian market.

Chapter IV describes the results of the probabilistic intake assessment of nutrients and contaminants via seafood consumption only. First, the current intake based on existing food consumption data for the Belgian population was described, followed by an evaluation of the benefits and risks related to the assessed intakes. Second, different consumption scenarios were considered in order to find out whether it is possible to meet the current

recommendation for LC n-3 PUFA intake by the consumption of seafood only, without increasing the intake of contaminants to levels of toxicological concern.

Important discussion points related to the subject of this thesis (i.e. seafood consumption and intake of n-3 PUFAs) but not investigated in detail in the discussion parts of the individual chapters are described in **chapter V**, for instance the intake of dioxin-like compounds via the total diet, consumers' perception related to seafood, the importance of fortified foods and supplements, and sustainable aspects related to fisheries.

Chapter II.

Overall dietary PUFA intake and importance of seafood as PUFA and vitamin D source

This chapter is based on the following papers:

1. Sioen I, Pynaert I, Matthys C, De Backer G, Van Camp J, De Henauw S. Dietary intakes and food sources of fatty acids for Belgian women, focused on n-6 and n-3 polyunsaturated fatty acids. *Lipids* 2006; **41**(5): 415-422.

2. Sioen I, Matthys C, De Backer G, Van Camp J, De Henauw S. Importance of seafood as nutrient source in the diet of Belgian adolescents. *Journal of Human Nutrition and Dietetics* 2007; In Press.

3. Sioen I, Huybrechts I, Verbeke W, Van Camp J, De Henauw S. Omega-6 and omega-3 poly-unsaturated fatty acid intakes of pre-school children in Flanders-Belgium. *British Journal of Nutrition* 2007; **98**(4): 819-825.

1. Introduction

Despite the potential favourable health effects, it has been repeatedly reported that modern diets in developed countries are low in omega-3 poly-unsaturated fatty acids (PUFAs) (Muskiet *et al*, 2006; Simopoulos, 2002). The diet in developed countries has evolved from a diet rich in α-linolenic acid (LNA) and long chain (LC) PUFAs to a modern Western diet in which linoleic acid (LA) is the main PUFA (Ruxton *et al*, 2004; Sontrop & Campbell, 2006). This is mainly due to intensified agricultural production processes and the increased consumption of margarines and food items containing vegetable oils that are rich in LA. A result of this dietary evolution is an increased ratio of omega-6 (n-6) over omega-3 (n-3) PUFAs, which has been suspected to promote the pathogenesis of many diseases, including cardiovascular and inflammatory diseases (Simopoulos, 2002).

In order to develop appropriate interventional strategies at the population level, it is important first to quantify the differences between current dietary intakes and recommendations (American Heart Association *et al*, 2006). Therefore, it was of interest to accurately estimate the current intakes of individual PUFAs of different subgroups of the Belgian population. Moreover, this PhD-thesis assesses quantitatively the seafood contribution to the total intake of LC n-3 PUFAs and vitamin D, based on available food consumption data.

The investigations described in this chapter focus on:

1. The dietary PUFA intake of **Flemish pre-school children,** the food sources that contribute to the intake of the individual n-6 and n-3 PUFAs as well as potential ways to bridge the gaps between current intakes and recommended levels. To the authors' knowledge, it was the first time that individual PUFA dietary intakes of pre-school children in Europe were described. In the past, only individual PUFA intake data of Australian, Canadian, and Chinese children have been published (Barbarich *et al*, 2006; Innis *et al*, 2004; Meyer *et al*, 2003).

2. The importance of seafood consumption as nutrient source in the diet of **Flemish adolescents**. The intake of individual n-6 and n-3 PUFAs as well as vitamin D was investigated in detail, the food sources of the considered nutrients were studied and it was

explored whether increased seafood consumption can be a solution for low intake of LC n-3 PUFAs and vitamin D.

3. The total intake of n-6 and n-3 PUFAs and the main food sources of these fatty acids in a population sample of **Flemish young women**, i.e. women of childbearing age**.**

For this investigation, available Flemish data were used. The results were enriched with data of the Belgian Health Interview Survey (Scientific Institute of Public Health, 2006) and the Belgian Food Consumption Survey (De Vriese *et al*, 2006) in order to generalise the results to the Belgian population.

2. Materials and methods

2.1. Population sample

2.1.1. Pre-school children

The first studied population included pre-school children living in Flanders (the northern, Dutch-speaking region of Belgium that includes approximately 60% of the total Belgian population). Representative samples of Flemish pre-school children aged 2.5 to 6.5 years were selected on the basis of random cluster sampling at school level, stratified by province and age. Details about the sampling strategy and the representativeness of the study sample have been described by Huybrechts *et al* (2006; 2007). A total of 2095 children were enrolled in this study. Their parents were asked to complete a food frequency questionnaire (FFQ), a structured three-day estimated diet record (EDR), and a general questionnaire on socio-demographic background, family composition, and child characteristics. Only the EDR data were used to assess the PUFA intake, since the FFQ was developed to assess calcium intake (Huybrechts *et al*, 2006).

Overall, 1052 subjects returned an EDR completed between October 2002 and February 2003. Only EDRs containing sufficiently detailed descriptions of the food products and the portion sizes consumed were used for analysis. The EDRs of 26 children were excluded due to missing data. Of the 1026 remaining subjects, 696 completed three-day diaries, 208 completed two-day diaries, and 122 completed only a one–day diary (Huybrechts *et al*, 2006; Huybrechts & De Henauw, 2007). In this study, only the data of children for whom three-day

diaries were available were used, since data from one day do not provide an accurate picture of usual intake on the individual level, due to within-individual diet variation (Willett, 1998). Since gender or age information was missing for 35 children, the EDRs of 661 children (338 boys and 323 girls) were included in the analyses.

2.1.2. Adolescents

The second subgroup of the population included Flemish adolescents. The survey was based on a sample of 341 adolescents (129 boys and 212 girls), aged 13–18 years, from the region of Ghent (± 250,000 inhabitants), a city in Flanders. Their food consumption data were collected between March and May 1997 (Matthys *et al*, 2003). Different educational options of the adolescents – 'classical' education (mainly theoretical courses) and vocational training (based on practical skills) - were represented in the sample. A seven-day EDR (semi-structured diary) was used to collect food consumption data. Instructions for the completion of the diary and regular checks for quality and completeness of the diaries were carried out by experienced dieticians. These data are the most recent available food consumption data on a seven-day basis for Flemish adolescents.

2.1.3. Young women

The data source for the Flemish young women is a large epidemiological survey that included women aged 18 to 39 years. A number of 4000 women were randomly selected from the population register of Ghent, in Flanders. They were invited to participate in the study by postal mail and were asked to reply by use of an enclosed postcard. Not less than 2634 subjects declined the invitation to participate and 424 invitation letters were declared undeliverable by postal service. Women who were pregnant, who had moved to another city, who did not speak Dutch, who were unable to come to the research centre, or who were unable to volunteer within the planned period of the fieldwork, were excluded from the study. In total, dietary data were collected for 641 women. The primary objective of the survey was to evaluate food and nutrient intake (in particular iron) in a group of Flemish women at reproductive age. Dietary assessment was done on the basis of a two-day EDR, using diaries with a semi-structured, open-entry format. In addition, a general socio-demographic questionnaire was administered. Data were gathered during the year 2002 (January 29th until December 22nd), thereby covering the different seasons. Only the data from the two-day EDR are used in this study for assessing the intake of total fat and important dietary fatty acids.

The three studies were approved by the Ethical Committee of the Ghent University Hospital. Additional information about the pre-school children, adolescent, and young women population has been previously published by Huybrechts *et al* (2006; 2007), Matthys *et al* (2003; 2006), and Pynaert *et al* (2005), respectively. Experienced dieticians used a standardized protocol, including a manual on household weights and measures to convert the estimated amounts in the EDRs into weights (Belgian Health Council, 2005). None of the individuals included in the studies reported the intake of supplements containing PUFAs.

2.2. Food composition database

The total fat content of the foods present in the food consumption databases of the three studied population groups was obtained from the Belgian and Dutch food composition database (FCDB) (NEVO Foundation, 2001; NUBEL, 1999). In the food consumption database of the Flemish pre-school children, 726 of the 936 food items (77.6%) contained fat. In the database of the adolescents and young women this was 70.6% (527 of the 745 food items) and 75.1% (700 of the 1063 food items), respectively.

To determine the **PUFA** concentration of the food items, a specific FCDB was developed that included the concentrations of linoleic acid (LA), α-linolenic acid (LNA), arachidonic acid (AA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA) as they were found in international literature sources. **Eight** publicly available FCDBs were used, and, in order of the number of items that were used, they were:

1. the Dutch FCDB (NEVO Foundation, 2006);
2. an extended French FCDB (Astorg *et al*, 2004);
3. the USDA National Nutrient Database (US Department of Agriculture and Agricultural Research Service, 2005);
4. the British McCance & Widdowson's FCDB (Food Standards Agency, 2002);
5. the Finnish FCDB (National Public Health Institute of Finland, 2004);
6. the Danish FCDB (Danish Institute for Food and Veterinary Research, 2005);
7. the Canadian Nutrient File (Health Canada, 2005); and
8. the German FCDB (Souci *et al*, 2000).

Furthermore, food composition information was obtained from food producers for specific food items, i.e. margarine, cheese, and dressings. For 46 composite food items, the fatty acid

(FA) composition was calculated using local recipes describing the different ingredients and their proportions, as well as the FA composition of the ingredients as found in one of the FCDBs. Detailed FA profiles of the fat-containing food items were then calculated by calculating the proportional share of each FA in the total fat content as reported in the newly compiled database and applying this proportional share to the total fat content of the food as listed in the Belgian and Dutch FCDB (NEVO Foundation, 2001; NUBEL, 1999).

For **vitamin D**, data of the Dutch FCDB were used (NEVO Foundation, 2001), completed with data from the Danish FCDB (Danish Institute for Food and Veterinary Research, 2005) describing the vitamin D concentration in five seafood items for which data were lacking in the Dutch data source. In total, 307 of the 745 food items (41.2%) in the adolescent food consumption database contained vitamin D.

2.3. Statistics

All statistical analyses were done using SPSS software version 12.0 (SPSS, Inc., Chicago, IL, USA). The average PUFA intakes were expressed in absolute amounts ((m)g/day) and in percentage of the total energy intake (%E). Normality of the intake distributions was tested using the Kolmogorov-Smirnov test ($p < 0.01$). A Mann-Whitney-U test was used to determine differences in vitamin D (only for adolescents) and PUFA intakes between boys and girls in the dataset of the pre-school children and the adolescents, as well as between different age groups in the pre-school children dataset ($p < 0.01$).

Percentages of individual PUFAs provided by the different foods were calculated as population proportions, as defined by Krebs-Smith *et al* (1989). The **population proportion** was calculated by summing the amount of a FA from a certain food item for all individuals and then dividing this number by the sum of that FA from all food items for all individuals. The formula for determining the population proportion is:

$$Population\ proportion = \frac{\sum_{i=1}^{n} F_i}{\sum_{i=1}^{n} T_i}$$

Where F_i = the amount of the fatty acid contributed by the particular food group for the i^{th} individual,

T_i = the total amount of the fatty acid from all food for the i^{th} individual,

n = the number of individuals in the total study population.

Using the adolescent food consumption data, the percentages were also calculated as mean proportions. The **mean proportion** of a FA from a certain food item for all individuals is determined by first calculating the contribution of a food item to the intake of that FA for each person and then taking an arithmetic mean of all proportions (Krebs-Smith *et al*, 1989). The formula for determining the mean proportion is:

$$Mean\ proportion = \frac{\sum_{i=1}^{n}(F_i / T_i)}{n}$$

The food items were grouped in 36 subgroups and 8 major groups, according to Astorg *et al* (2004). A Mann-Whitney-U test was used to look for significant differences between boys and girls in the contributions of different food groups to the nutrient intake (calculated as mean proportion).

2.3.1. Pre-school children

The average PUFA intakes were calculated as the mean of the three-day period. The usual PUFA intakes were computed using the NUSSER-method, a statistical method developed at Iowa State University, based on the advice of the American Institute of Medicine (IOM) with respect to the need to determine the distribution of usual nutrient intakes when assessing diets of population groups in relation to the recommended levels (Institute of Medicine, 2005). The NUSSER-method estimates the usual intake distributions by accounting for within-individual variation in nutrient intakes, while requiring relatively few days of intake data per individual (Nusser *et al*, 1996). C-side software was used for the NUSSER-method (Iowa State University, 2006). Moreover, exploratory calculations were done to assess the amount of seafood that should be consumed by the pre-school children to achieve the recommended amount of LC n-3 PUFAs.

2.3.2. Adolescents

The average vitamin D and PUFA intakes were calculated as the mean of the seven-day period. For this subpopulation, no extra analyses were done to account for the within-individual variation. In contrast, some additional analyses were done to study the importance of seafood as a nutrient provider of vitamin D (in µg/d) and EPA plus DHA (in mg/d). The study population was divided in tertiles based on the intake of these considered nutrients. This division was done separately for boys and girls. A Kruskal-Wallis test was used to test the importance of the different food groups between the tertiles ($p < 0.01$).

2.3.3. Young women

The average intakes of individual PUFAs of the group of young women were calculated based on the mean of two days per individual, without correction for within-individual variation.

2.4. Evaluation of the assessed intakes

To evaluate the intakes of the individual PUFAs and vitamin D (only assessed for adolescents), the recommendations formulated by the Belgian Health Council were applied (Belgian Health Council, 2007). The recommendations for **PUFAs** for children older than three years of age on the one hand and for adults on the other hand are given in Table II.1, expressed in %E. The PUFA recommendations for adults were applied to evaluate the intakes of adolescents and young women. For **vitamin D**, the Belgian Health Council has formulated a recommended daily intake range for adolescents and adults equal to 2.5 to 10 µg.

Table II.1: Recommendations for PUFAs for children older than three years of age and adults (expressed as percentage of the total energy intake (%E)) (Belgian Health Council, 2007)

	Recommendations (%E)	
	Children older than three years of age	Adults
Total PUFAs	> 8.0	5.3 - 10.0
LA	2 - 5	> 2.0
AA	0.10 - 0.25	-
Total n-6 PUFAs	-	4.0 – 8.0
LNA	0.45 - 1.50	> 1.0
EPA	0.05 - 0.15	-
DHA	0.10 - 0.40	-
EPA + DHA	-	> 0.3
Total n-3 PUFAs	-	1.3 – 2.0

3. Results

3.1. Seafood consumption

Being of special interest in this PhD-thesis, the consumption of total seafood for the three studied population groups is reported here. Most details are provided on the seafood consumption data of the adolescents, since these data were used further on in the evaluation of risk and benefits related to seafood consumption (chapter IV). Moreover, for the two other population groups, consumption data were only available on two or three days, which is not sufficient to give a good picture of episodically consumed food items such as seafood.

For the **pre-school children** it was found that only 207 of the 661 (31.3%) children did consume seafood during the three days included in the study, with only 50 children consuming fatty fish (salmon being the most important species). The mean seafood consumption over the three days was 8.6 g/d for the total population sample and 27.4 g/d for the subsample who consumed seafood.

The seafood consumption of the total group of studied **adolescent population** and the seafood consumers only is summarized in Table II.2. From the 341 respondents, 63.9% did consume seafood during the week of the study, respectively 81 boys and 137 girls (Table II.2). The weekly amount of seafood consumption for boys was higher than for girls, but not significantly different. The mean seafood eating occasions per week was 1.14, with a maximum of five times a week. In total, 32 different seafood species and two seafood products (caviar and surimi) were consumed by the adolescents. The most important species

were cod, saithe & Alaska Pollack, and salmon, accounting for more than half of the amount of seafood consumed.

Table II.2: Seafood consumption of the studied adolescent population (g/week) and the subpopulation of seafood consumers only

	Seafood consumption (g/week)					
	Whole population			Seafood consumers only		
	All (n=341)	Boys (n=129)	Girls (n=212)	All (n=218; 64%)	Boys (n=81; 63%)	Girls (n=137; 65%)
Mean	106.8	119.5	99.0	167.0	190.2	153.3
25th percentile	0.0	0.0	0.0	65.0	80.7	50.0
Median	55.0	70.0	48.8	148.0	150.0	125.0
75th percentile	183.0	184.5	184.0	221.0	226.3	219.5

Only 301 of the 641 (47.0%) of the **Flemish women** in the dataset consumed seafood during the two-day study period. The average seafood consumption (calculated as a mean of the two days) was 27.0 g/d for the whole group and 57.5 g/d for the seafood consumers only.

3.2. Assessed PUFA intakes

The intakes of LA, LNA, AA, EPA, DHA, total n-6 PUFA, and total n-3 PUFA for Belgian pre-school children, adolescents, and young women and the corresponding recommendations are shown in the figures below (Fig. II.1-3). The intakes as presented in the histograms were not corrected for within-individual variation.

Fig. II.1 shows the intake of **LA and LNA** of the three studied population groups. The resulting LA over LNA ratio is quite high: being 9.1 for the pre-school children and adolescents and 8.7 for the young women.

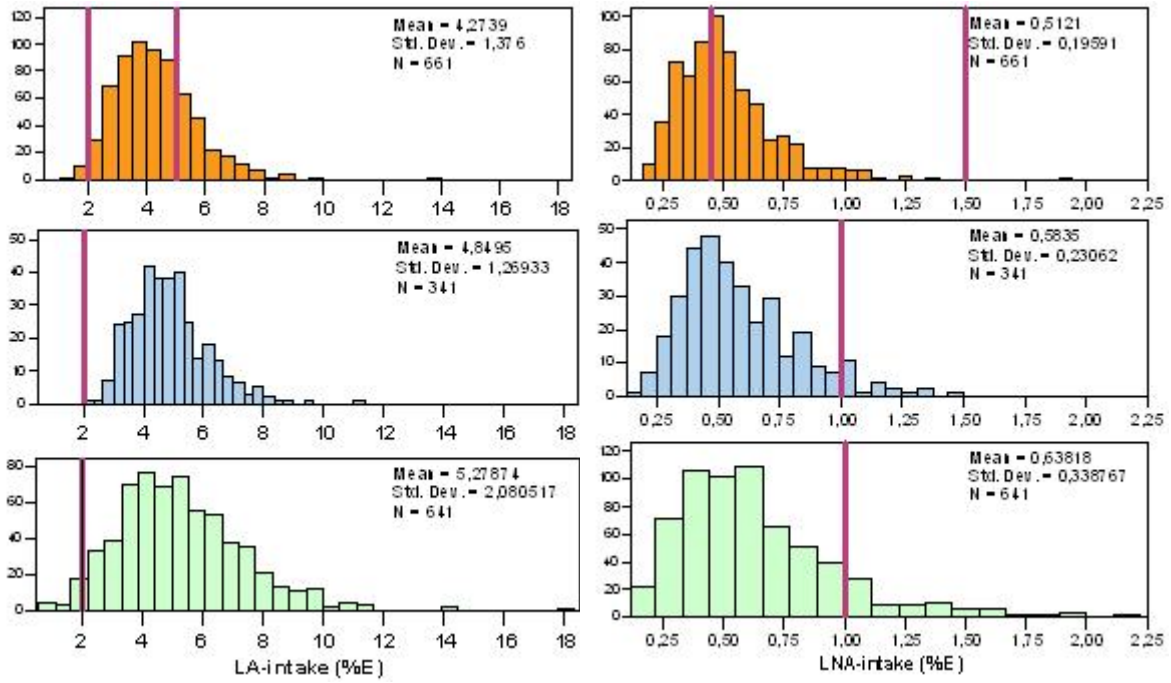


Fig. II.1 Histograms of the LA (left) and LNA (right) intake of pre-school children (orange), adolescents (blue), and young women (green), as well as the recommended intake (red), expressed as percentage of the total energy intake (%E)

Fig. II.2 shows the intake of **AA** only for the pre-school children as well as the intake of the sum of **LA and AA** (further referred to as total n-6 PUFA intake or **Σn-6PUFA**) for the three different subpopulations studied. The corresponding recommendation is also indicated in the histograms, apart of the recommendation for total n-6 PUFA intake for pre-school children because it is not specified by the Belgian Health Council (Table II.1). In general, the contribution of AA to the ∑n-6PUFA was very low.

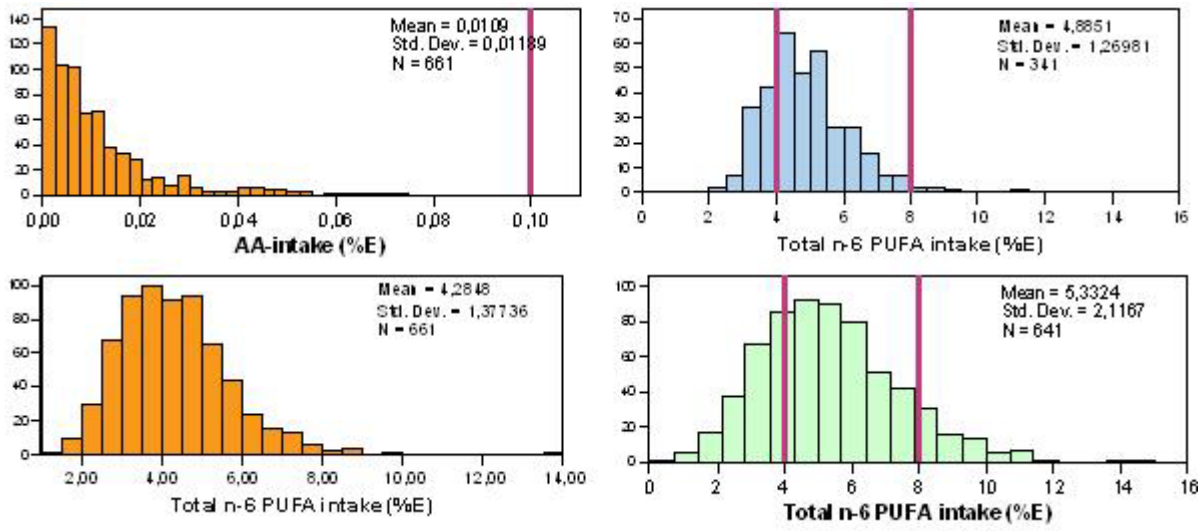


Fig. II.2 Histograms of the AA (for pre-school children only) and Σn-6PUFA (LA+AA) intake of pre-school children (orange), adolescents (blue), and young women (green), as well as the recommended intake (red), expressed as percentage of the total energy intake (%E)

In Fig. II.3 the intake of **EPA and DHA** is represented separately for the pre-school children, since a separate recommendation was formulated for both fatty acids. In contrast, for adolescents and young women the intake is expressed as the sum of both fatty acids to permit an easy comparison with the recommendations.

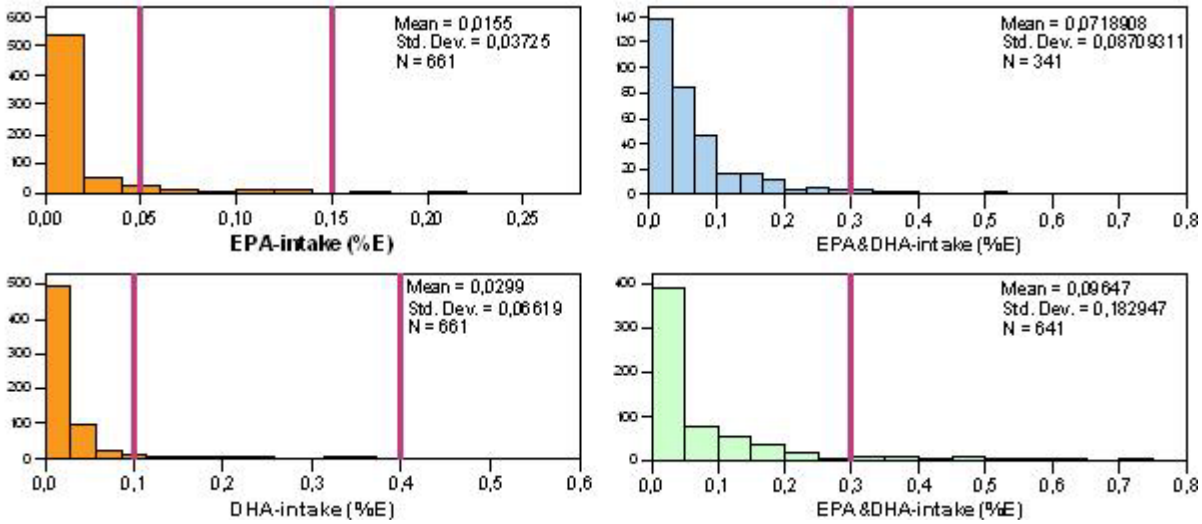


Fig. II.3 Histograms of the intake of EPA and DHA separately for pre-school children (orange) and the sum of EPA and DHA for adolescents (blue) and young women (green), as well as the recommended intake (red), expressed as percentage of the total energy intake (%E)

Fig. II.4 shows the intake of the sum of **LNA, EPA, DPA, and DHA** (further referred to as total n-3 PUFA intake or **Σn-3PUFA**) for the adolescents and the young women. This was not shown for the pre-school children because no recommended intake was specified for them.

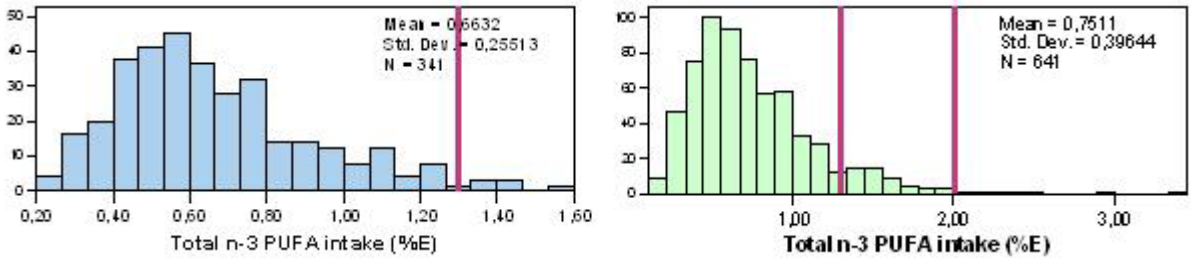


Fig. II.4 Histograms of the Σn-3PUFA (LNA+EPA+DPA+DHA) intake for adolescents (blue) and young women (green), as well as the recommended intake (red), expressed as percentage of the total energy intake (%E)

Table II.3 shows the mean **Σn-6PUFA/Σn-3PUFA ratio** of the three studied subpopulations.

Table II.3: The mean Σn-6PUFA/Σn-3PUFA ratio for the three studied subpopulations

	Mean Σn-6PUFA/Σn-3PUFA ratio
Pre-school children	8.5
Adolescents	8.1
Young women	7.8

In most cases, the PUFA intake distributions of the intakes were skewed to the right. Moreover, a considerable part of the studied population groups had during the study period an intake of LC PUFAs equal to zero. **Normality** was tested for the intake distributions expressed in mg/day as well as in %E. In the group of pre-school children, only the intake distribution of LA and ∑n-6PUFA expressed in %E, of the LA/LNA ratio, and of the ∑n-6PUFA/∑n-3PUFA ratio were normally distributed. For the adolescents, only the intake distributions of LA and ∑n-6PUFA expressed in mg and in %E, of LNA expressed in mg, and of the ∑n-6PUFA/∑n-3PUFA ratio seemed to be normally distributed. In the group of young women, only the intake distributions of LA and ∑n-6PUFA expressed in %E were normally distributed.

3.2.1. The PUFA intakes of the pre-school children: additional results

The **NUSSER-method** applied to assess the usual PUFA intakes of the pre-school children turned out to be only applicable for LA, LNA, Σn-6PUFA, and Σn-3PUFA, but not for individual LC PUFAs: AA, EPA, DPA, and DHA (data not shown). The reason is that the intake of these PUFAs over the three days was equal to zero for many children (Fig. II.2 and II.3). For none of the calculated PUFA intakes significant differences were found between boys and girls, neither between the children younger and older than four years of age.

Exploratory calculations were performed to assess the amount of seafood that should be consumed to achieve the recommended amount of LC n-3 PUFAs. Starting from the Belgian nutrient recommendations for EPA and DHA and a mean energy intake of 6543 kJ for boys and 5757 kJ for girls (Huybrechts & De Henauw, 2007):

- the EPA intake has to be 608-1824 and 535-1605 mg/week, respectively for boys and girls,
- the DHA intake has to be 1216-4865 and 1070-4281 mg/week, respectively for boys and girls.

Based on the food composition data, it was calculated that two portions of 50 gram fatty fish (e.g. mackerel, sardines, salmon) per week are needed to fulfil the requirements, assuming that seafood is the only source of EPA and DHA. On the basis of the three-day EDRs, it was found that only 50 of the 661 children consumed fatty fish (salmon being the most important specie).

3.2.2. The vitamin D and PUFA intakes of the adolescents: additional results

The adolescents' intakes of **vitamin D** (in µg/day as in µg/kJ/day) are given in Table II.4, separately for boys and girls.

Table II.4: Intake of vitamin D (µg/day and µg/kJ/day) for Flemish adolescents

	Mean	5th percentile	Median	95th percentile
Vitamin D ♂ (µg/d)	4.0	1.8	3.6	7.6
Vitamin D ♂ (µg/kJ/d)	0.37	0.19	0.35	0.64
Vitamin D ♀ (µg/d)	2.5	1.3	2.5	4.9
Vitamin D ♀ (µg/kJ/d)	0.35	0.18	0.33	0.56

None of the vitamin D intake distributions were normally distributed. The vitamin D intake was significantly higher for boys compared to girls when expressed in µg/day; after dividing the vitamin D intake by the energy intake, no significant difference was found between both genders.

For the **PUFA intakes**, significant differences were found between boys and girls for LA, LNA, AA, ∑n-6PUFA, and ∑n-3PUFA when expressed in g/d, with the intakes of the boys higher than of the girls (data not shown). When comparing the intakes expressed as %E no significant differences were detected. This is explainable by the correction made by using the energy intake that was significantly higher in boys (10625 kJ) as compared with girls (8030 kJ) (Matthys *et al*, 2003).

3.3. Food sources of the individuals PUFAs

3.3.1. The PUFA food sources for the pre-school children

Fig. II.5 shows the **dietary sources** of the different PUFAs for the Flemish pre-school children calculated as population proportions.

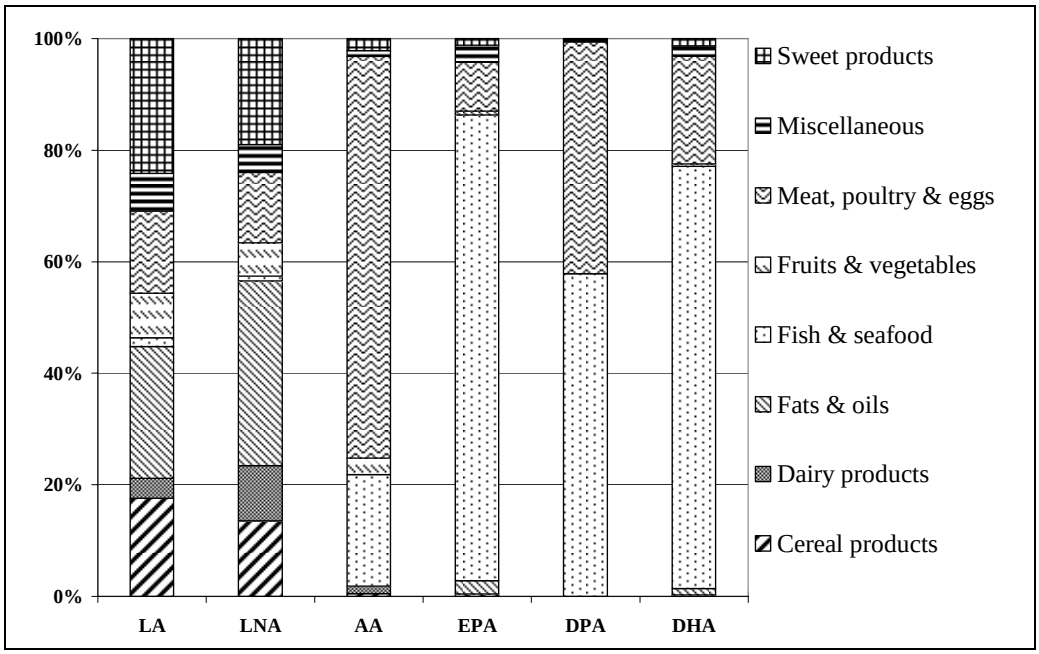


Fig. II.5 Food sources of the different PUFAs for the studied pre-school children (% of the total intake of each FA brought by each food group for the whole population. The group 'Miscellaneous' contains salty & other snacks, soy drinks, vegetarian substitute, and spices & condiments)

- It is remarkable that the contribution of sweet products (including biscuits, chocolate products, pastry & desserts, and sugar & sweets) to the **LA intake** was as high as the contribution of fats & oils (including fatty sauces, margarines, vegetable oils, and mixed fats). Within the group of sweet products, the most important contributor was chocolate products (11.6%), followed by biscuits (8.3%). Two other important LA contributors were cereal products (with bread as most important contributor) and meat, poultry & eggs.
- The contribution pattern of the different food groups for **LNA** was quite similar to the one found for LA, with almost one third from the consumption of fats & oils, with margarines being the most important subgroup (Fig. II.5).
- The **AA intake** was mainly contributed by meat, poultry & eggs (32.6% by poultry and 34.3% by meat & meat dishes). The second important contributor was fish & seafood (11.1% from lean fish and 7.4% by molluscs & crustaceans).
- For the **LC n-3 PUFAs**, fish & seafood was the major source, with fatty fish being the most important subgroup (a contribution of 53.4%, 42.8%, and 48.1% for EPA, DPA, and DHA, respectively). A substantial part of the DPA intake came from poultry (23.5%) and meat & meat dishes (18.1%). Owing to fortification of margarines and the use of eggs in the preparation of certain sweet products and snacks, these products also contributed to the LC n-3 PUFA intake (a contribution of 1.2%, 0.1%, and 1.3% by sweet products and of 1.5%, 0.5%, and 1.9% by snacks for EPA, DPA, and DHA, respectively).

3.3.2. The vitamin D and PUFA food sources for the adolescents

Table II.5 shows the proportional contribution of the different food groups to **vitamin D** and **PUFA** intake of the studied adolescents, calculated as population proportions.

Table II.5: Contribution of food groups to vitamin D (VitD), n-6 and n-3 PUFA intakes (% of the total intake of each FA brought by each food group for the whole population) for the Flemish adolescents

Food groups	VitD	LA	LNA	AA	EPA	DPA	DHA	∑n-6PUFA	∑n-3PUFA
Bread & rusks	0.12	18.21	12.97	0.00	0.00	0.00	0.00	18.08	11.45
Breakfast Cereals	0.00	0.51	0.21	0.00	0.00	0.00	0.00	0.51	0.18
Cereal based dishes	1.30	1.25	1.77	0.75	0.00	0.00	0.17	1.24	1.57
Pasta, rice & other cereals	0.73	1.77	1.12	0.32	0.02	0.00	0.00	1.76	0.99
Total cereal products	**2.16**	**21.74**	**16.06**	**1.07**	**0.02**	**0.00**	**0.17**	**21.59**	**14.18**
Butter	0.69	0.16	0.64	0.68	0.00	0.00	0.00	0.16	0.56
Cheese	4.90	1.02	3.56	2.22	0.00	0.00	0.00	1.03	3.14
Cream	0.34	0.05	0.17	0.00	0.00	0.00	0.00	0.05	0.15
Milk	0.82	0.37	1.08	0.42	0.00	0.00	0.00	0.37	0.95
Yogurts	0.24	0.05	0.16	0.02	0.00	0.00	0.00	0.05	0.14
Total dairy products	**7.00**	**1.65**	**5.60**	**3.34**	**0.00**	**0.00**	**0.00**	**1.66**	**4.94**
Fatty sauces	0.44	10.54	32.86	0.00	0.44	0.00	0.00	10.47	29.01
Margarines	36.43	19.23	15.25	0.00	0.00	0.00	0.00	19.09	13.45
Mixed fats	0.02	5.15	0.08	0.02	0.00	0.00	0.00	5.11	0.07
Vegetable oils	0.00	8.55	0.82	0.00	0.00	0.00	0.00	8.49	0.73
Total fats & oils	**36.89**	**43.47**	**49.00**	**0.02**	**0.44**	**0.00**	**0.00**	**43.16**	**43.25**
Fatty fish	8.28	0.21	0.44	1.23	42.36	40.68	29.87	0.21	4.47
Fish products	1.45	0.21	0.07	0.47	4.62	2.28	4.02	0.21	0.54
Half-fatty fish	0.58	0.03	0.05	0.73	4.28	3.51	9.39	0.04	0.90
Lean fish	2.57	0.01	0.03	2.09	17.17	8.51	16.56	0.02	1.90
Molluscs & crustaceans	0.27	0.01	0.03	3.16	15.68	4.29	5.55	0.03	1.02
Total fish & seafood	**13.15**	**0.46**	**0.62**	**7.68**	**84.11**	**59.27**	**65.40**	**0.51**	**8.83**
Fruits	0.00	0.03	0.31	0.00	0.00	0.00	0.00	0.03	0.28
Legumes	0.00	0.02	0.13	0.00	0.00	0.00	0.00	0.02	0.12
Nuts & seeds	0.00	1.42	0.45	0.00	0.02	0.00	0.00	1.41	0.40
Potatoes	12.72	0.06	0.21	0.00	0.00	0.00	0.00	0.06	0.18
Soups	0.39	0.10	0.06	0.03	0.00	0.00	0.00	0.10	0.05
Vegetables	0.00	0.19	0.68	0.02	0.06	0.00	0.00	0.19	0.60
Total fruits & vegetables	**13.11**	**1.83**	**1.84**	**0.04**	**0.08**	**0.00**	**0.00**	**1.81**	**1.63**
Eggs	5.25	1.29	0.41	20.43	0.34	10.22	8.54	1.42	1.10
Meat & meat dishes	9.65	7.61	8.25	35.59	4.29	6.78	9.03	7.80	8.15
Poultry	3.00	2.26	1.72	25.11	5.36	19.60	10.35	2.42	2.66
Total meat, poultry & eggs	**17.90**	**11.16**	**10.38**	**81.13**	**9.99**	**36.60**	**27.92**	**11.65**	**11.91**
Biscuits	2.38	3.51	2.68	0.27	0.00	0.46	0.07	3.49	2.38
Chocolate products	0.53	8.39	5.77	0.00	0.00	0.00	0.00	8.34	5.09
Pastry & desserts	5.89	2.73	3.00	4.32	0.06	0.00	1.07	2.74	2.72
Sugar & sweets	0.00	0.03	0.02	0.00	0.00	0.00	0.00	0.03	0.02
Total sweet products	**8.80**	**14.66**	**11.47**	**4.60**	**0.06**	**0.46**	**1.14**	**14.59**	**10.21**
Miscellaneous	0.00	0.01	0.02	0.00	0.00	0.00	0.00	0.01	0.02
Salty snacks	0.02	2.99	1.10	0.00	0.00	0.57	0.08	2.97	0.99
Snacks	0.97	1.64	3.41	2.13	5.19	3.10	5.29	1.65	3.60
Spices & condiments	0.00	0.09	0.32	0.00	0.00	0.00	0.00	0.09	0.28
Vegetarian substitute	0.00	0.31	0.18	0.00	0.10	0.00	0.00	0.31	0.16
Total miscellaneous	**0.99**	**5.04**	**5.03**	**2.13**	**5.30**	**3.67**	**5.36**	**5.02**	**5.05**

∑n-6PUFA= LA+AA; ∑n-3PUFA= LNA+EPA+DPA+DHA

- The major sources of **vitamin D** were fats & oils, with margarines as most important subgroup. Other important contributors were meat, poultry & eggs, fish & seafood, and prepared potatoes (e.g. mashed potatoes, containing eggs and margarine).
- Fats & oils were also the main source for **LA**, followed by cereal products. The contribution of sweet products to the LA intake was less important than for the pre-school children (Fig. II.5). In the group of fats & oils, margarines, and fatty sauces (dressings, etc.) were the major sources (Table II.5).
- For **LNA**, again a quite similar result as for LA was found, with fats & oils being the major contributors, followed by cereal products. In contrast, where dairy products were of negligible importance for the LA intake, they counted for 5.6% for the LNA-intake, with cheese being the most important subgroup. In the group of cereal products, bread & rusks were most important.
- The intake of **AA** was mainly contributed by meat, poultry & eggs.
- For the three different **LC n-3 PUFAs**, fish & seafood contributed for 84.1%, 59.3%, and 64.4% respectively for EPA, DPA, and DHA. The most important subgroup was fatty fish. For **EPA**, molluscs and crustacean were also quite important. A substantial part of the **DPA** intake was contributed by poultry, meat & meat dishes, and eggs. They contributed also to the **DHA** intake, with poultry as major subgroup. Consequently, we can conclude that for the LC PUFAs the food sources are similar for the pre-school children as for the adolescents.

The contribution of each food group to the nutrient intake was also calculated on individual level (mean proportion) and then compared between boys and girls (data not shown). No relevant differences were found on that level. More detailed information about the actual consumption of the different food groups is previously published (Matthys *et al*, 2006).

Table II.6 indicates the energy and fat intake and the consumption of some relevant food items for the **tertiles** based on the vitamin D and EPA&DHA intake. The consumption of the food items is expressed in g/d as in contribution to the total energy intake (%E); the latter to correct for the overall energy intake of the individuals.

*Table II.6: Consumption of different food items (in g/day and in contribution to the total energy intake (%E)) for the different tertiles based on the vitamin D intake and the EPA&DHA intake, separately for boys and girls **

		♂ (n=120)			♀ (n=212)		
Tertiles of vitamin D intake (µg/d)		<3.00	[3.00-4.33]	>4.33	< 2.25	[2.25-3.05]	>3.05
Energy intake	kJ	**9761**	**10553**	**11937**	**6847**	**8083**	**9122**
Fat intake	g/d	**89.6**	**103.5**	**122.8**	**61.2**	**77.3**	**92.0**
	%E	**34.5**	**36.9**	**38.7**	**33.4**	**36.1**	**38.0**
Margarine	g/d	**8.3**	**16.7**	**36.2**	**6.4**	**11.9**	**20**
	%E	**2.4**	**4.2**	**7.4**	**2.5**	**4**	**5.3**
Total fats & oils	g/d	**7.8**	**12.7**	**16.6**	**6.4**	**8.5**	**11.6**
	%E	**2.3**	**3.4**	**3.7**	2.7	3	3.4
Lean fish	g/d	21.2	23.3	27.7	18.8	19.4	29.7
	%E	0.9	0.8	0.8	1	1	1.3
Half-fatty fish	g/d	16.5	18.6	13.7	8.2	10.4	10.6
	%E	1	1.1	0.5	0.6	0.7	0.5
Fatty fish	g/d	**7.6**	**8.2**	**22.8**	**5.5**	**7.2**	**22.4**
	%E	0.6	0.7	1.7	**0.9**	**0.9**	**2.8**
Total fish & seafood	g/d	14.1	15.7	20.4	**11.4**	**13**	**18.8**
	%E	0.7	0.9	1	0.8	0.8	1.3
Tertiles of EPA&DHA intake (mg/d)		<70	[70-180]	>180	<54	[54-143.6]	>143.6
Energy intake	kJ	10848	10537	10864	7740	7924	8377
Fat intake	g/d	105.9	104.3	105.2	73.4	75.9	80.9
	%E	36.7	37.3	36.2	35.4	35.8	36.3
Lean fish	g/d	**6.7**	**17.8**	**31**	**8.3**	**14.4**	**26.2**
	%E	**0.2**	**0.6**	**1**	**-**	**0.7**	**1.2**
Half-fatty fish	g/d	2.9	14.5	17.5	**3.9**	**8.5**	**11.7**
	%E	0.1	0.8	0.9	0.2	0.6	0.7
Fatty fish	g/d	**8.2**	**3.6**	**17.8**	**2.1**	**4.9**	**18.7**
	%E	**0.5**	**0.3**	**1.4**	**0.3**	**0.7**	**2.3**
Molluscs & crustacean	g/d	3.6	6.5	12.6	**3.8**	**7**	**15.9**
	%E	0.1	0.2	0.4	**0.2**	**0.4**	**0.7**
Fish products	g/d	15	18.5	18.5	9.7	14.9	14.4
	%E	1.2	1.4	1.3	0.9	1.3	1.3
Total fish & seafood	g/d	**8.3**	**12.9**	**20.1**	**6**	**10**	**18.6**
	%E	**0.5**	**0.6**	**1.1**	**0.5**	**0.7**	**1.3**

For the figures indicated in bold, a significant difference between the tertiles was found ($p < 0.01$)
* The amounts consumed of the different food items reported in g/day and in %E are calculated as the mean only for those adolescents that consumed the food item during the week of the study and not as a mean for all members of the tertile. In other words, it is a mean of the consumer population only. As a result, the sum of the mean consumption of the different fish types is not equal to the consumption of total fish & seafood, since a different number of consumers is accounted for.

- For both genders, higher energy and fat intake as well as higher consumption of margarine and total fats & oils was found for the higher **vitamin D tertiles**. This was also the case for fatty fish, expressed in g/day (Table II.6). Moreover, for the girls, significant differences were found for the consumption of pastry & desserts, potatoes, and poultry (in g/d), with a higher consumption for the higher vitamin D tertiles (data not shown).
- When considering the **EPA&DHA tertiles**, significant differences were only found for food items belonging to the fish & seafood group, showing that higher EPA&DHA intake was related with higher fish & seafood intake; the intake of energy and fat did not differ

significantly. For non-mentioned food groups (e.g. fruits & vegetables), no significant differences were found over the different tertiles.

3.2.3. The PUFA food sources for the young women

Table II.7 shows the proportional contribution of the different food groups to **PUFA intake** of the studied young women, calculated as population proportions.

As for the adolescents, fats & oils were the main sources for LA and especially for LNA, followed by cereal products. In the group of fats & oils, margarines and fatty sauces (dressings, etc.) were the major subgroups. Compared to the adolescents, a more important contribution of nuts and seeds to the LA and LNA intake was found for the young women.

Again, the intake of AA was mainly contributed in the diet by meat, poultry & eggs, however, for this population group eggs was the most important subgroup. As found for the two other studied population groups, fish & seafood are the most important food sources of EPA, DPA, and DHA, even more important for the young women than for the adolescents.

Table II.7: Contribution of food groups to n-6 and n-3 PUFA intakes (% of the total intake of each FA brought by each food group for the whole population sample) for the Flemish young women

Food Groups	LA	LNA	AA	EPA	DPA	DHA	∑n-6PUFA	∑n-3PUFA
Bread & rusks	12.9	9.9	0.0	0.0	0.0	0.0	12.8	8.5
Breakfast Cereals	1.1	0.5	0.2	0.0	0.0	0.0	1.1	0.4
Cereal based dishes	0.8	1.4	0.7	0.0	0.0	0.1	0.8	1.2
Pasta, rice & other cereals	1.6	1.3	0.6	0.4	0.0	0.2	1.6	1.2
Total cereal products	**16.4**	**13.1**	**1.6**	**0.4**	**0.0**	**0.3**	**16.3**	**11.3**
Butter	0.3	0.9	1.2	0.0	0.0	0.0	0.3	0.8
Cheese	1.2	4.4	4.3	0.0	0.0	0.0	1.3	3.7
Cream	0.1	0.5	0.0	0.0	0.0	0.0	0.1	0.4
Milk	0.2	0.6	0.4	0.0	0.0	0.0	0.2	0.6
Yogurts	0.1	0.2	0.0	0.0	0.0	0.0	0.1	0.2
Total dairy products	**1.9**	**6.6**	**5.9**	**0.0**	**0.0**	**0.0**	**2.0**	**5.6**
Fatty sauces	11.3	25.1	0.0	0.1	0.1	0.0	11.2	21.6
Margarines	15.7	17.7	0.0	0.9	0.0	0.5	15.6	15.3
Mixed fats	0.1	0.2	0.0	0.0	0.0	0.0	0.1	0.1
Vegetable oils	4.3	1.8	0.0	0.0	0.0	0.0	4.3	1.6
Total fats & oils	**31.4**	**44.7**	**0.0**	**1.1**	**0.1**	**0.5**	**31.2**	**38.6**
Fatty fish	0.4	0.6	5.0	47.4	49.8	48.1	0.4	7.3
Fish products	0.4	0.1	0.4	3.2	1.9	3.0	0.4	0.5
Half-fatty fish	0.0	0.0	1.2	5.2	3.8	6.6	0.0	0.8
Lean fish	0.0	0.0	2.2	7.6	4.5	9.5	0.0	1.2
Molluscs & crustaceans	0.0	0.1	8.9	23.9	6.1	12.9	0.1	2.3
Total fish & seafood	**0.8**	**0.9**	**17.8**	**87.3**	**66.0**	**80.0**	**0.9**	**12.1**
Fruits	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.4
Legumes	0.1	0.3	0.0	0.0	0.0	0.0	0.1	0.3
Nuts & seeds	6.6	3.5	0.0	0.6	0.0	0.0	6.6	3.1
Potatoes	5.5	1.7	0.0	0.0	0.0	0.0	5.5	1.5
Soups	0.6	0.6	0.8	0.1	0.0	0.2	0.6	0.5
Vegetables	0.6	1.8	0.0	0.1	0.0	0.0	0.6	1.5
Total fruits & vegetables	**13.4**	**8.4**	**0.8**	**0.8**	**0.0**	**0.2**	**13.4**	**7.2**
Eggs	1.1	0.3	25.5	0.2	6.2	6.1	1.2	0.9
Meat & meat dishes	8.4	8.3	17.9	2.4	12.1	2.0	8.5	7.6
Poultry	1.6	1.2	15.3	2.7	12.9	3.7	1.6	1.7
Total meat, poultry & eggs	**11.1**	**9.9**	**58.7**	**5.2**	**31.2**	**11.8**	**11.3**	**10.1**
Biscuits	2.9	2.7	0.2	0.0	0.0	0.0	2.8	2.4
Chocolate products	5.4	3.7	0.0	0.0	0.0	0.0	5.4	3.2
Pastry & desserts	4.5	4.4	8.7	0.1	0.1	0.9	4.5	3.8
Sugar & sweets	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.0
Total sweet products	**12.8**	**10.8**	**9.0**	**0.1**	**0.1**	**0.9**	**12.8**	**9.3**
Dietetic products	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Miscellaneous	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.0
Salty snacks	2.2	0.7	0.0	0.0	0.2	0.0	2.1	0.6
Snacks	6.8	2.9	6.2	4.3	2.3	6.2	6.8	3.2
Soydrink	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Spices & condiments	0.5	0.8	0.0	0.0	0.0	0.0	0.5	0.7
Vegetarian substitute	2.6	1.3	0.0	0.8	0.0	0.0	2.6	1.2
Total miscellaneous	**12.1**	**5.8**	**6.2**	**5.1**	**2.5**	**6.2**	**12.1**	**5.7**

∑n-6PUFA= LA+AA; ∑n-3PUFA= LNA+EPA+DPA+DHA

4. Discussion

4.1. Methodological limitations

4.1.1. Food consumption data

A first methodological limitation was related to the **food consumption data** used. A weakness of the EDR method is underreporting or underestimation of the food intake. In general, food items rich in fat and/or carbohydrates (such as butter, sweet products, and snacks) are reported less frequently and/or in smaller quantities than actually consumed. This has an influence on the assessed nutrient intake. Moreover, the number of recorded days is restricted for the food consumption dataset of the pre-school children and the young women. A three-day and two-day EDR for the pre-school children and the young women, respectively, does not necessarily reflect usual intake of individuals and is likely to lead to more extreme high and low intakes, especially for nutrients abundantly present in foods that are not consumed on a daily basis, such as seafood (Willett, 1998). This is illustrated in Fig. II.1-4, indicating the smaller ranges for the assessed intakes of the adolescent population compared to those of the studied pre-school children and young women. The main reason is a high within- and between-individual variation for these nutrient intakes over different days.

Nelson *et al* (1989) calculated the days needed to assess the individual PUFA intake with a good correlation ($r \geq 0.9$) between the observed and the true mean intake. At least 15, 18, 28, and 30 days should be included for toddlers (1-4 years), male children (5-17 years), female children (5-17 years), and women older than 18 years, respectively. However, this is not feasible in practice. As an alternative, different methodologies are developed to take into account the within-individual variation. These methodologies include statistical modelling to estimate long-term usual intake from food consumption data recorded on a limited number of days (Dodd *et al*, 2006). Applying such statistical methodologies should be a more efficient solution than asking respondents to report their consumption pattern during more than seven days. It is known that increasing the number of days per study person leads to high respondent burden and risks to decrease the quality of the reported information.

One of the existing modelling methods is the NUSSER-method, which was applied to the dataset of pre-school children in order to assess usual PUFA intakes. However, the NUSSER-method turned out to be inapplicable for these PUFAs for which a substantial part of the population had a zero intake, being the major disadvantage of this method. Nevertheless, the mean PUFA intakes on population level, resulting from the three-day EDRs did not differ much from the usual nutrient PUFA estimates assessed by the NUSSER-method (data not shown). This might be due to rather small within-individual variation of the consumption pattern of young children. Recently, improved methods for estimating usual intake distributions for episodically consumed foods are being developed, but were not yet available on the moment of the study (Dodd *et al*, 2006). Nevertheless, it was assumed that the three- and two-day EDRs yielded unbiased estimates on population level of the mean food consumption and nutrient intakes. Of course, the data can not be used to evaluate the intake on individual level.

In contrast to the datasets of the pre-school children and the young women, the adolescent food consumption data describe the consumption during seven consecutive days. The important advantage of these consumption data gathered over a longer period involves that they allow assessing the intake of nutrients present in relatively few foods that are not eaten on daily basis, such as seafood (Lamberg-Allardt, 2006). However, the disadvantage of this dataset is that it concerns rather old data (1997).

4.1.2. Food composition data

Another limitation of this study is that no own analytical **concentration data** for vitamin D and PUFA were at our disposition. Hence, several previously published data were used, but the derivation of this data is unknown. Additionally, it is noticeable that in individual foods PUFAs, in particular the LC PUFAs, are present in only small amounts, but accumulate to significant levels of biological importance in the context of a whole diet. For most foods, these low values often round down to zero when reported on a single decimal place and so it is likely that PUFA concentrations will be consistently underreported in FCDBs (Mann *et al*, 2003) leading to an underestimation of the assessed PUFA intake. Furthermore, for some items it was not evident to find the PUFA composition because new (highly processed or imported) food items are available on the market on a regular basis. Quinoa (a South-

American crop primarily grown for its edible seeds which are prepared and eaten as a cereal), for example, was only mentioned in the German FCDB (Souci *et al*, 2000). Eight different FCDBs were necessary to determine the PUFA composition of all consumed food items, which is not an ideal situation since different protocols can be hidden behind the data and since the origin of the published data was hard to trace for some FCDBs.

Moreover, a very recent Australian study noted that the meat & poultry was a significant source of LC n-3 PUFA (Howe *et al*, 2006). The authors stated that the modest amount of PUFA in muscle tissue phospholipids of lean meat needs to be taken into account when determining dietary PUFA intakes. The majority of LC n-3 PUFA in meat is DPA. According to Howe *et al* (2006) the LC n-3 PUFA content of meat products are underestimated up to now. Since the limitations of available data about the fatty acid content of these food items, LC n-3 intake from meat, poultry & eggs, especially DPA intake, could have been underestimated. Nevertheless, the Belgian recommendation considers only EPA and DHA for the evaluation of LC n-3 PUFA intake (Belgian Health Council, 2007). Therefore, the evaluation of the intake data against the Belgian recommendations is not influenced by the possible underestimation of DPA during the intake assessment.

4.2. Evaluation of the intakes

4.2.1. Observations versus recommendations

4.2.1.1. LA and LNA

Fig. II.1 shows that the intake of **LA** of almost all individuals of the three studied population groups exceeded the recommendation, indicating that the risk of LA deficiency is extremely low. This confirmed the abundance of LA sources in the modern Western diet (Sontrop & Campbell, 2006). Therefore, it can be concluded that no dietary shifts are needed to increase the current LA intake. In contrast, a non-negligible part of the three considered population groups had a **LNA** intake lower than the recommended level (Fig. II.1). Since the food sources of LA and LNA are quite similar (Fig. II.5, Table II.5 and II.6), a general shift in the consumption of a certain food group to increase the LNA intake will simultaneously have an effect on the LA intake. Therefore, a shift between foods within a certain food group is needed. The consumption of LNA rich foods by the Flemish population should be

encouraged, specifically by encouraging the consumption of food items with a lower LA/LNA ratio e.g. rapeseed oil, linseed oil, soy bean oil, and walnut (oil), either by direct consumption of these foods or by increased use of these ingredients in processed foods, such as margarines and biscuits, which must be possible by new and highly-developed techniques in food manufacturing. Considering margarine, food industries must try to keep the trans fatty acid concentration of their products as low as possible, since these fatty acids are known to increase the risk of coronary heart disease (Korver & Katan, 2006; Upritchard *et al*, 2005).

4.2.1.2. AA

Fig. II.2 shows that the AA intake of pre-school children is extremely low compared to the recommendation. An in-depth discussion on the AA intake is given below (paragraph 4.2.2.1.). On the other hand, Fig. II.2 indicates that the mean ∑n-6 PUFA intake of the studied adolescents and young women fall within the recommended range, i.e. 4-6 %E.

4.2.1.3. LC n-3 PUFA

Fig. II.3 and II.4 indicate clearly that the intakes LC n-3 PUFA (EPA and DHA) as well as the ∑n-3 PUFA fall well below the recommended intake. As such, it can be concluded that all studied population groups have an important deficit for these fatty acids.

4.2.1.4. Flemish versus Belgian data

As stated in the introduction of this chapter, the available food consumption data used for the PUFA intake assessment were limited to the Flemish population. Nevertheless, the Belgian Health Interview Survey (HIS) (Scientific Institute of Public Health, 2006) and the Belgian Food Consumption Survey (FCS) (De Vriese *et al*, 2006) provided data about seafood consumption and these surveys had sampled through all inhabitants of Belgium. However, both datasets are limited to persons over 15 years.

- **Data of the HIS** of 2004 indicated that the percentage of people eating at least as much fish as recommended was higher in the Flemish region (66.0%) than in the Walloon region (54.9%), and that the Brussels region had the highest percentage (67.0%). Unfortunately, it was not indicated what was meant by 'at least as much fish as recommended' and it was not clear whether only fish or also other seafood was considered.

- In contrast, the **data of the FCS** indicated that almost 70% of the Belgian population consumed less seafood than recommended. Here, a recommendation of 30 g/day was applied. Despite of the different results, the FCS data also indicated that the seafood consumption in the Flemish region is higher than the seafood consumption in the Brussels and Walloon region.

Therefore, it can be assumed that the conclusion that the intake of LC n-3 PUFA is very low compared to the recommendations is true for the Flemish region as well as for the other Belgian regions.

4.2.1.5. Seafood consumption recommendations

Increased seafood consumption is one of the possibilities to increase the LC n-3 PUFA intake. Currently, the Belgian Health Council advices the population to consume seafood one to two times a week (Belgian Health Council, 2004b). Moreover, seafood has a prominent place in the Flemish food triangle and the Walloon food pyramid. The Belgian Health Council underlines the recommendation to consume different species from various origins. Moreover, they advise pregnant and lactating women to consume only one portion of tuna per week (Belgian Health Council, 2004a). In chapter IV of this PhD-thesis, it is investigated whether this recommendation is sufficient to achieve the DRI for EPA and DHA and whether an upper limit is needed related to contaminant intake.

Nevertheless, 36.1% of the studied adolescents did not consume any seafood in the week of the study. An evaluation of the seafood consumption for the two other studied populations is difficult since only short term consumption data were available. Nevertheless, a more regular replacement of meat & meat products by seafood should, above an increased intake of LC n-3 PUFAs and vitamin D, also be beneficial to decrease the too high saturated fatty acid (SFA) intake of the studied populations, as previously reported (Huybrechts & De Henauw, 2007; Matthys *et al*, 2006; Sioen *et al*, 2006a). Additionally, replacement of high-fat cheese regularly consumed during bread meals, with seafood like mackerel and sardines can lower the SFA intake and at the same time increase the intake of vitamin D and LC n-3 PUFAs. These arguments are also a counter-argument to the intake of supplements. While LC n-3 PUFA supplements can seem to be an easy solution for low LC n-3 PUFA intake, the presence of other nutrients in food sources of EPA and DHA must also be considered, such as important amino-acids and trace elements together with a low concentration of SFA and cholesterol.

4.2.1.6. Seafood consumption barriers

Nevertheless, one could identify different barriers to recommend increased seafood consumption:

1. There are concerns that higher fatty fish consumption could impact on intakes of **contaminants** such as dioxin-like compounds and heavy metals. But, currently, more and more studies indicate that the potential benefits from LC n-3 PUFAs outweigh the risk of exposure to toxicological levels of contaminants (American Heart Association *et al*, 2006; Ruxton *et al*, 2004; SACN/COT, 2004). A more detailed evaluation of the risks and benefits related to seafood consumption can be found in the following chapters of this PhD-thesis.
2. The **food choice** of young people is mostly driven by taste, smell, and convenience, and seafood does not have a high preference rate (Diehl, 1999). Additionally, in the case of pre-school children and adolescents, it will be in the first place the decision of the parents or school catering services whether or not seafood will be regularly placed on the menu. Furthermore, although parent's food consumption decisions are shaped by personal norms or moral obligation to serve their children a nutritious meal, children's preferences and the desired compliance with these play a role as well (Kelly *et al*, 2006). Recent empirical evidence suggests that the presence of young children in the family acts as an important barrier to increased seafood consumption, particularly in countries with a relatively weak seafood consumption tradition such as Belgium. The presence of young children in Belgian households has been associated with a lower impact of external social norms, i.e. parents' interest and willingness to take into account the advice from external information sources in their social environment such as medical sources, in determining seafood consumption intention (Verbeke & Vackier, 2005).
3. Belgian adults' **awareness** of both the fact that seafood is an important dietary source of LC n-3 PUFA, and the fact that these PUFAs have a potential beneficial impact on human health, is rather limited (Verbeke *et al*, 2005).
4. Last but not least, fish and/or seafood **allergies** or total **dislike** of seafood will influence the actual consumption. In the latter case, other strategies, e.g. fortified foods and supplements, can be used as alternatives to increase the LC n-3 PUFA intake.

4.2.2. Evaluation of the results and comparison with international data

In the paragraphs below, the intakes of the studied subgroups of the Flemish population are compared to international data. Without doubt, different food composition data and different methodologies to collect food consumption data were used in the different studies hampering the comparability of the data. Moreover, non-negligible differences exist in the age and the total energy intake of the population. Yet, it was worth to compare the results to evaluate the PUFA intake data of the Flemish population groups in an international context.

4.2.2.1. PUFA intakes of pre-school children

The PUFA intakes of the pre-school children were compared to results from other studies:

1. Canadian children (n=84; 1.5-5 years), based on an FFQ;
2. Australian children (2-3 years (n=383) and 4-7 years (n=799)), based on one 24-h recall;
3. children in rural China (n=196; 1-5 years) based on three 24-h recalls (Table II.8).

*Table II.8: PUFA intakes of pre-school children from different studies **

	LA g/d	LA %E	LNA g/d	LNA %E	AA mg/d	AA %E	EPA mg/d	DPA mg/d	DHA mg/d	DHA %E	LA/ LNA
Flemish† 2.5-3y	6.71	4.29	0.81	0.51	17	0.01	22	10	43	0.03	9.1
Flemish† 4-6.5y	7.12	4.27	0.86	0.51	18	0.01	26	10	49	0.03	9.1
Australian‡ 2-3y	6.10	-	0.68	-	16	-	10	5	24	-	-
Australian‡ 4-7y	7.50	-	0.81	-	22	-	19	10	47	-	-
Canadian§ 2y	9.04	-	2.02	-	260	-	57	-	95	-	5.2
Canadian§ 3-5y	9.39	-	1.72	-	226	-	60	-	96	-	6.6
Chinese‖ 1-3y	2.08	2.9	0.28	0.4	55	0.08	-	-	34	0.05	-
Chinese‖ 4-5y	2.27	2.5	0.34	0.4	50	0.06	-	-	23	0.02	-

† This study; ‡ (Meyer et al, 2003); § (Innis et al, 2004); ‖ (Barbarich et al, 2006)
** Since values expressed as %E were not found in other studies for EPA and DPA, these were not given in the table.*

- Comparison of the **LA** intakes illustrated that the intake of Flemish pre-school children was similar to the intake of Australian, lower than that of Canadian, but much higher than that of Chinese children (Table II.8).
- The **LNA** intake of Flemish pre-school children was - as for the LA intake - comparable to that of Australian, but much lower than that of Canadian children. The LNA intake of Chinese children seemed to be very low when expressed in g/d, but only a bit lower than

the Flemish intakes when expressed in %E, due to the low energy intake of rural Chinese children.

- The rather high LA intake and the too low LNA intake of Flemish pre-school children resulted in a high **LA/LNA ratio**, being much higher than the LA/LNA ratio of Canadian children. This confirms that the consumption of food items with lower LA/LNA ratio by Flemish pre-school children (and by the Flemish population in general) should be stimulated and that food industries must be encouraged to lower the LA/LNA ratio of their products, if possible.
- All Flemish pre-school children had an **AA** intake lower than the recommended lower level. Different possible explanations or influencing factors can be put forward:
  1. the overall diet truly contain very little AA,
  2. the recommended AA intake range set by the Belgian Health Council is too high,
  3. the AA concentrations in the foods were underestimated in the FCDB used.

  Considering the second explanation, no specific AA recommendations are given by the EU (Commission of the European Communities, 1993) or the American Institute of Medicine (IOM) (Institute of Medicine, 2005), hence hampering a comparison. However, the IOM indicates that 5 to 10 %E should come from n-6 PUFAs, with approximately 10% from LC n-6 PUFAs. Considering the third explanation, literature appears to be equivocal. An Australian study stated that AA values in FCDB are too high, leading to an overestimation of the AA intake (Mann *et al*, 1995). In contrast, an American study concluded that AA values in the American FCDB (version HB-8) were significantly lower compared to their analytical results (Taber *et al*, 1998). The AA intakes of Flemish and Australian pre-school children were comparable. That of Chinese children were more than two times higher, and that of Canadian more than 10 times higher (chicken was the major source of dietary AA for many of the Canadian children). Possible ways to increase the current AA intake are increased consumption of poultry meat. Nevertheless, when recommending increased meat consumption, attention must be paid not to increase the overall saturated fatty acid intake.
- Canadian children had the largest **LC n-3 PUFA** intake being twice as high as the intake of the Flemish ones (Table II.8). Exploratory calculations showed that stimulation of fatty fish consumption among Flemish pre-school children is a possible solution to bridge the gap between the intake and the recommendation.

4.2.2.2. Vitamin D and PUFA intakes of adolescents

The mean and median **vitamin D** intakes of the adolescent boys were situated within the recommended range. In contrast, the results showed that half of the studied girls had an intake lower than 2.5 µg/d. The most important dietary source of vitamin D for the adolescents was margarine, due to the mandatory vitamin D fortification of margarine in Belgium. Food fortification is widely used in many industrialised countries for increasing vitamin D intake (Ovesen *et al*, 2003). As the consumption of fatty fish was significantly higher for the highest vitamin D tertiles, a lot of girls, and also boys, would benefit from higher fatty fish consumption. On the other hand, it must be taken into account that advising increased consumption of fat-rich food items to increase the vitamin D intake will simultaneously increase the total fat intake. When considering vitamin D, it must also be mentioned that ultraviolet-induced skin production constitutes the main contributor to vitamin D in humans, making oral intake nonessential in principle (Sichert-Hellert *et al*, 2006). In contrast, Ovesen *et al* (2003) stated that skin synthesis of vitamin D may not compensate for the low nutritional intake in Europe. Moreover, Lamberg-Allardt (2006) suggested that the recommendation should be increased to be at least 10 µg/d in all age groups when solar UVB is scarce. In this study population, even the 95th percentile does not reach this level, with the 95th percentile of the girls being only 4.9 µg/d. Nevertheless, Ricketts disease, the direct consequence of vitamin D deficiency, is not a major public health problem in Belgium. But this does not mean that a long term, too low intake of vitamin D will not create health problems.

The results of the **PUFA** intake of the studied Flemish adolescents were compared to other intake data of (LC) n-6 and n-3 PUFAs for adolescents from

- Australia (1086 children; 12-18y; one 24-h recall plus a food frequency questionnaire (FFQ); 1995) (Howe *et al*, 2006) and
- the USA (581 boys and 536 girls; 14.8±0.02y; one 24-h recall; 2001) (Harel *et al*, 2001).

The latter only described the intake of n-3 PUFAs. The LA and ∑n-6 PUFA intake was higher in Belgium than in Australia (respectively, 11.7 and 11.8 versus 11.1 and 11.4 g/d). The LNA intake was highest in Belgium, followed by Australia (1.18 g/d) and the USA (0.35 g/d). Nevertheless, the sum of EPA, DPA, and DHA was highest in Australia (195.0 mg/d), followed by Belgium (185.7 mg/d) and the USA (38.5 mg/d).

4.2.2.3. PUFA intakes of young women

International data of mean dietary intake of PUFA in adult women, expressed in g or mg/day, are presented in Table II.9.

Table II.9: Fat and PUFA intake data of different other larger and smaller population studies with women (w)

Country	Population	Method	Fat	LA	LNA	AA	EPA	DPA	DHA	∑ n-6 *	∑ n-3 #	LA/ LNA	n-6/ n-3
	Larger sample			g/d			mg/d			g/d			
Belgium (this study)	641 w (18-39y)	2-day record	77.6	11.6	1.4	56	78	25	131	12.0	1.7	8.7	7.8
Australia (Howe *et al*, 2006)	5770 w (+19y)	24h-recall	NA	8.7	0.9	117	60	52	83	8.9	1.1	NA	NA
France (Astorg *et al*, 2004)	2,785 w (35-63y)	6x 24h-record	73.6	8.1	0.7	152	118	56	226	NA	NA	11.1	NA
Germany (Linseisen *et al*, 2003)	898 w (35-64y)	24h-recall	76.1	11.6	1.5	140	80	NA	140	11.7	1.7	NA	7.2
Germany (Linseisen *et al*, 2003)	1,078 w (35-64y)	24h-recall	78.0	10.9	1.3	160	70	NA	140	11.0	1.5	NA	8.0
Australia (Meyer *et al*, 2003)	3178 w (19-64y)	24h-record	NA	9.4	1.0	41	46	21	89	9.4	1.2	8.0	NA
Japan (Tokudome *et al*, 1999)	180 w (middle-aged)	1-day record	57.7	11.2	1.7	139	242	NA	469	NA	NA	NA	NA
Norway (Johansson *et al*, 1998)	1627 w (16-79y)	FFQ	67.0	8.8	1.2	120	270	60	400	NA	NA	NA	4.2
	Smaller sample												
USA (Loosemore *et al*, 2004)	31 w (mean:25y)	2x 24h-recall	74.0	11.6	1.3	155	16	NA	68	11.7	1.5	9.0	4.5
Japan (Tokudome *et al*, 2003)	71 w (40-49y)	7-day record	NA	10.5	1.6	135	278	75	497	10.6	2.4	NA	4.6
Canada (Innis & Elias, 2003)	55 pregnant w (20-40y)	FFQ	79.8	11.2	1.6	121	78	NA	160	NA	NA	NA	NA
Belgium (De Vriese *et al*, 2001)	26 pregnant w; 1st trim	FFQ	85.9	12.9	1.3	130	170	NA	300	13.2	1.8	NA	NA
Belgium (De Vriese *et al*, 2001)	26 pregnant w; 3rd trim	FFQ	90.2	13.7	1.5	130	150	NA	300	14.0	2.0	NA	NA
Nether-lands (Otto *et al*, 2001)	19 pre-pregnant w (mean:30.7y)	FFQ	88.2	13.2	1.1	30	50	10	90	13.2	1.3	NA	11.3
Nether-lands (Otto *et al*, 2001)	20 pregnant w (mean:30.7y)	FFQ	86.9	13.2	1.0	20	80	20	140	13.2	1.3	NA	12.0

∑n-6 * and ∑n-3 # = depending on the study; NA= not available; w = women; trim = trimester

- The women of the studies in France, Norway, and Australia seemed to consume less **LA**, whereas the women in Germany and Japan consumed quite similar levels as the Flemish women (Astorg *et al*, 2004; Howe *et al*, 2006; Johansson *et al*, 1998; Linseisen *et al*, 2003; Meyer *et al*, 2003; Tokudome *et al*, 2003). Conversely, higher absolute LA intake levels

were found in another Belgian and a Dutch study with a smaller sample size (De Vriese *et al*, 2001; Otto *et al*, 2001) (Table II.9).

- Another pattern was found when comparing the **LNA** intake. The absolute intake of LNA determined for the Flemish women is quite high when compared to other studies, with exception of the Japanese and German study (Loosemore *et al*, 2004; Tokudome *et al*, 2003) and a study done in pregnant Canadian women (Innis & Elias, 2003). In all the other countries, a lower LNA intake was determined. This is remarkable, since a comparison of the LNA intake with the Belgian recommendations showed a deficit of this fatty acid in the diet of the Belgian women. Astorg *et al* (2004) translated the mean LNA intake of the female population group in %E, being 0.38 %E. Comparing to the Belgian recommended minimum level, the French women have even a worse deficit than the Flemish women. Correspondingly, mean LA/LNA ratio of the French women is higher (11.1) than the one found for the Flemish women.
- Most of the other studies found a mean absolute **AA** intake, being two or three times higher than the mean AA intake of the Flemish women. Nevertheless, the most contributing food sources for AA are equal compared to those found in the French and the Australian study (Astorg *et al*, 2004; Meyer *et al*, 2003), being meat, poultry & eggs (counting for more than 50% of the AA intake) and fish & seafood. So a probable explanation is that the AA concentration of these food items is underestimated in the FCDBs that were used in this study.
- A large variation is found in the intake estimations of **LC n-3 PUFAs** between studies and countries. As for LA and LNA mean intake, the mean LC n-3 PUFAs intake of Flemish women is comparable with the results found in the German study (Loosemore *et al*, 2004) as well as with the results of the Dutch study (Otto *et al*, 2001). A larger absolute intake for EPA, DPA and DHA is found in France and especially in Norway and Japan, probably due to larger seafood consumption. In this study, the average seafood consumption of the women (calculated as a mean of the two days) was 27.0 g/d. Welch *et al* (2002) reported the seafood consumption in 10 European countries for men and women between 35 and 74 years old. For women, a mean seafood consumption of 15.9 g/d and 19.9 g/d was found in two German cities; and a mean of 13.3 g/d and 13.4 g/d in two Dutch cities (Welch *et al*, 2002). In comparison, French women have a mean seafood consumption ranging between 35.0 g/d and 52.4 g/d, depending to the region where they live; and the mean intake of Norwegian women was 42.9 g/d in South & East Norway and 63.3 g/d in North & West Norway (Welch *et al*, 2002). Johansson *et al* (1998)

even reported a mean intake of 57 g fish per day for Norwegian women. These results show that the higher seafood consumption in the latter two countries is clearly the main reason for the higher LC n-3 PUFA intake.

Comparison of **food sources** of nutrients over different studies is not straightforward, since the composition of food groups are not the same in the different studies. The composition of food groups in this study is based on the French study (Astorg *et al*, 2004), so the results of these two studies are quite comparable. The comparison with other results must be seen as an indication.

- The main sources for **LA** intake of Flemish women were fats & oils (31.4%), followed by cereal products (16.4%). The important contribution of fats & oils to the LA intake was also found in the French and the Australian studies (counting for 33.5% and 21.9%, respectively) (Astorg *et al*, 2004; Meyer *et al*, 2003) as well as in the Japanese study (Tokudome *et al*, 1999).
- Considering the **LNA** intake of Flemish women, fats & oils were by far the major contributor (45%), especially fatty sauces and margarines. This is at variance with the French study, where fats & oils were only a minor contributor (10%), the major ones being dairy products, fruits & vegetables and meat, poultry & eggs (Astorg *et al*, 2004). Fatty sauces and margarines seem to be much less consumed in France than in Flanders.
- For **AA**, the intake of Flemish women was mostly contributed by meat, poultry & eggs, followed by fish & seafood. The same results were found by the French and Australian authors: meat, poultry & eggs counted for 67.2% and 70.2%, respectively; followed by fish & seafood (11.1% and 27.2%, respectively). Also in the Japanese study, chicken eggs and pork together contributed for 50.6% to the AA intake.
- In this study, fish & seafood were the major contributors for the **EPA, DPA, and DHA** intake counting respectively for 87.3%, 66.0%, and 80.0%. In Norway, fish was also the major source (respectively counting for 55%, 43% and 57%), followed by cod liver oil, which was used by 36% of the survey subjects (Johansson *et al*, 1998). In contrast, cod liver oil, being rich in LC n-3 PUFA, was not consumed by the Flemish women included in this study. In the French data, fish & seafood were the major sources for EPA and DHA, respectively for 72.0% and 64.7% (Astorg *et al*, 2004), but meat, poultry & eggs were the first contributor of DPA intake (55%). In Norway, meat and fish were the main sources of DPA (43% each) (Johansson *et al*, 1998). In this study, the contribution of meat, poultry & eggs to the DPA intake was lower (31%). A very recent Australian publication

described in detail the contribution of meat sources to the intake of LC n-3 PUFA (Howe *et al*, 2006). Their analyses showed that fish & seafood contributed for 49.7%, 15.4%, and 69.9% to the intake of respectively EPA, DPA, and DHA. On the other hand, meat, poultry & game products counted for respectively, 44.8%, 73.2%, and 19.6%. This shows that an underestimation of the LC n-3 PUFA content of meat can be crucial, since the consumption of meat & meat products is large compared to that of fish & seafood.

5. Conclusion

The intake of LA for the studied Flemish population groups fell within the recommended range. In contrast the intake of LNA was rather low and as a result, the mean LA/LNA ratio was high. Therefore, the Flemish population would benefit from a higher consumption of LNA rich foods e.g. by replacement of n-6 rich oils by n-3 rich oils such as linseed and rapeseed in food formulations.

Moreover, dietary shifts are necessary to bridge the gap between the intakes and the recommendations of LC n-6 and n-3 PUFAs. Regular replacement of meat products rich in SFA by poultry meat is a possible solution to increase the AA intake, which was evaluated to be very low for the studied pre-school children. Fatty fish consumption should be stimulated since it is a rich source of LC n-3 PUFAs of which the current intakes are far below the recommendations. Seafood consumption data of the overall Belgian adult population indicated that low intakes of LC n-3 PUFAs are omnipresent. Increased seafood consumption can, additionally, lead to higher vitamin D intake and can replace SFA-rich food items. Nevertheless, due to contamination, seafood consumption can not be increased without limitation. In chapter IV of this thesis it was studied how much seafood can be consumed per week without leading to contaminant intake of toxicological concern.

However, further investigation is necessary to explore the best way to convince the Flemish population of these health beneficial steps. Since a previous study in Belgium showed that consumer awareness and beliefs related to n-3 PUFA, their health benefits and their food source are poor and often wrong (Verbeke *et al*, 2005), nutrition education should play an important role in order to convince people of this dietary shift.

Chapter III.

Traceability and nutrient and contaminant content of seafood on the Belgian market: elaboration of databases

This chapter is based on the following papers:

1. Sioen I, Verbeke W, De Henauw S, Parmentier K, Raemaekers M, Willems JL, Van Camp J. Traceability of seafood products on the Belgian market. *Fisheries Research*. Submitted.

2. Sioen I, De Henauw S, Verdonck F, Van Thuyne N, Van Camp J. Development of a nutrient database and distributions for use in a probabilistic risk-benefit analysis of human seafood consumption. *Journal of Food Composition and Analysis* 2007; **20**(8): 662-670.

3. Sioen I, Van Camp J, Verdonck F, Van Thuyne N, Willems JL, De Henauw S. How to use secondary data on seafood contamination for probabilistic exposure assessment purposes? Main problems and potential solutions. *Human and Ecological Risk Assessment* 2007; **13**(3):632-657.

1. Introduction

One of the main objectives of this PhD-thesis was to execute an intake assessment of nutrients and contaminants via seafood consumption in order to evaluate the risks and benefits related to seafood consumption in Belgium and to formulate recommendations concerning seafood consumption. This intake assessment is based on combining seafood consumption data with nutrient and contaminant concentrations – if possible taking into account the origin of the seafood species – by using a probabilistic approach, which takes into account the existing variability of the different parameters (consumption data, body weight data, and concentration data). Taking into account the variability of parameters is relevant in the case of contaminant and nutrient concentrations in seafood as well as for consumption patterns and body weights. In the probabilistic intake assessment executed in this PhD-thesis and described in chapter IV, the variability of the seafood consumption by the population is taken into account in a non-parametric way (i.e. using all the individual data as such and without assuming any underlying probability model), whereas the variability of the nutrient and contaminant concentrations is taken into account in a parametric way (i.e. by applying and assuming probability distributions). Moreover, special attention was given to the origin and traceability of the seafood consumed in Belgium.

Nutrient and contaminant concentration data can be collected:

1. by performing laboratory analyses in representative samples, or
2. by using published literature data (articles, reports, food composition tables, etc.).

In this PhD-thesis, the second approach – retrieving literature data - is applied. This is in accordance with the strategy recently proposed by Brüders *et al* (2005), stating that existing data should be used in the most effective way as collecting samples and analyzing them is expensive. Nevertheless, the comparison of data from different sources is always difficult especially if one only has access to aggregated data without having sufficient information about the collecting scheme, location data, sampling procedure, or laboratory procedures (Brüders *et al*, 2005). Moreover, necessary data are sometimes lacking, which hampers the intake assessment.

Different databases were constructed:

1. a first database attempting to describe the origin of the seafood products available on the Belgian market (described in part 2 of this chapter),
2. a second database describing the nutrient concentrations of seafood species relevant for Belgian consumption (described in part 3 of this chapter), and
3. a third one describing the contaminant concentrations in the relevant species (described in part 4 of this chapter).

This chapter starts by presenting an overview of the species that were relevant for Belgian consumption and are as such considered in this study. On the basis of the food consumption databases used further on in this PhD-thesis, 41 seafood species and two seafood products (caviar and surimi) were considered. Five fat groups (FG) were determined in order to group the species according to their fat concentration (expressed on fresh weight basis) (Table III.1).

Table III.1: The 41 seafood species and two seafood products taken into account because of their relevance for consumption by the subgroups of the population studied

Fat Group 1: < 1.0% fat	Fat Group 2: 1.0% ≥ fat < 2.5%	Fat Group 3: 2.5% ≥ fat < 5.0%	Fat Group 4: 5.0% ≥ fat < 10%	Fat Group 5: fat ≥ 10%
Anglerfish	Common shrimp	Anchovy	Milkfish	Eel
Brill	Common whelk	Caviar	Sardine	European catfish
Cod	European plaice	Conger	Trout	Herring
Crab	John dory	Halibut		Mackerel
Haddock	Lobster	Sea bream		Salmon
Ling	Mussel	Swordfish		Sprat
Saithe&Pollack	Nile perch	Tuna		
Scallop	Norway lobster	Wolf fish		
Skate	Oyster			
Surimi	Redfish			
Whiting	Scampi			
	Sole			
	Squid			
	Tilapia			
	Turbot			

In Annex III.1 of this chapter, a table is given with the English, Dutch, French, and scientific names of the 41 seafood species.

2. Traceability of seafood products on the Belgian market

2.1. Introduction on traceability

Contaminant concentrations in seafood species depend strongly on the species itself, its metabolism, its feed, and the environmental conditions, i.e. chemical contamination of the sediment and suspended particulate matter of the region(s) where it was living before its catch or during its production (Domingo *et al*, 2007a; Judd *et al*, 2003a). Since Belgium is a small country with a limited coastal region and only three commercial harbours, a large part of the seafood available on the Belgian market is imported from other countries. Moreover, the current seafood supply and food supply in general is characterized by an increasing globalization, with increasing import and export, hampering adequate food traceability. Specifically, seafood is the most traded of all food commodities (Nierentz, 2006). Hence, it is a substantial challenge to improve the seafood traceability. Traceability is defined as 'the ability to trace the history, application, or location of what is under study' (ISO 9000) (Frederiksen & Gram, 2003; Thompson *et al*, 2005) and is part of any good quality management system. The Commission of the Codex Alimentarius defined traceability or product tracing as 'the ability to follow the movement of a food through specified stage(s) of production, processing, and distribution' (FAO/WHO, 2004).

The purpose of this traceability study was to trace the origin of seafood products available on the Belgian market, in order to link them with the corresponding contaminant concentrations. As such, one may regard a traceability study as a part of an epidemiological investigation (Frederiksen & Gram, 2003). The introduction of traceability into the food supply chain is a relatively new concept, whereas traceability systems have been used for many years in several other sectors such as aviation, automobile, and pharmaceutical industry (Frederiksen & Gram, 2003). Globalization of trade and the lack of international standards made it difficult to identifying the origin and history of seafood products, raising concerns from retail, food services, and consumers about the safety of their seafood supplies. It is clear that traceability could be an important strategy to address consumer concerns about quality of the supplied seafood and declining fish populations as well as to address growing pressure from consumers to produce sustainable food (Thompson *et al*, 2005).

In 2001, the European Commission published a regulation laying down detailed rules about the information that has to be supplied to the consumer of fishery and aquaculture products (Commission Regulation EC 2065/2001) (European Commission, 2001). This regulation determines that appropriate marking or labelling for seafood products has to indicate:

1. the commercial designation of the species,
2. the production method (caught at sea or in inland waters or farmed), and
3. the fishing ground where it was caught or produced (i.e. traceability).

The latter must be documented as follows:

- for fish caught at sea, the FAO area (for more details see later) must be stated;
- for fish from inland waters the country of origin must be given;
- for farmed fish the country of the final development of the product must be given (Frederiksen & Gram, 2003).

This information must be indicated on the label or posted up in the case of fresh fish sold in bulk, e.g. in retail or fish shops.

Up to now, no database is available describing quantitatively the origin of the seafood species available for consumption in Belgium; and to the authors' knowledge no publications describe the existence of such a database in other countries. Moreover, a pan-European study by Euroconsumers showed that incorrect labelling of seafood products is the rule rather than the exception: almost 90% of the samples were labelled incorrectly (Jooken & Lauryssen, 2006). Also a recent Norwegian study evaluated the traceability systems in the supply chain of the Norwegian fish industry and food retail trade and showed that traceability labelling is unsatisfactory (Karlsen & Senneset, 2006).

The aim of the work reported in this part of chapter III was to investigate the traceability and origin of the seafood products available on the Belgian market, with the purpose of using this information for assessing contaminant intake of the Belgian population via seafood consumption. However, during the study, it became clear that many impediments exist which made the detailed execution of this work difficult. The text, therefore, focuses on the attempts made, the results obtained, and recommendations related to extra data needed to improve seafood traceability and any subsequent analyses related to benefits and/or risks depending on seafood origin. Probably, the origin of the seafood products also influence the nutrient content, but the information on nutrient concentrations was not detailed enough to take this into account in the same way as was done for the contaminant concentrations.

2.2. Materials and methods

In order to gather information about the origin of the 41 commercial seafood species available on the Belgian market (Table III.1 given in the introduction part of chapter III), four different data sources were combined:

Two **national** databases:

1. An economic database from the Central Economic Council (**CEC**) being part of the Ministry of Economic Affairs of Belgium (received in November, 2004);
2. Data on **landings in Belgian harbours**, provided by the Sea Fisheries Department, Agricultural Research Centre (received in November, 2004);

And two **international** databases:

3. The landings/production databases of seafood from the Food and Agriculture Organisation (**FAO**) (together with the software FishStat Plus Version 2.3) (http://www.fao.org; consulted in January 2005);
4. Catch data from the International Council for the Exploration of the Sea (**ICES**) (http://www.ices.dk; consulted in January 2005).

The economic **CEC-database** resulted in an Excel-file containing Belgian import and export data of all seafood products (fresh, frozen, canned). The database contained for each seafood product the flow (import or export), the product name (mentioning one or more scientific names of seafood species), the Dutch name, the country of import or the country to which it was exported, the amount in tons imported or exported, and the value in euros. The **FAO** provided data about the annual catch and production data of all different seafood species by all countries. **ICES** collected data on annual landings officially submitted by 19 ICES Member States in the Northeast Atlantic Sea including over 200 species. For this study, a combination was made of different FAO-datasets and the ICES-dataset in order to link this combined datasets with the CEC-data and describe as detailed as possible the catching and production of seafood with respect to the various fishing grounds.

The FAO classification was applied to describe the origin of the seafood species. The FAO defined worldwide 24 different fishing grounds. Six codes describe a zone of inland waters and 18 describe a sea or a part of an ocean (Table III.2 and Fig. III.1).

Table III.2: Area codes and names of the 24 international fishing grounds all over the world (www.fao.org)

	Area code	Area name
Continents	1	Africa - Inland waters
	2	America, North - Inland waters
	3	America, South - Inland waters
	4	Asia - Inland waters
	5	Europe - Inland waters
	6	Oceania - Inland waters
Parts of an ocean	21	Atlantic, Northwest
	27	Atlantic, Northeast
	31	Atlantic, Western Central
	34	Atlantic, Eastern Central
	37	Mediterranean and Black Sea
	41	Atlantic, Southwest
	47	Atlantic, Southeast
	48	Atlantic, Antarctic
	51	Indian Ocean, Western
	57	Indian Ocean, Eastern
	58	Indian Ocean, Antarctic
	61	Pacific, Northwest
	67	Pacific, Northeast
	71	Pacific, Western Central
	77	Pacific, Eastern Central
	81	Pacific, Southwest
	87	Pacific, Southeast
	88	Pacific, Antarctic

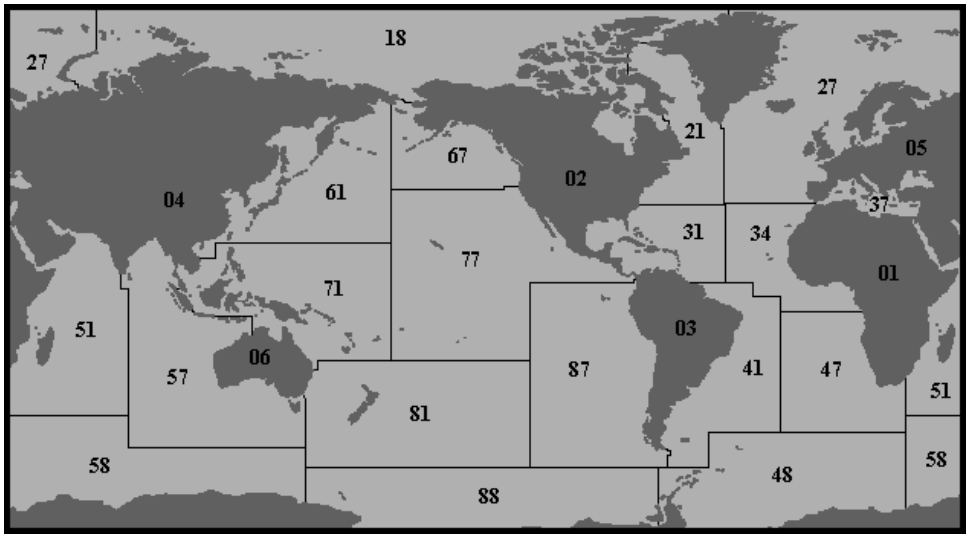


Fig. III.1 The 24 international fishing grounds as defined by FAO (www.fao.org)

In addition, it was of interest to subdivide four fishing grounds and define the origin by their subdivisions, since they are important regions for the seafood on the Belgian market:

1. the North-eastern Atlantic Ocean,
2. the Eastern Central Atlantic Ocean,
3. the South-eastern Atlantic Ocean,
4. the Mediterranean & Black Sea.

As such, smaller seas, e.g. the Baltic Sea and the North Sea, became separated entities and were not just considered together with a lot of other seas as the North-eastern Atlantic Ocean.

The applied **methodology** to determine the origin of the available seafood products consisted of two consecutive steps:

1. In a **first step**, the countries of origin were determined for the seafood products on the Belgian market.
2. In a **second step**, an attempt was made to express their origin in terms of fishing grounds.

2.2.1. STEP 1: Defining the countries of origin

With respect to seafood imports, only the import data from other countries into Belgium were taken into account in this phase (step 1) of the data management process. Thus, Belgian landing data (i.e. catching data of the Belgian fleet) were excluded at this stage, since these data were provided by the Belgian Sea Fisheries Department already containing details about the origin (expressed on the basis of the FAO classification). Moreover, only data of the year 2000 were used, describing quantitatively the different countries of import for each seafood product on the Belgian market in 2000.

Considering the seafood imported from other countries to Belgium, a large amount of the imported seafood is again exported, which need to be taken into account in step 1. Lacking more detailed information, the **assumption** had to be made that exporting seafood products from Belgium to other countries (whether it were own landings or imports) did not change the country's proportional import share. The result of this first step was that for each seafood product available on the Belgian market the ratio coming from each country of interest was calculated in terms of percentages.

2.2.2. STEP 2: Defining the fishing grounds of origin

In this second step, the relative amounts per country were converted to relative amounts per fishing ground by applying the combined dataset of the FAO data and the ICES data. To do so, the data were sorted per species (based on the scientific name) and per country (result of step 1) and for each of these species-country combinations the amounts caught or produced per fishing ground were given in tons (result of step 2). In step 2, a **second assumption** had to be made, considering that the country of import is the same as the country of origin (assuming that there was no transit).

In practice, two databases were constructed: CEC2000 and FAO2000. **CEC2000** describes the amount imported from all relevant countries (species-country combination), for each seafood product on the Belgian market (defined by a product name). **FAO2000** describes the amount caught or produced in each relevant fishing ground (species-country-fishing ground combination), for each seafood species and all countries all over the world. These two databases were then linked to each other at the level of species and country by creating 101 Sp-codes (*Species*-codes) and 1022 unique SpC-codes (*Species-Country*-codes). As such, the species-country combinations in both files could be described by that code. Subsequently, the distribution per SpC-code out of CEC2000 over the different fishing grounds was calculated case by case by multiplying the amount imported in ton by the relative percentage caught or produced by that country over the different fishing grounds (found by looking up the corresponding SpC-code in FAO2000).

Up to this point in the procedure, the data describing the landings in the Belgian harbours in 2000 were not taken into account. These data were available in a database already split up per fishing ground. Subsequently, these data were added to the newly composed database. Thereupon, for each Sp-code the distributions over the fishing grounds were summed in order to get the overall division per species without the separation per country. Finally, the relative percentages for each species per fishing ground were calculated to reach the objective of the study.

2.3. Findings

2.3.1. Countries of origin (result of step 1)

From the CEC2000 data, it appeared that in the year 2000 219,000 tons of seafood was imported from 116 countries to Belgium, spread over the five continents and 26,000 tons seafood was landed in Belgian harbours. This yields a total amount of 245,000 tons of seafood entering Belgium in 2000 from which 89% was imported. Of the total of Belgian imports and landings, 90% was obtained from only 22 countries (Belgium inclusive) (Table III.3), 71% of which originated from European countries. When different seafood groups are considered, it appeared that 98% of the shellfish was imported, and imported finfish accounted for 85% of the total finfish supply. As shown in Table III.3, more than 50% is supplied by Belgium and three European countries: The Netherlands, France, and Denmark. It is important to indicate that the table describes the countries of import, not of origin. Of the total amount of 245,000 tons of seafood entering in Belgium, 40% (99,000 tons) were subsequently exported to other countries, leading to 146,000 tons available on the Belgian market for consumption. This is roughly 14.6 kg/year/caput or 280 g/week/caput.

Table III.3: The 22 most important countries supplying seafood for the Belgian market, with their percentage supplied relative to the total amount (% of 245,000 tons)

The Netherlands	23.9	Vietnam	2.0	Thailand	1.1
Belgium	10.6	China	1.9	Senegal	1.0
France	9.3	India	1.7	Ireland	1.0
Denmark	7.7	Sweden	1.6	Uganda	0.9
Germany	7.0	United States of America	1.6	Indonesia	0.9
Tanzania	6.4	Canada	1.5	Ecuador	0.9
United Kingdom	3.7	Spain	1.5		
Iceland	2.4	Bangladesh	1.3		

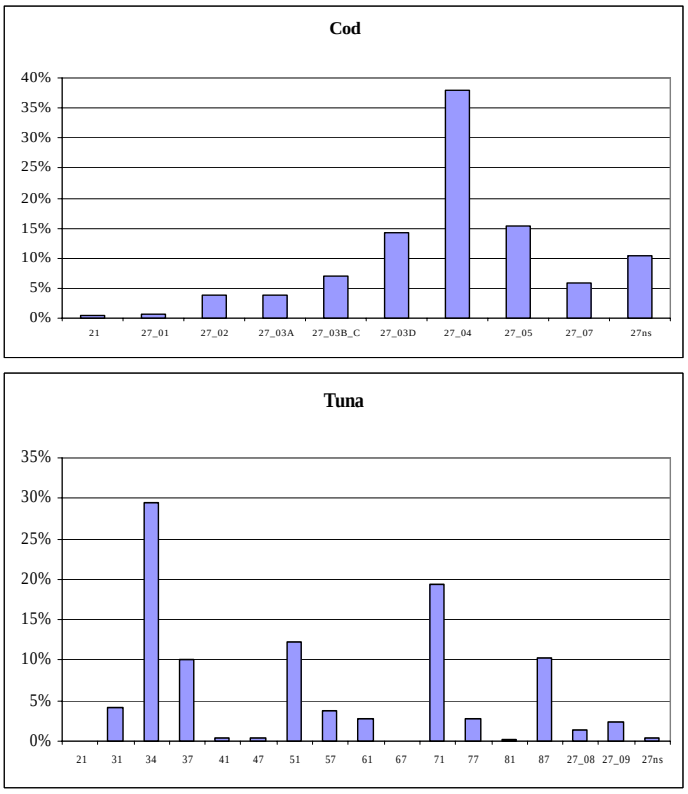
2.3.2. Fishing grounds of origin (result of step 2)

Two assumptions had to be made before distributing the imported seafood products on the Belgian market over the different fishing grounds expressing their origin.

1. The **first** one involved assuming that the country of import did not differ from the country where the seafood was captured or produced. It should be noted that this is not correct in all cases since it is known that several countries import raw fish from a country with an extensive seafood capture or production capacity to process them (peeling of shrimps, filleting of fish …) and export post processing. But as no quantitative information was available concerning transit of seafood products, there was no alternative for this assumption.

2. A **second** assumption was related to the observation that a large amount of fish imported or caught in Belgium is exported once again to other countries. This hampers the determination of the origin of marine food products, since it is unknown what the origin of the exported products really is: they may be landed or imported from various countries. The assumption was made that exporting a certain seafood species out of Belgium to other countries (whether it were own landings or imports) did not change the ratio describing the different countries of origin for that species.

Within the assumptions made, the combination of the estimated data indicated that more than 50% of the seafood products on the Belgian market originated from the Northeast Atlantic Area, with the North Sea being the most important sub area (accounting for 13%). Fig. III.2 shows the results for two individual seafood species frequently consumed in Belgium. This figure indicates that almost all cod available on the Belgian market origins from the Northeast Atlantic Sea. Tuna originates from many different FAO areas, with the Eastern Central Atlantic Sea contributing the most.



Area code	Area name (FAO)
21	Atlantic, Northwest
27	Atlantic, Northeast
27_01	Barents Sea
27_02	Norwegian Sea, Spitsbergen & Bear Island
27_03A	Skagerrak and Kattegat
27_03B_C	Sont and Belten
27_03D	Baltic Sea
27_04	North Sea
27_05	Iceland and Faroer Islands
27_07	Irish Sea and coast
27_08	Gulf of Biskaje
27_09	Portuguese coast
31	Atlantic, Western Central
34	Atlantic, Eastern Central
37	Mediterranean and Black Sea
41	Atlantic, Southwest
47	Atlantic, Southeast
51	Indian Ocean, Western
57	Indian Ocean, Eastern
61	Pacific, Northwest
67	Pacific, Northeast
71	Pacific, Western Central
77	Pacific, Eastern Central
81	Pacific, Southwest
87	Pacific, Southeast

Fig. III.2 The calculated origin of two frequently consumed fresh fish species in Belgium (in %)

2.4. Discussion and conclusion

Pàlsson *et al* (2000) reported that the fish industry mainly uses paper based traceability systems. Although this is gradually changing owing to information technology advancements (Cibot & Kolypczuk, 2003), important barriers to its application in the field persist. Software systems allowing for the integration of financial and production data in one software package are typically too costly for the small business units in the fish industry (Frederiksen & Gram, 2003). Questions rise as to how artisanal and small exploitations at the primary production level can cope with regulatory and contractual traceability requirements (Lupin, 2006). Even when these practical issues would be solved, the lack of an electronic database bringing together all relevant information on (inter)national level remains a major challenge related to the traceability of commercial seafood. This lack of one database causes problems to determine the origin of the products and might also hamper withdrawal or recall of defective or hazardous products. Moreover, even if the data resulting from European legislation had led to a consistent database, the enforced level of detail might not be sufficient, e.g. the Baltic Sea cannot be distinguished from the North Sea, since both fishing grounds are located in the FAO area North-eastern Atlantic Ocean.

Lupin (2006) notes that 'despite the noticeable development of traceability systems, some important questions remain open, particularly at the level of international food and fish trade'. Apart from regulatory issues, approvals of principles and practical implications, one of these open questions relates to the establishment of centralised databases. This traceability study made clear that it is very hard – even in the case of a small and well-defined market – to trace the origin of commercial seafood products until consumers' dishes; this, in spite of all existing regulations concerning traceability of food items and marking and labelling of commercial food products. Examples of voluntary chain traceability systems including continuous recording and maintaining of all relevant information on a central database are available (e.g. TRACEFISH). Nevertheless, to the authors' knowledge, no existing market-level databases covering fish species, volumes, and origin are available. In absence of a clear and useable database, this study aimed at establishing the origin of seafood products on the Belgian market. Therefore, efforts were made to collect data from different sources and to link them.

Even though the market under consideration is rather small and well defined, important problems were encountered. **First**, the information needed came from different, non related sources, which can hardly be linked. **Second**, countries of import did not necessarily define fishing grounds or product sites and several assumptions had to be made in this respect. **Third**, even when one is able to retrieve information about the origin of seafood in terms of fishing grounds, the relevance of this information remains uncertain since seafood species caught at a certain fishing ground might have transited many others during their life. **Finally**, the purpose of studying the origin of seafood products was to use the numerical results in the assessment of the intake of contaminants via seafood consumption. This also necessitates the availability of contaminant concentration data in seafood products specifying the related fishing grounds. In contrast to contaminants, for nutrient concentration in seafood not enough detailed information was available specifying the related fishing grounds. Therefore, for nutrients the origin was not taken into account.

It is indeed a challenge to investigate the traceability of seafood on national markets, since seafood is the most traded food item and given its substantial health benefits and eventual safety risks for consumers. Moreover, since regulations exist on European level to trace and label seafood with its origin, some extra efforts on the level of data collection can hopefully lead to (inter)national databases making seafood much more traceable. When the latter efforts are performed, it will be of interest to label the origin of the seafood on such a level of detail that it becomes more meaningful for consumers, policy makers, and researchers in the field of public health. This exercise has exemplified some of the difficulties encountered in determining the origin of seafood products on a particular market and aimed at identifying limitations and questions, which hopefully can be solved when traceability systems become a truly operative reality after some transitory period (FAO, 2004).

3. Development of a nutrient database for use in a probabilistic risk-benefit analysis of human seafood consumption

3.1. Introduction

The probabilistic approach for the intake assessment of contaminants in foods is increasingly being used (Carrington & Bolger, 2002; Gibney & van der Voet, 2003; Gilsenan *et al*, 2003; Lopez *et al*, 2003; Matthys *et al*, 2005; Solomon *et al*, 2000; Tran *et al*, 2004; Vrijens *et al*, 2002; Wenning, 2002). In contrast, for the assessment of the nutrient intake it is still rather uncommon (Rubingh *et al*, 2003). However, since many foods show considerable variability in nutrient concentrations, this approach is for many purposes more informative than the deterministic approach. Particularly for seafood, the fat concentration and the fatty acid composition is highly variable and largely depending on environmental factors like temperature of the water, season, trophic conditions in various habitats, feed, etc. (Copeman & Parrish, 2004; Mattila *et al*, 1997). By using probability distributions to describe the fat concentration, the fatty acid composition and other nutrients, this environmental variability will maximally be incorporated into the intake assessment. Moreover, as about 89% of the seafood products on the Belgian market are imported from a range of countries (part 2 of this chapter), it seems useful to take into account data from different national food composition databases (FCDBs).

A key component in a probabilistic intake assessment is the selection of the most appropriate probability distributions for any given data set, whether it be nutrients, contaminants, or other parameters (Gilsenan *et al*, 2003). It is recognized that the determination of such distributions will have to rely on the collection of quantitative data being accurate (i.e. agreeing with the actual concentration) and representative (i.e. reflecting the concentration of the whole group, in this case the species in general). This chapter part describes the problems encountered when nutrient concentration data for seafood were brought together in a new extensive database in order to fit useful probability distributions. The next part of this chapter (part 4 of chapter III) handles the same topics related to the contaminant concentration data.

3.2. Materials and methods

An Excel®-database containing nutrient concentrations for seafood species was build in order to have a dataset for the distribution fitting. Total fat content, EPA plus DHA concentration, vitamin D, and iodine have been taken into consideration. The following sources were used:

- fourteen FCDBs (Carnovale & Marletta, 2000; Danish Institute for Food and Veterinary Research, 2005; Favier *et al*, 1995; Food Standards Agency, 2002; Health Canada, 2005; Holland *et al*, 1993; Institut Paul Lambin, 2004; National Public Health Institute of Finland, 2004; NEVO Foundation, 2001; NUBEL, 1999; Salvini *et al*, 1998; Souci *et al*, 2000; Sugiyama Jogakuen University, 2004; US Department of Agriculture and Agricultural Research Service, 2005),
- 44 peer reviewed research papers (mostly about original research),
- two books (Ackman, 2000; Sondergaard & Leerbeck, 1984),
- own analytical data.

The selection of the FCDBs is based on their availability, either as a book or free available via internet (found via LanguaL-website maintained and supported by the EuroFIR Consortium and funded under the EU 6th Framework Food Quality and Safety Programme). In the new compiled nutrient database, all relevant information was included: commercial name, scientific name, culinary processing procedure (if relevant), farmed or wild fish (if known), number of samples, number of individual sample units per sample in the case of composite samples, (mean) fat content of the fish, and (mean) nutrient content, with extra statistical data if available (standard deviation, minimum and maximum). In contrast to the contaminant database, no distinction was made according to fishing grounds because not enough detailed information was available to do so.

In order to perform a probabilistic intake assessment of nutrients (and contaminants) via seafood for the Belgian populations, nutrient concentration data for all considered seafood species were needed and distributions to describe those concentrations had to be selected. The distribution fitting program BestFit® Version 4.5 (Palisade Corporation, Newfield, NY, USA, 2002) was used to determine the distribution model and parameters that describe the nutrient concentrations as well as possible. For each specie and nutrient, the concentration data points of the new developed database were entered in BestFit® in combination with a weighing factor, expressing the cumulative probability of occurrence for each concentration. To do so, BestFit® needs at least three data pairs. The program identifies the best describing

distributions to a given dataset using goodness-of-fit tests (the method of the least squares). The final selection of the distribution model was based on this test as well as on visual evaluation of the probability plot (Palisade Corporation, 2002). Distribution models that can give rise to unrealistic high or low concentrations were truncated as follows: on half of the all-time lowest concentration at the lower end of the distribution and on the double of the all-time highest concentration at the higher end of the distribution.

3.3. Results and discussion

3.3.1. Building up a database

During the development of the database, a number of problems with respect to methodological issues were encountered and are presented here, along with the proposed procedures to solve them.

A **first** problem was related to the **description of the food items**, in this case the seafood species. From country to country, inconsistency exists in naming fish and other seafood. Seafood can be sold in different regions or countries under the same name, but being entirely different species, even from a different genus, and consequently having a different nutrient composition, e.g. salmon can be used to describe *Salmo salar* and *Oncorhynchus spp*. To further complicate matters, the opposite situation also exists, i.e. that different common names may be applied to describe one single seafood species, e.g. dab and flounder are used as common name to describe *Pleuronectus limanda*. Therefore, an unequivocal food description is of primary importance to avoid confusion between different species when bringing data from different sources together. In the compiled database, it was aimed to describe all seafood species by their scientific name. In most of the publications used, the scientific name of the species for which data were reported was given. In contrast, in some of the applied FCDBs the food description lacked this level of detail. In these cases, an assumption had to be made about the scientific name of the species on the basis of the common name indicated. Based on these findings, the importance of an unequivocal food description in FCDBs and other publications reporting nutrient composition data is once again demonstrated.

A **second** problem was that **different sampling strategies** (sampling plans) were used over the different publications reporting nutrient contents in seafood. According to Holden *et al* (2002) an ideal sampling plan should address a demographic framework as well as the profiles and attributes of the food sampled in order to obtain nationally representative samples. When looking to the articles used:

- some researchers obtained their samples from local fish wholesalers (Mattila *et al*, 1995; Sérot *et al*, 1998),
- others purchased them from various markets or retail stores over a certain region (Piironen *et al*, 2002; Takeuchi *et al*, 1984),
- others tried to cover different fishing grounds (Mattila *et al*, 1997),
- etc.

Holden *et al* (2002) set up a range of criteria in order to evaluate the data according to their sampling plan. In this study, no weighting of the data was applied according to the representativeness of the sampling procedures since no objective, quantitative criteria were available to do so. A difference is only made between the results of individual sample units and composite samples. Compositing of sample units is a possible way to guarantee the value of an individual measurement as it represents a mean of several units. It can, however, result in the compression and underestimation of the variability (World Health Organization, 2000). Only a minority of the articles used reports the application of composite samples and reports the number of individual units in each sample. Nevertheless, a distinction between both types of data (composite samples and individual sample units) was made by weighing the data according to the number of sample units, such that concentrations measured in composite samples get a higher probability of occurrence. A weighing factor $\mathbf{W_{i,unit}}$ for each concentration data point x_i was created. $W_{i,unit}$ was equal to one in the case of an individual sample unit and equal to the number of units per sample in the case of composite samples. Hence, the concentration measured in a composite sample of n individual units was considered as the mean of n individual samples (nevertheless, this ignores the fact that n fish samples caught together are likely to have more similar concentrations than the same number of fish samples caught separately). When the number of individual sample units per composite sample was unknown, $W_{i,unit}$ was assumed to be one, leading to the assumption that the values from publications not giving the appropriate details have a lower weight.

Third, analytical results in different publications are based on a different number of samples and are **reported in different ways**. In some publications, all individual measurement results are reported, whereas others only report mean values of a group of samples (aggregated results). As indicated by the WHO, the preferred method for generating a distribution curve for a nutrient or contaminant in food is to use data on individual measurements. When data on individual measurements are not available in sufficient quantities (or quality) to generate a distribution, the next preferable option suggested by the WHO – and adopted in this study - is to combine data from different sources, from both individual and aggregated results (World Health Organization, 2000). For the purpose of using the collected data in an appropriate way as basis for the fitting of the most suitable distribution, a second weighing factor $\mathbf{W_{i,meas}}$ was applied for each data point x_i as a function of the number of measurements on which it was based. When the number of measurements was not known, $W_{i,meas}$ was assumed to be one, thereby assigning a lower weight in the overall distribution to results reported without detailed information. In the case of FCDBs, less than half of the FCDBs used mentioned the number of samples used for the nutrient content determination. According to Greenfield and Southgate (2003), the number of samples should be at least 10 for primary produce for FCDBs. Holden *et al* (2002) suggested even that more than 25 analytic sample units would be needed to improve the reliability of the estimated mean. Because this is a theoretical situation and not reality, the number of samples – when not mentioned in the FCDB - was assumed to be one. Eventually, for each data point x_i in the compiled database, the second weighing factor $W_{i,meas}$ (expressing the number of measurements) was multiplied with the first weighing factor $W_{i,unit}$ (expressing the number of individual sample units – see above), in order to have an overall weighing factor:

$$W_{i,total} = W_{i,meas} \cdot W_{i,unit}$$

Subsequently, after ranking all the available data points x_i per species and per contaminant, the cumulative probability of occurrence of each data point x_i was calculated as:

$$F(x_i) = \frac{\sum_{i} W_{i,\,total}}{\sum_{n} W_{i,\,total}}$$

where i is the rank number, $W_{i,total}$ is the overall weighing factor, and n is the total number of data points. Afterwards, using BestFit®, the data were administered together with the weighing factor expressed as a cumulative probability of occurrence. An example of a distribution showing the variability of a nutrient concentration as a function of the probability of occurrence is given in Fig. III.3. Extra information about the influence of using the weighing factor is given in the next part about the contaminant database.

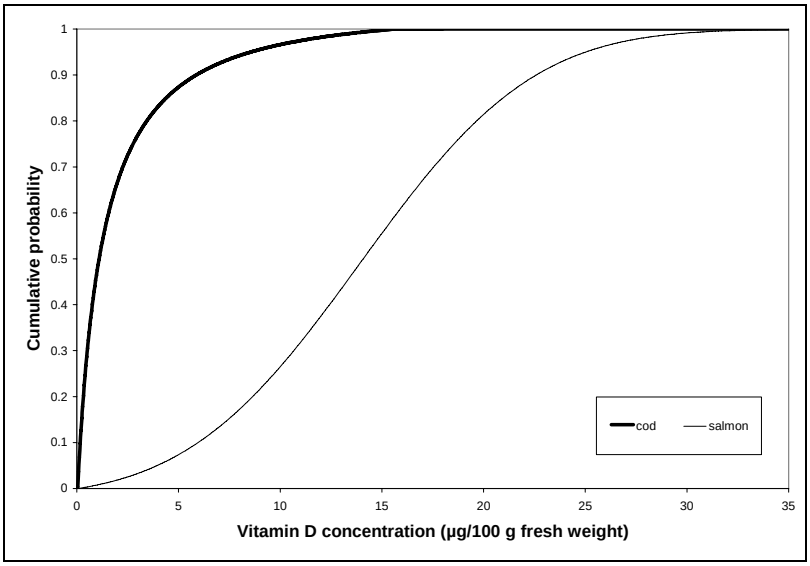


Fig. III.3 Cumulative probability function of the vitamin D concentration in cod and salmon

Fourthly, FCDBs report **not only values from analyses** done in own laboratories. Data in FCDBs are generated:

- by chemical analyses of food samples (from the food industry, from government agencies, …),
- by calculations (by recipes or formulations),
- by expert estimation or by copying from other FCDBs, or
- from scientific literature.

In practice:

- five of the 14 FCDBs used did so and mentioned for each value the source (Danish Institute for Food and Veterinary Research, 2005; Health Canada, 2005; National Public Health Institute of Finland, 2004; NEVO Foundation, 2001; Salvini *et al*, 1998)
- four others mentioned that some values in the database came from other sources without reporting the specific references and without marking the copied values (Favier *et al*, 1995; Food Standards Agency, 2002; Holland *et al*, 1993; US Department of Agriculture and Agricultural Research Service, 2005)
- five FCDBs did not give any detailed information about the sources of their data (Carnovale & Marletta, 2000; Institut Paul Lambin, 2004; NUBEL, 1999; Souci *et al*, 2000; Sugiyama Jogakuen University, 2004).

On the one hand, values were copied without any modification. But on the other hand, values were copied from one publication to another for applying them for a similar but not identical species or the copied values were used after recalculation. For example, the vitamin

D content of anchovy in the Italian FCDB (Salvini *et al*, 1998) was taken over as such from the vitamin D content of sardine given in the English FCDB (Holland *et al*, 1993). An example of the recalculation method is the vitamin D content of raw red salmon in the Canadian FCDB (Health Canada, 2005), which was calculated based on the known fat content of that salmon and the vitamin D (in µg/g fat) content of that species given in the USDA FCDB (US Department of Agriculture and Agricultural Research Service, 2005). When detailed source information was available, it was possible to take this information into account and then the following rules were applied:

- when a value did not contain any new information (used for the same species without recalculation), the value was not taken into consideration a second time;
- as soon as a copied value contained new information (used for another species or recalculated), it was included for further use.

When no detailed information was available about the sources of the data, no distinction could be made between copied and non-copied values and they were taken over and considered as new values, leading to a potentially erroneous higher weighing factor.

A **fifth** remark concerns the application of **different analytical methods** for the determination of a same nutrient over the different publications. During the last thirty years, different analytical methodologies were applied for the determination of vitamin D. The older fish vitamin D data are based on the application of biological methods (Sondergaard & Leerbeck, 1984). The vitamin D contents of Sondergaard and Leerbeck (1984) based on biological methods, varied according to the species from 0.5 to 30 µg/100 g fresh weight. Takeuchi *et al* (1984) - using an HPLC method to determine the cholecalciferol (one of the vitamin D steroids) content in fish - obtained a range from 0 to 132 µg/100 g fresh weight. Matilla *et al* (1995; 1996) also applied HPLC to determine the vitamin D content in fish species used in Finland, analysing cholecalciferol as well as 25-hydroxycholecalciferol and ergocalciferol. The variation found over the different fresh fish species ranged from 0.3-47.7 µg/100 g fresh weight. Mattila (1995) reported that many discrepancies occurred in the vitamin D concentrations of fish and fish products given in the English FCDB (Food Standards Agency, 2002; Holland *et al*, 1993) compared to their own studies e.g. the English FCDB indicated that cod contains only traces of vitamin D, whereas Matilla (1995) measured 6.9±0.2 µg vitamin D per 100 g raw cod. According to Mattila (1995), the English information is based on the estimation of Holland *et al* (1993) that all the fish species with white flesh contain only traces of vitamin D and this information is taken over in several other FCDBs,

which brings us again to the already mentioned problem of copying values. This creates a bias when comparing the data and using them all together for fitting one distribution expressing the nutrient concentration and its variability in a species. Moreover, this implies another problem about how to deal with the indications 'traces'. When FCDBs report 'traces', this means that the species contains amounts of the nutrient being below the limit of quantification (LOQ), i.e. the minimal amount necessary for quantification. A problem arises if no numerical value of the LOQ is mentioned in the publication. In this study, this was a frequent problem for vitamin D concentrations (17% of the data). To replace the indication 'traces' by real figures that can be used for the distribution fitting procedure, an assumption had to be made about the LOQ. Salo-Väänänen *et al* (2000) reported LOQ of 0.01 µg/100 g for cholecalciferol (defined as three times the detection limit (LOD), being the lowest level at which a nutrient can be detected), whereas a report of the FSA indicates a LOD of 0.05 µg/100 g and a LOQ of 0.1 µg/100 g (defined two times the LOD) (Lawrance, 2002). Based on that recent report, half of the LOQ (0.05 µg/100 g) is taken to replace the indications 'traces'.

A **sixth** and last encountered problem concerns the nutrient data of **raw versus processed** seafood products. For **vitamin D** concentrations, different studies showed that the effect of processing on vitamin D is very small (Aminullah Bhuiyan *et al*, 1993; Lawrance, 2002; Mattila *et al*, 1999; Suzuki *et al*, 1988). For instance, Matilla *et al* (1999) found that the losses of vitamin D were smaller than 10% after household cooking. Therefore, all available vitamin D concentration data of raw and processed seafood products are used without distinction. Only those, describing the content in seafood products also containing other ingredients (flower, milk, tomato sauce, …) were excluded. The same approach was applied for the **iodine** concentrations.

There is quite some knowledge that processing influences the **fatty acid** composition of seafood products (Agren & Hanninen, 1993; Al Saghir *et al*, 2004; Candela *et al*, 1997; Candela *et al*, 1998; Gall *et al*, 1983; Gokoglu *et al*, 2004; Mai *et al*, 1978; Sanchez-Muniz *et al*, 1992; Sioen *et al*, 2006b). Also own research investigated the effects of pan frying cod and salmon fillets in margarine and olive oil on the fatty acid composition. The results showed that the effect of pan frying of fish fillets depends on the total fatty acid content of the fish species and on the fatty acid profile of the culinary fat used. In lean fish a significant increase in the amount of fatty acids was detected, leading to a fatty acid profile similar to the profile of the culinary fat

used. In fatty fish, there was an insignificant decrease in total fatty acid content. The alterations in the fatty acid profile of the fatty fish also changed the fatty acid profile in the direction of that of the culinary fat. Therefore, control over the fatty acid content and composition of the consumed food can be influenced by the selected culinary fat (Sioen *et al*, 2006b).

However, the existing knowledge is not extensive enough to build a database with conversion factors for all species, all preparing methods, and all culinary fats. Moreover, taking into account such information in the intake assessment would only be possible if this information was also known at the consumer side. Due to this lack of knowledge, the same approach as applied for vitamin D and iodine was also applied for the fat and EPA plus DHA concentration data, i.e. that **no distinction was made for processing**. Additionally, it was found that the variability in the EPA plus DHA concentration data of the raw samples in the developed database was higher than the changes induced by processing, in other words, the overall variability did not change by taking the data of processed seafood products into account. For example, the EPA content in raw trout ranged from 100 mg/100 g fresh weight (Hepburn *et al*, 1986) to 606 mg/100 g fresh weight (National Public Health Institute of Finland, 2004). The values of the EPA content in prepared trout varied between 200 mg/100 g prepared weight (Holland *et al*, 1993) and 468 mg/100 g fresh weight (US Department of Agriculture and Agricultural Research Service, 2005). Also Stolyhwo *et al* (2006) found that the variability in raw herring and sprats was larger than the changes induced by smoking or by storage.

3.3.2. Collected data and fitted distributions

The result of the collected data and their weighing factors formed the basis for the distribution fitting and selection process. The available data are presented here as box plots. In such a figure, each box represents the interquartile range (25^{th} to 75^{th} percentile). The bold square in the box expresses the median value (50^{th} percentile). The whiskers extend from the boxes and indicate the upper and lower values not classified as statistical outliers or extremes. Open circles are statistical outliers.

3.3.2.1. EPA & DHA

Fig. III.4 shows box plots of the published EPA plus DHA concentrations in the different species of interest, where four or more data points were available. The species are sorted according to their median EPA plus DHA concentration.

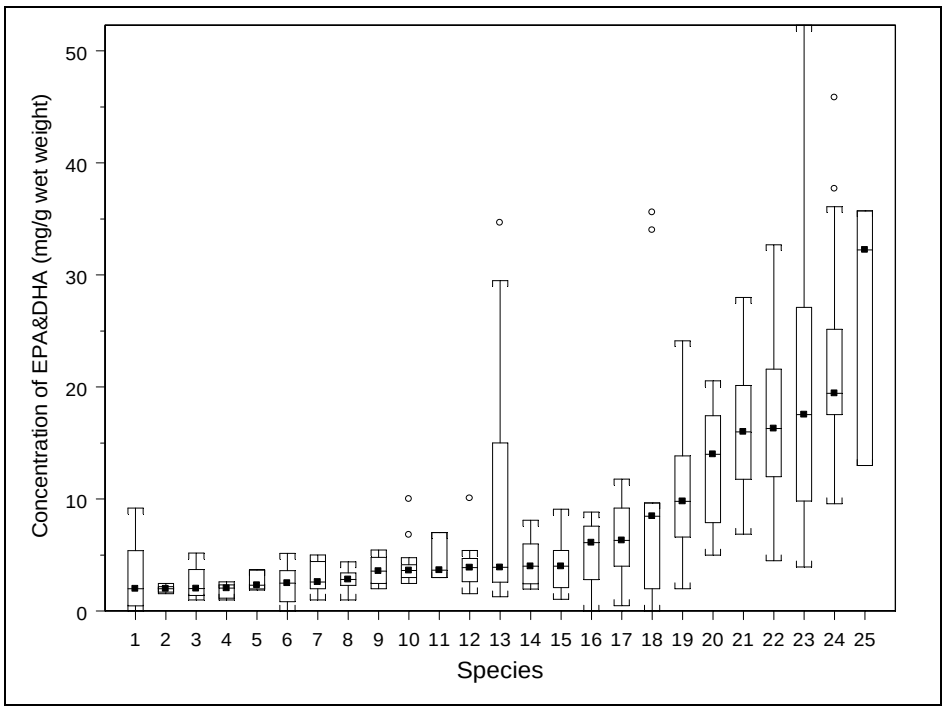
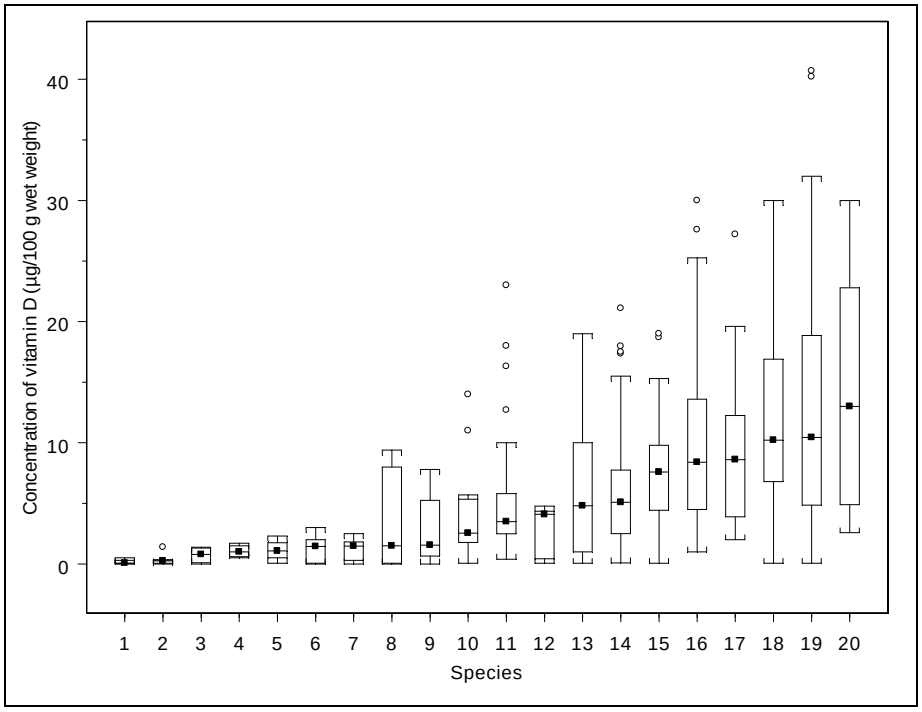


Fig. III.4 Concentrations of EPA plus DHA in different species, box plots. Data available from the open literature and from national and international reports

The figure shows that trout, anchovy, herring, salmon, sardines, mackerel, and sprat contain a high concentration of LC n-3 PUFA. Nevertheless, the figure makes clear that a high within variability exists in the EPA plus DHA concentrations. In other words, not only the fish species, but a lot of other factors influence the estimated PUFA concentration, leading to high within-species variability. For three species (skate, John dory and conger) only two data points were available and are, therefore, not shown in the box plots. Their EPA concentration is defined by a uniform distribution, using the two data points as minimum and maximum. For three other seafood species: Norway lobster, Nile perch, and sea bream, no EPA plus DHA concentrations could be found. Their EPA plus DHA concentration is therefore described by these of lobster, sole, and wolf fish, respectively, because of the similar fat content. The distribution of the EPA plus DHA concentrations for the considered species as well as the number of available data points per species can be found in Annex III.2 of this chapter.

3.3.2.2. Vitamin D

Fig. III.5 shows the published data of vitamin D for different species, ordered by the median vitamin D concentration.



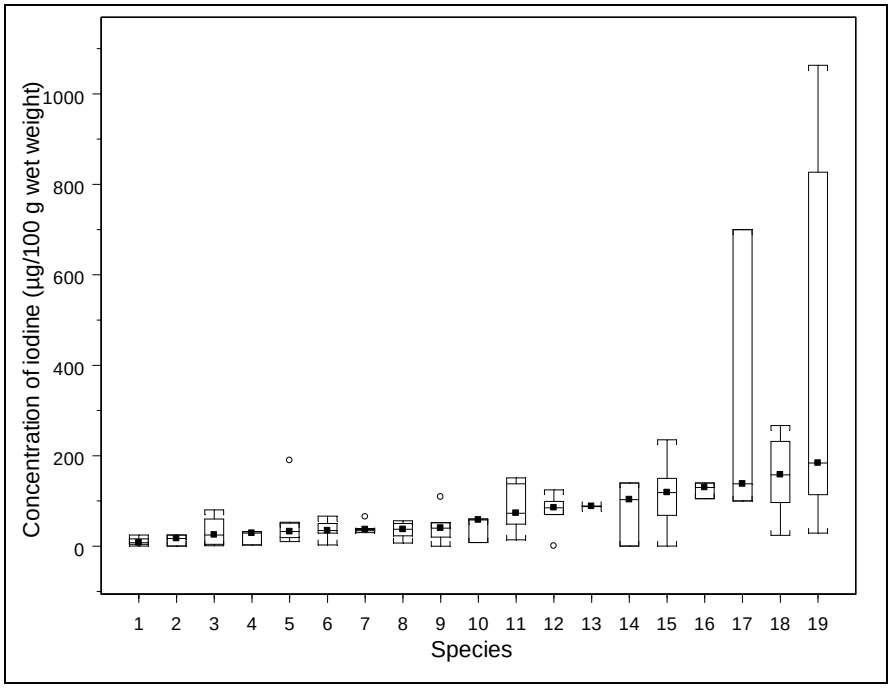
1.Scampi;
2.Haddock;
3.Mussel;
4.Wolf fish;
5.Sea bream;
6.European plaice;
7.Saithe and Pollack;
8.Sole;
9.Cod;
10.Anchovy;
11.Tuna;
12.Whiting;
13.Halibut;
14.Mackerel;
15.Trout;
16.Sardine;
17.Caviar;
18.Salmon;
19.Herring;
20.Eel

Fig. III.5 Concentrations of vitamin D in different species, box plots. Data available from the open literature and from national and international reports

Sardine, salmon, herring, and eel are the species with a rather high vitamin D concentration, but all with high within-species variability. Also caviar contains quite high vitamin D concentrations. Since only few data were available for sprat (therefore, not shown in the box plot), and since it is of the same family as herring, the distribution of herring is also used to describe the vitamin D content of sprat. For eight non-fish seafood species (crustacean, shellfishes and molluscs) and six fishes with a rather low fat content, the vitamin D concentration was described by a uniform distribution with zero as minimum and the LOD (0.1 µg/100 g) as maximum, since the available published concentration data only indicated 'traces'. Annex III.3 shows for the relevant fish species the distribution of their vitamin D concentration as well as the number of available data points per species.

3.3.2.3. Iodine

In contrast to EPA plus DHA as well as vitamin D, iodine is a water soluble nutrient. Nevertheless, literature data showed that the iodine concentration is influenced by the fat concentration of the fish, as well as by its habitat (saltwater fish versus freshwater fish). Fig. III.6 shows box plots of the iodine concentration in different species.



1.Trout;
2.Sole;
3.Eel;
4.Sardine;
5.European plaice;
6.Herring;
7.Salmon;
8.Tuna;
9.Halibut;
10.Oyster;
11.Mackerel;
12.Red fish;
13.Tilapia;
14.Caviar;
15.Saithe and Pollack; 16.Mussels;
17.Lobster;
18.Haddock;
19.Cod

Fig. III.6 Concentration data of iodine in different species, box plots. Data available from the open literature and from national and international reports

High within species variability was found for cod and lobster. Due to lack of data for some seafood species, data of different species with similar characteristics were aggregated and distributions were fitted based on the aggregated data. Two times five groups were made, related to the fat groups but separated for saltwater versus freshwater species. The distributions as well as the number of available data points per species can be found in Annex III.4.

3.3.2.4. Fat

The most important reason to gather data on the total fat content of the different seafood species was to study the correlation between the intake of fat and the intake of other nutrients and contaminants via seafood consumption. This information also formed the basis for the determination of the different fat groups. Data about the fat content are not shown

here, but Annex III.5 shows the distribution of the fat concentration for the relevant seafood species.

3.4. Conclusion

A key component in probabilistic intake assessments is the selection of the most appropriate input distributions to describe the parameters. This part of chapter III described the construction of a nutrient database, pooling fat, EPA plus DHA, vitamin D, and iodine concentrations in seafood from different FCDBs and publications together with the encountered problems related to a lack or inconsistency of information given in these publications: food description, number of samples, sampling plan, sources of the values, LOQ, etc. Different solutions have been proposed and the work resulted in a huge database allowing to describe distributions of fat, EPA plus DHA, vitamin D, and iodine concentrations and their variability in seafood species relevant for Belgian consumption. The distribution fitting and selection procedure resulted in different distribution models for the different species and nutrients.

4. Development of a contaminant database for use in a probabilistic risk-benefit analysis of human seafood consumption

4.1. Introduction

Different seafood contamination databases existed already (e.g. NIFES (2004); ICES (Raemaeckers, 2005); CFSAN (US Department of health and human services and US Environmental Protection Agency, 2004)), but none of them as such contained enough information to describe the concentrations in all species available on the Belgian market. Therefore, a de novo establishment of a contaminant database for use in this PhD-thesis was envisaged. In line with the previous description of the elaboration of the nutrient database, this part of chapter III describes the methods that were used, the problems that were encountered, and the solutions that have been proposed in the course of

1. the development of an extensive contaminant database, using published sources only,
2. the characterization of appropriate input distributions describing the variability of contaminant concentrations in different seafood species from different fishing grounds, relevant for Belgian consumption patterns.

4.2. Materials and methods

The following contaminants have been included: mercury, indicator PCBs (iPCBs), dioxin-like PCBs (dl PCBs), PCDDs plus PCDFs (PCDD/Fs), and total TEQ-content (sum of toxicity normalized PCDDs, PCDFs, and dl PCBs). In the database, all relevant information is included: commercial name, scientific name, farmed or wild seafood (if known), period of capture, age of the sampled seafood, fishing ground where the samples were caught or produced, number of samples, number of individual sample units per sample in the case of composite samples, mean fat content of the seafood, and mean contaminant content, with extra statistical data if available (standard deviation, minimum and maximum). Concentration data of dioxin-like compounds in seafood are expressed in pg TEQ/g fresh weight, iPCB concentrations and mercury concentrations in ng/g fresh weight. In a first phase, all available data were entered in the Excel®-database. Later, a selection was made

based on temporal and geographical arguments: (1) It was decided to consider only data points from analysis in the period 1995-2005; (2) Only those species coming from fishing grounds or countries relevant for the Belgian market were taken into account when characterizing the variability by probability distributions.

The database search (exploring PubMed, Web of Science and Google) has retrieved 127 relevant data sources:

- 30 reports and/or databases of governments/research institutes;
- two important national data sources: a confidential database from the Belgian Food Safety Agency, and data analysed at the Fisheries Department of Oostende, Belgium (in the framework of an MSc thesis);
- 80 peer reviewed research papers; and
- 15 proceedings from the International Symposia on Halogenated Environmental Organic Pollutants and POPs (Persistent Organic Pollutants).

Based on the available Belgian food consumption data, 41 seafood species and two seafood products were retained as relevant for the average Belgian diet (Annex III.1 given at the end of this chapter). For these species, the contaminant distributions and corresponding parameters were examined using the BestFit® distribution fitting program Version 4.5 (Palisade Corporation, Newfield, NY, USA). In the applied statistical procedure, available data per species were weighed for their cumulative probability of occurrence (for details, see below). The selection of the best fit was based on a goodness-of-fit test (the method of the least squares) as well as on visual evaluation of the probability plot (Palisade Corporation, 2002), as was done for the nutrient distributions.

4.3. Results and discussion

This section is essentially organised around five main topics that have been identified as potential sources of bias in the process of establishing the contaminant database and characterizing the appropriate input distributions. For each topic, it was attempted to include:

1. a description of the problem as such,
2. a proposal for a strategy to deal with the problem,

3. a justification for the proposed strategy, when relevant supported with literature data,
4. tailored recommendations for future reporting of contaminant data in order to make them more useful for other applications.

4.3.1. Building up a database

In July 2006, the seafood database contained:

- 1177 indicator PCB (iPCB, congeners 28, 52, 101, 118, 138, 153, 180) concentration data,
- 1254 dl PCB (congeners 77, 81, 126, 169, 105, 114, 118, 123, 156, 157, 167, 189) concentration data,
- 1615 PCDD/F concentration data,
- 1139 total dioxin-like compounds concentration data, and
- 2082 mercury concentration data.

After a selection based on the seafood species/products relevant for Belgian consumption (Annex III.1 given at the end of this chapter), 978, 1045, 1367, 982, and 1410 data were retained, respectively.

The retrieval, storage, and characterization of contamination data from the above mentioned literature sources was hampered by a number of very specific problems, mainly related to:

1. the sampling plan;
2. the sample handling prior to analysis;
3. the analytical methodologies applied;
4. the format of reporting the results (individual versus aggregated results); and
5. missing data.

These aspects influence the accuracy and representativeness of the data when applied for human intake assessment. Where possible, solutions and weighing procedures were applied to minimize the impact of these problems. The different procedures are described below.

First, different studies in the database used a variety of **sampling schemes** for the collection of seafood samples. Some samples came from local markets, others from wholesalers; some were caught by the research group while other samples originated from existing monitoring programs in specific regions. Although the sampling plan directly affects the representativeness of the samples, no weighing or selection of the data in relation to differences in sampling validity has been applied, as no objective criteria exist for that

purpose. This approach is similar as for the nutrient database. It emphasizes the need for standardization of sampling procedures to achieve contaminant levels which are comparable over different studies, a need that is also expressed by the European Food Safety Authority (EFSA, 2005a). Another problem at the level of the sampling plan, which was also encountered for the nutrient database, involves the application of individual sample units versus composite samples. Compositing of sample units is a possible way to assure the representativeness of the mean, without the need to increase the number of measurements. However, this results in underestimation of the true variability (World Health Organization, 2000). As in the nutrient dataset, a distinction between both types of data (composite samples and individual sample units) was made by weighing the data according to the number of sample units, such that concentrations measured in composite samples get a higher probability of occurrence. A weighing factor $\mathbf{W_{i,unit}}$ for each concentration data point x_i was created. $W_{i,unit}$ was equal to one in the case of an individual samples unit and equal to the number of units per sample in the case of composite samples. When the number of individual sample units per composite sample was unknown, $W_{i,unit}$ was assumed to be one, leading to the assumption that the values from publications not giving the appropriate details, have a lower weight. This is in line with the recommendations by Judd *et al* (2003b) based on the consideration that the absence of details about the methodological approaches inevitably introduces increased uncertainty in the dataset. In the example given in Table III.4, $W_{i,unit}$ is equal to one in all cases, which means that no composite samples were used, or that no information about it was given.

Table III.4: Data about the mercury concentration in sardines from two different origins, with the different weighing factors ($W_{i,meas}$, $W_{i,unit}$, $W_{i,total}$) and the cumulative probability of occurrence ($F(x_i)$); the fitted distributions are shown in Fig. III. 12

Sardines from the Eastern Atlantic Sea *

Hg concentration (ng/g fresh weight)	$W_{i,meas}$	$W_{i,unit}$	$W_{i,total}$	$\sum_i W_{i,\,total}$	F(xi)
5	1	1	1	1	0.06
6	1	1	1	2	0.11
7	1	1	1	3	0.17
12	1	1	1	4	0.22
12	1	1	1	5	0.28
13	1	1	1	6	0.33
13	1	1	1	7	0.39
14	1	1	1	8	0.44
18	1	1	1	9	0.50
19	1	1	1	10	0.56
23	1	1	1	11	0.61
30	1	1	1	12	0.67
38	1	1	1	13	0.72
40	1	1	1	14	0.78
47	1	1	1	15	0.83
51	1	1	1	16	0.89
88	1	1	1	17	0.94
104	1	1	1	18	1.00
			$\sum_n W_{i,\,total} = 18$		

Sardines from the Mediterranean Sea $

Hg concentration (ng/g fresh weight)	$W_{i,meas}$	$W_{i,unit}$	$W_{i,total}$	$\sum_i W_{i,\,total}$	F(xi)
63.6	11	1	11	11	0.03
75.7	24	1	24	35	0.10
130	300	1	300	335	0.94
142	10	1	10	345	0.96
156	3	1	3	348	0.97
208	10	1	10	358	1.00
			$\sum_n W_{i,\,total} = 358$		

* The data about the mercury concentration in sardines from the Eastern Atlantic Sea origin from one publication (Knowles *et al*, 2003).

$ The data about the mercury concentration in sardines from the Mediterranean Sea origin from four different publications (Juresa & Blanusa, 2003; Plessi *et al*, 2001; Sanzo *et al*, 2001; Storelli *et al*, 2003a).

Secondly, the tissue selected for analysis and the preparation methods influence the value of the concentration data for human exposure assessment. Proper and comparable **handling of samples** prior to analysis is critical (Holden *et al*, 2002). In the final database, only concentrations measured in muscle tissues of fish are used, since fish liver (often used for

ecological measurements) is rarely consumed in Belgium. A similar problem is that, in some studies, the skin of the fish was removed prior to analysis whilst in others it was left in the sample. Some studies do not mention this detail at all. However, it has been shown that this difference in manipulation of the sample may affect the contaminant concentration (Bayen *et al*, 2005; Hamre *et al*, 2003; Salama *et al*, 1998). For the current database, no data selection was made in this regard. The rationale for this was that, by including both kinds of data (with and without skin) the variability on consumption level is covered to some extent, as some consumers will remove the skin before consumption, while others will not. Overall, these very specific sources of variability at the level of the sample handling, again point at the importance of a maximally detailed description of the preparation of samples. This was also recently emphasized by Törnkvist *et al* (2005), summarizing the importance of specific and clear instructions for sample preparation when analyzing organochlorine compounds in seafood. Their research also addressed the need to have clear regulations and to carefully describe the seafood sample preparation procedure.

Third, different **analytical methodologies** can be used for determining the same substance. In the case of (dl) PCBs, some measured individual congeners, whereas others quantified Aroclor mixtures, i.e. commercial mixtures of different PCB congeners. It is under the form of Aroclor mixtures that PCBs were previously marketed, e.g. Aroclor 1016, 1260, 1254, etc. When the PCB level was quantified as Aroclor mixtures, it was sometimes difficult to estimate the contribution of the individual PCB congeners. These different approaches in the quantification of PCBs influence the comparability of the concentrations reported in different publications. For example, it is difficult to compare the data of the salmon study of Hites *et al* (2004a) to the salmon contamination data reported by Easton *et al* (2002), because Hites *et al* (2004) measured Aroclor 1254 whereas Easton *et al* (2002) measured 112 unspecified PCB congeners. Aroclor concentrations were not included in the database, unless it was possible to convert them to concentrations of the seven indicator PCBs, e.g. with the data published by Kodavanti *et al* (2001). In order to avoid the creation of biases, only the concentration data of publications clearly mentioning that the seven indicator PCBs were measured (iPCBs, congeners 28, 52, 101, 118, 138, 153, 180; (EFSA, 2005c)) and/or the sum of the four non-ortho and eight mono-ortho dl PCBs (congeners 77, 81, 126, 169, 105, 114, 118, 123, 156, 157, 167, 189; (EFSA, 2005c)) were used in the newly developed database and applied when characterizing the probability distributions. At the level of dioxin-like compounds, only data expressed as pg TEQ/g fresh weight were used (where possible, concentration data

expressed in g/g wet weight were converted to TEQs using the TEF-values). A problem directly related to the reading of the results from the analysis is the statistical treatment of concentrations below the limit of detection or quantification (LOD or LOQ), a problem that was also encountered for the nutrient database. Nevertheless, this did not cause many problems in our study because the published data for the sum of iPCBs, sum of dl PCBs, PCDD/F, and total sum of dl-like compounds were all present at detectable levels. The exception is for some ($n < 10$) of the mercury concentrations. These have been systematically replaced by the LOD reported in the considered publication (as worst case scenario approach). The effect of this replacement is not explicitly studied, but assumed to be negligible as it was applied to so few cases. On the other hand, it is likely that different approaches have been applied in different publications in the calculation of aggregated data. To give an example: when some of the PCB congeners were lower than the LOD during the determination of the sum of the seven iPCBs, one author may have replaced them by zero to calculate the reported sum of PCB congeners, while another author may have replaced it by the LOD before adding up. Obviously, such particularities regarding the data are hidden behind the aggregated figures in the published reports and cannot be dealt with in the process of compiling new databases. It is nevertheless useful to consider the potential effect of this phenomenon. This has been explicitly studied by Storelli *et al* (2003b) investigating the effect of replacing the non-detectable congener concentrations by respectively zero, half of the LOD, and the LOD, in the calculation of the dl PCB intake. By assuming a seafood consumption of 60 g seafood per person and per day, an intake of 0.01-6.20 pg TEQ/kg body weight (bw)/day, 0.29-6.23 pg TEQ/kg bw/day, and 0.56-6.26 pg TEQ/kg bw/day, respectively, was obtained. This illustrates that the approach used leads only to a slight difference in the higher percentiles of the intake distribution. It can be assumed that similar effects apply to other aggregated data in this context.

Fourth, as also encountered for the nutrient database, analytical results in literature are **reported in many different ways**. Again, a second weighing factor $\mathbf{W_{i,meas}}$ was applied for each data point x_i as a function of the number of measurements on which it was based. When the number of measurements was not known, $W_{i,meas}$ was assumed to be one. As explained for the nutrient database, for each data point x_i in the compiled database, the second weighing factor $W_{i,meas}$ was multiplied with the first weighing factor $W_{i,unit}$, in order to have an overall weighing factor ($W_{i,total} = W_{i,meas} \cdot W_{i,unit}$). The example given in Table III.4 clarifies this approach. The way in which this weighing procedure influences the eventual shape of

the fitted distribution is explained further on in this text. Another problem at the level of reporting is that some of the published concentrations were expressed per gram fat, without concomitant reporting of the corresponding total fat concentration in the analyzed samples. Such data preclude the calculation of concentrations per gram fresh weight, and were not included in the database. Finally, a considerable amount of governmental publications reported concentration data without mentioning the origin of the seafood (because in most of the cases it concerns commercial samples with undefined origin), creating a lack of essential information. But as in the end no distinction according to origin was made for most species, these data were yet included in the database.

The **fifth** problem is the **lack of data**. It is generally known that – for many contaminants – analytical data are often not published in open literature and therefore are difficult to access. Some of them are available via scientific publications or via websites of national food (safety) agencies, but in most cases these data are published in 'grey' reports, which are difficult to trace and retrieve. Consequently, contaminant data could often not be found for commercially relevant regions; e.g. no dl PCB concentration data were found for halibut from the Northwest Atlantic Sea. Overall, for most of the seafood species, not enough data were available to make a difference between the different fishing grounds of origin, obliging us to group the data over the different fishing grounds. In addition, data of different species had to be aggregated (for dioxin-like compounds and PCBs according to fat content) in cases where no sufficient data on species level were available for the distribution fitting procedure and similar probability distributions were determined for these grouped species (Annex III.6-11 of this chapter). Judd *et al* (2003a) encountered a similar problem, when carrying out an intake assessment of PCBs via seafood for two specific American population groups with high seafood diets, the Squaxin Tribe and the Asian Pacific Islanders. For these population groups, appropriate total PCB data were available for respectively 58% and 4% of the seafood species consumed, as for such data aggregation was the only solution.

Finally, for several ecological or monitoring studies, it was not clear whether the samples analyzed are **representative** for human consumption. Therefore, more detailed information about the seafood samples is essential in order to avoid that data collected for one purpose are incorrectly applied in other situations. Judd *et al* (2003b) indicated that use of data collected in a context of ecological evaluation for the purpose of human health risk assessments may lead to substantial risk assessment errors.

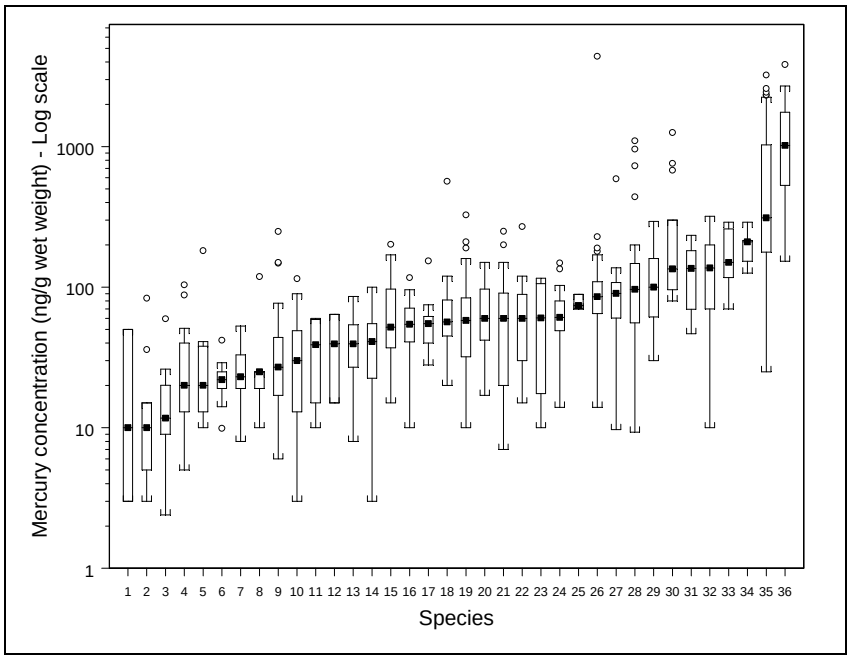
4.3.2. Observed variability

The result of the data collection is summarized in box plots, visualizing the observed variability within the species (Fig. III.7-11). Species for which only a low number of data points was available, were not plotted. Contaminant concentrations ranged per gram fresh weight from 2.4 to 4390.0 ng for mercury, from 0.1 to 5736.6 ng for the sum of the iPCBs, from 0.002 to 115.000 pg TEQ for the sum of dl PCBs, from 0.002 to 34.400 pg TEQ for PCDD/Fs, and from 0.006 to 126.000 pg TEQ for the total sum of dioxin-like compounds.

4.3.2.1. Mercury

Fig. III.7 shows the published mercury data for different seafood species, sorted according to their median mercury concentration. To protect public health, maximum levels of mercury in fishery products are laid down by the European Commission (Commission Regulation (EC) No 1881/2006 of 19 December 2006) (European Commission, 2006). The levels should be as low as reasonably achievable, taking into account that for physiological reasons certain species concentrate mercury more easily in their tissues than others. Mercury limit for fishery products in general is 500 ng/g fresh weight. It is 1000 ng/g fresh weight for anglerfish, Atlantic catfish, bass, blue ling, bonito, eel, halibut, little tuna, marlin, pike, plain bonito, Portuguese dogfish, rays, redfish, sail fish, scabbard fish, shark, snake mackerel, sturgeon, swordfish, and tuna. In the compiled database, these limits were exceeded for five species: cod, common shrimp, tuna, swordfish, and Nile perch; for respectively 1.0%, 2.4%, 28.7%, 51.1%, and 7.7% of the concentration data.

An origin-based distinction could be made to separate the seafood coming from the Mediterranean Sea and the other fishing grounds (possible for mackerel, squid, and sardine). To describe the mercury content in salmon, a distinction was made between farmed salmon and wild Pacific salmon. The box plots show that tuna and swordfish have the highest mercury load and that mackerel, squid, and sardines from the Mediterranean Sea contain more mercury compared to other catching areas. It was observed that mollusks and crustacean have rather low mercury concentrations. Moreover, the box plots show the within-species variability of the mercury concentrations, e.g. the lowest measured mercury concentration in tuna (Fig. III.7, n°35) falls below the 75th percentile of the mercury concentrations in halibut (species with the lowest median; Fig. III.7, n°1).



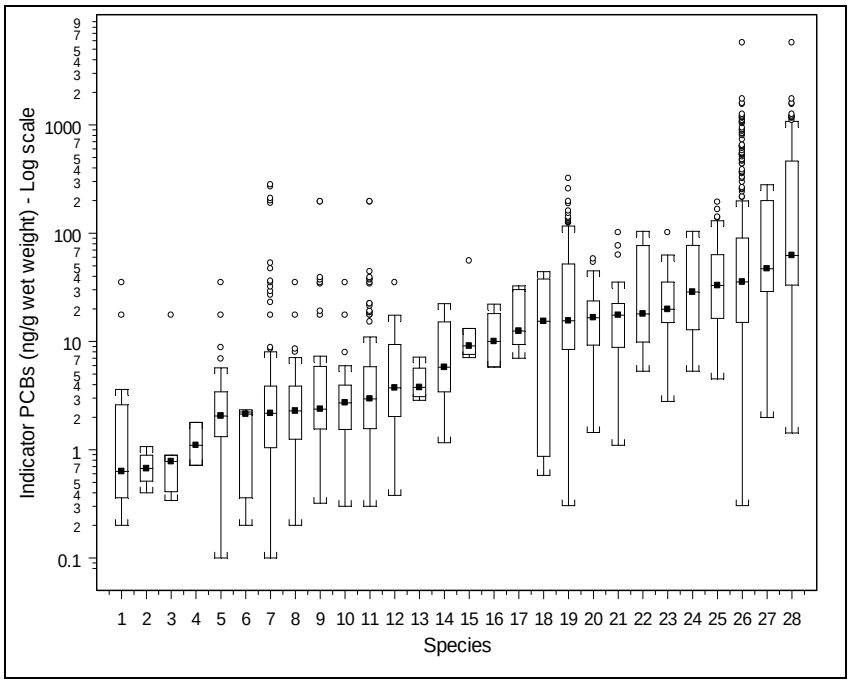
1. Halibut; 2. Squid, non-Mediterranean; 3. Tilapia; 4. Sardine, Eastern Central Atlantic Sea; 5. Mussel; 6. Scallops; 7. Oyster; 8. Norway lobster; 9. Scampi; 10. Herring; 11. Sprat; 12. Brill; 13. Salmon, farmed; 14. Mackerel, Northeast Atlantic Sea; 15. European plaice; 16. Salmon, Pacific, wild; 17. Anchovy; 18. Common shrimp; 19. Sole; 20. Crab; 21. Saithe and Pollack; 22. Haddock; 23. Redfish; 24. Trout; 25. Squid, Mediterranean Sea; 26. Cod; 27. Nile perch; 28. Skate; 29. Whiting; 30. Anglerfish; 31. Sardine, Mediterranean Sea; 32. Eel; 33. Ling; 34. Mackerel, Mediterranean Sea; 35. Tuna; 36. Swordfish

Fig. III.7 Mercury concentrations in different species, box plots. Data available from the open literature and from national and international reports

For the goal of this PhD-thesis, mercury concentrations were converted to methyl mercury, since this organic form is the major chemical form in which mercury is present in seafood and it is the most toxic form of the element (Clarkson & Magos, 2006). The majority of the mercury released in the marine environment is inorganic mercury, but this can then be converted to methyl mercury by anaerobic bacteria in sediments (Storelli *et al*, 2002). On the basis of literature data, it was assumed that 80% of the total mercury in fish is present in methylated form (Baeyens *et al*, 2003; Capelli *et al*, 2004; Easton *et al*, 2002; Foran *et al*, 2004; Forsyth *et al*, 2004; Storelli *et al*, 2002; Storelli *et al*, 2003a; Storelli *et al*, 2003c; Storelli *et al*, 2005), being 68% and 34% for molluscs and crustacean (Baeyens *et al*, 2003; Foran *et al*, 2004), respectively.

4.3.2.2. iPCBs

Fig. III.8 shows that lean seafood species have generally a lower iPCB concentration, only crab (Fig. III.8, n°27) seems to be an exception. Large within-species variability can be observed. The only species for which a distinction according to different catching areas was made is herring form the Baltic Sea (Fig. III.8, n°25) versus non-Baltic (Fig. III.8, n°15), with the first having a higher contamination load.



1.Skate;
2.Scampi;
3.Scallop; 4.(Alaska) pollack and saithe;
5.Whiting;
6.Haddock;
7.FG1;
8.Cod;
9.Common shrimp;
10.European plaice;
11.FG2;
12.Sole;
13.Redfish;
14.Mussels;
15.non-Baltic Herring;
16.Halibut;
17.Sprat;
18.Squid;
19.Salmon;
20.Mackerel;
21.FG3; 22.FG4; 23.Anchovy;
24.Sardine;
25.Herring, Baltic Sea;
26.FG5;
27.Crab; 28.Eel

Fig. III.8 Concentrations of the sum of iPCBs in different species, box plots. Data available from the open literature and from national and international reports. For an explanation of FG1 to FG5 see Table III.1

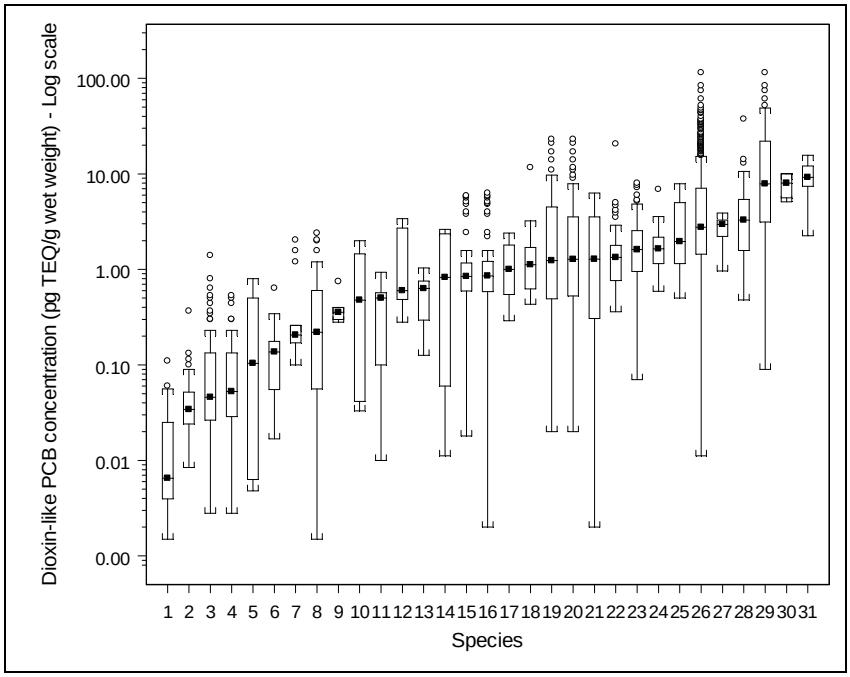
4.3.2.3. dl PCBs, PCDD/Fs, total dioxin-like compounds

The European Commission published a regulation (Commission Regulation (EC) No 1881/2006 of 19 December 2006) setting maximum levels for certain contaminants in foods as regards PCDD/Fs and dl PCBs (European Commission, 2006). Concerning muscle meat of fish, fishery products and products thereof, the maximum levels for PCDD/Fs is 4.0 pg TEQ/g fresh weight and the maximum level for the sum of PCDD/Fs and dl PCBs is 8.0 pg TEQ/g fresh weight, with exception of eel which may contain 12.0 pg TEQ/g fresh weight. In the compiled database, concentrations exceeding the limit were found for seven species. Table III.5 gives the percentages of those data in relation to the total data.

Table III.5: Percentage of data and number of data points exceeding the limits for dioxin-like compounds

Species	PCDD/Fs	Total dioxin-like compounds
	% of the data exceeding the limit (number of data points)	
Eel	4.9 (4)	24.5 (26)
Halibut	2.6 (1)	10.0 (1)
Herring, Baltic Sea	48.7 (127)	50.0 (93)
Herring, non-Baltic	4.5 (3)	13.4 (9)
Mackerel	1.6 (1)	1.9 (1)
Salmon, Baltic Sea	66.7 (34)	96.0 (48)
Salmon, farmed	15.4 (8)	4.5 (8)
Salmon, Pacific, wild	0.0	0.0
Trout	3.3 (2)	6.3 (5)
Tuna	2.4 (1)	22.0 (9)

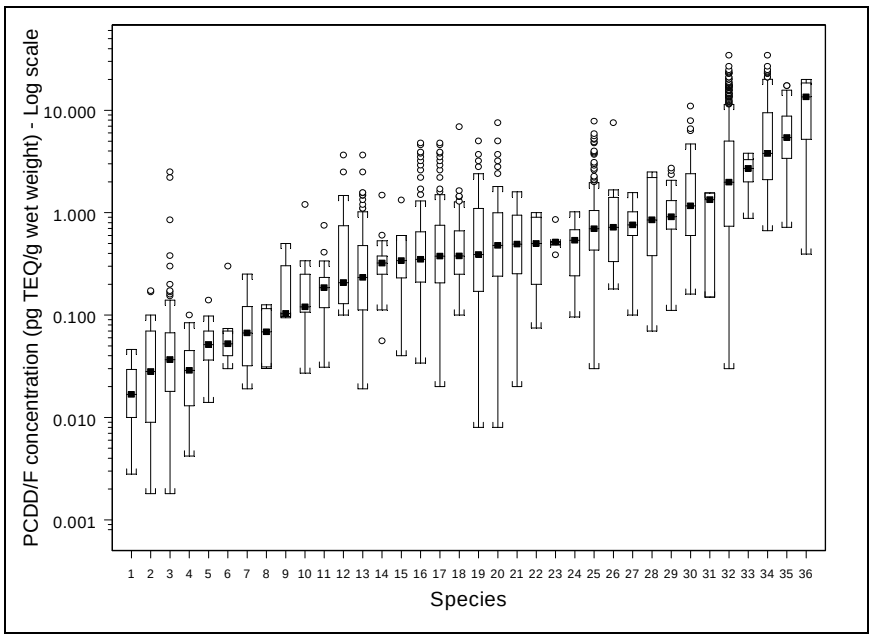
Fig. III.9 visualizes that the fatty fish species have the highest dl PCB load: herring, salmon, eel and sprat, but again with high within-species variability.



1.Scampi;
2.Haddock;
3.FG1; 4.Cod;
5.(Alaska) Pollack and saithe;
6.Whiting; 7.Mussels;
8.FG2; 9.Redfish;
10.Squid; 11.Common shrimp;
12.Swordfish;
13.European plaice;
14.Salmon, Pacific Ocean;
15.Trout; 16.FG4;
17.Turbot; 18.Halibut;
19.Tuna; 20.FG3; 21.Sardine;
22.Mackerel;
23.Farmed salmon;
24.Herring (North Sea);
25.Anchovy; 26.FG5; 27.Sprat;
28.Herring (Baltic Sea);
29.Eel; 30.Herring (others);
31.Salmon (Baltic Sea)

Fig. III.9 Concentrations of the sum of dl PCBs in different species, box plots. Data available from the open literature and from national and international reports. For an explanation of FG1 to FG5 see Table III.1

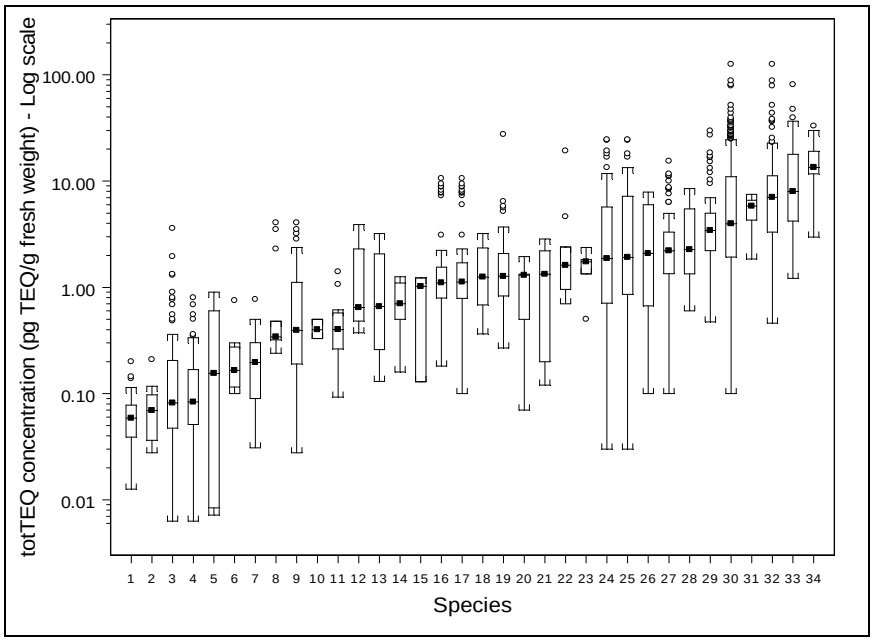
Fig. III.10 shows the PCDD/F concentrations of the different species of interest, ordered by their median PCDD/F concentrations, leading to a clear gradient, but again with high within-species variability.



1.Cod, Iceland and Faroer;
2.Saithe and Pollack; 3.FG1;
4.Haddock; 5.Whiting;
6.Cod, North Sea; 7.Scampi;
8.Cod, other Nortwest Atlantic seas; 9.Swordfish;
10.Squid; 11.Redfish;
12.Mussels; 13.FG2;
14.European plaice;
15.Anchovy; 16.Trout; 17.FG4;
18.Mackerel; 19.Tuna; 20.FG3;
21.Sardines; 22.Turbot;
23.Wolf fish;
24.Halibut, Northeast Atlantic;
25.Salmon, non-Baltic;
26.Halibut, Northwest Atlantic; 27.Common shrimp;
28.Crab;
29.Herring, North Sea; 30.Eel;
31.Sole; 32.FG5; 33.Sprat;
34.Herring, Baltic Sea;
35.Salmon, Baltic Sea;
36.Herring, other Northwest Atlantic seas

Fig. III.10 PCDD/F concentrations in different species, box plots. Data available from the open literature and from national and international reports. For an explanation of FG1 to FG5 see Table III.1

Fig. III.11 shows the concentrations of the total sum of dioxin-like compounds (total TEQ) in the different species. A clear gradient appears when ordering the different seafood species according to their median total TEQ concentration. The species with the highest median concentration are herring and salmon of the Baltic Sea (Fig. III.11, n°33, 34). In this context, it is important to note that different methodologies can be applied to measure the concentrations of total dioxin-like compounds, other than summing the measured PCDD/F and dl PCB concentrations. In other words, the results in Fig. III.11 are not simply the sum of those in Fig. III.9 and III.10.



1.Haddock;
2.Scampi;
3.FG1;
4.Cod;
5.Saithe and Pollack;
6.Salmon, Pacific, wild;
7.Whiting; 8.Mussels;
9.FG2; 10.Sole;
11.Redfish; 12.Swordfish;
13.Squid;
14.European plaice;
15.Lobster;
16.Trout; 17.FG4;
18.Turbot; 19.Mackerel;
20.Scallop;
21.Common shrimp;
22.Halibut;
23.Oysters; 24.FG3; 25.Tuna;
26.Sardines; 27.Salmon, farmed; 28.Anchovy;
29.Herring, non-Baltic; 30.FG5;
31.Sprat; 32.Eel;
33.Herring, Baltic Sea;
34.Salmon, Baltic Sea

Fig. III.11 Total TEQ concentrations in different species, box plots. Data available from the open literature and from national and international reports. For an explanation of FG1 to FG5 see Table III.1

For the dl PCBs and the total TEQ concentration, a distinction according to fishing ground could only be made for the species herring and salmon, making clear that the Baltic herring and salmon are more contaminated (Fig. III.9 and III.11). A distinction between different fishing grounds could be made for the PCDD/F concentration distributions for cod, halibut, herring, and salmon (Fig. III.10).

4.3.3. Distribution fitting

For each contaminant, the concentration data together with their cumulative probabilities of occurrence, $F(x_i)$, were used per species and – where possible - per fishing ground, in order to characterize distributions using BestFit® Version 4.5 (see Table III.4 given previously in combination with Fig. III.12 and explanatory notes later on). On the basis of the suggested distributions and goodness-of-fit test statistics delivered by BestFit®, it has been decided which kind of distribution with matching parameters could be applied to adequately describe the data. Annex III.6 to III.11 give the selected distributions for all different species and the five contaminants.

Annex III.6 and III.7 give the distributions and parameters of the **mercury and methyl mercury** concentration in different fish species. The tables show that a distinction according to origin of the seafood product and its mercury concentration could be made for mackerel, sardine, squid, and salmon. The percentages given in the second column are a result of the work executed in the traceability study and give an indication of the importance of each catching area (part 2 of this chapter). The data in the table also indicate that no data were available for three seafood products: caviar, conger, and surimi. In those cases, the mercury contamination was assumed to be negligible. The underestimation of the total mercury intake caused by this assumption will be very small, due to the low amount of these products that is consumed.

Annex III.8 gives the distributions and parameters of the **iPCB** concentration in different fish species. As a solution to fill the gaps of lacking data, the different concentrations data of all species belonging to the same fat group were pooled and used for distribution fitting. When no data were available for an individual species, the distribution of the corresponding fat group was used. This approach was also applied for dl PCBs, PCDD/Fs, and total dioxin-like compounds.

Annex III.9 to III.11 present the distributions and parameters of the different fish species for the different **dioxin-like compounds**. Also here, aggregation per fat group was used to fill data gaps.

Overall, six different **distribution models** were selected: a normal distribution, a lognormal distribution, a logistic distribution, a loglogistic distribution, a 4-parametrical beta distribution, and a uniform distribution. Since the normal, lognormal, logistic, and loglogistic distributions have open ends to infinity, they were in this application truncated to avoid unrealistic high or low concentrations. However, it is difficult to quantify biologically plausible lower and upper bounds. Yet, they can be higher than the highest value in the available database, especially when the quantity of data is rather limited. In general, maximum values in the data are not suitable upper bounds, and it seems inappropriate to exclude the possibility of more extreme values (Paulo *et al*, 2006). As was done for the nutrient distributions, the imposed minimum was defined as half of the lowest observed concentration and the imposed maximum as double of the highest observed concentration. A uniform distribution was applied in the cases where only one or two concentration data were available and no possibility for aggregation existed (in the case of two available data points, they were used as minimum and maximum of the uniform distribution; in the case of only one available data point, the minimum and maximum were defined as respectively the concentration minus/plus half of that concentration). Due to the uncertainty related to the low number of data points, the latter approach was only applied when no alternative was available.

Fig. III.12 to III.14 show some **examples of the selected distributions** to describe the contaminant concentration for a certain seafood species from a certain fishing ground, as well as the original data points. Both representations together give a visual idea of the goodness of fit.

Fig. III.12 (together with Table III.4 given previously) indicated that more data points were available for the mercury concentration in sardines from the Eastern Central Atlantic Sea than from the Mediterranean Sea. Moreover, it is clear that the latter has the highest mercury contamination. The sudden rise in the Mediterranean Sea data points (from 75.5 ng/g with a cumulative probability of 0.10 to 130.0 ng/g with a cumulative probability of 0.94) is caused by the high weight of the latter caused by the high number of measurements ($W_{i,meas}$) behind that concentration (Table III.4).

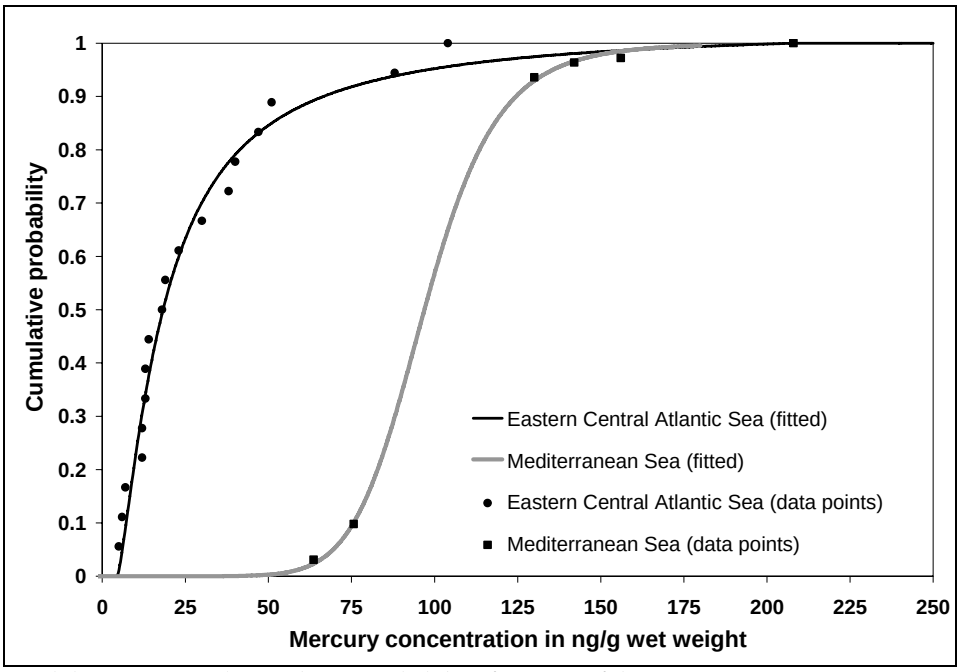


Fig. III.12 Used data points and cumulative probability function of the mercury concentration in sardines from the Eastern Central Atlantic Sea and the Mediterranean Sea; detailed information about the data points is given in Table III.4

Fig. III.13 shows a case where a rather low number of data points was available. Consequently, each data point has a high influence on the final distribution and parameter selection.

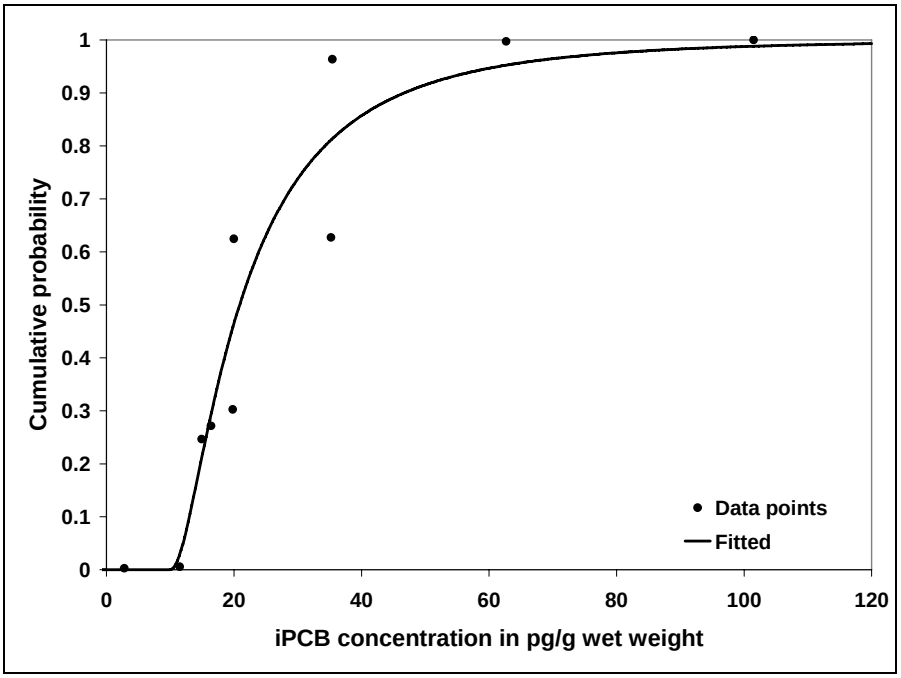


Fig. III.13 Used data points and cumulative probability function of the iPCB concentration in anchovy (no distinction was made between different origins)

Fig. III.14 shows the PCDD/F concentration in herring from two different fishing grounds, showing a higher contamination level in Baltic herring as compared to the North Sea.

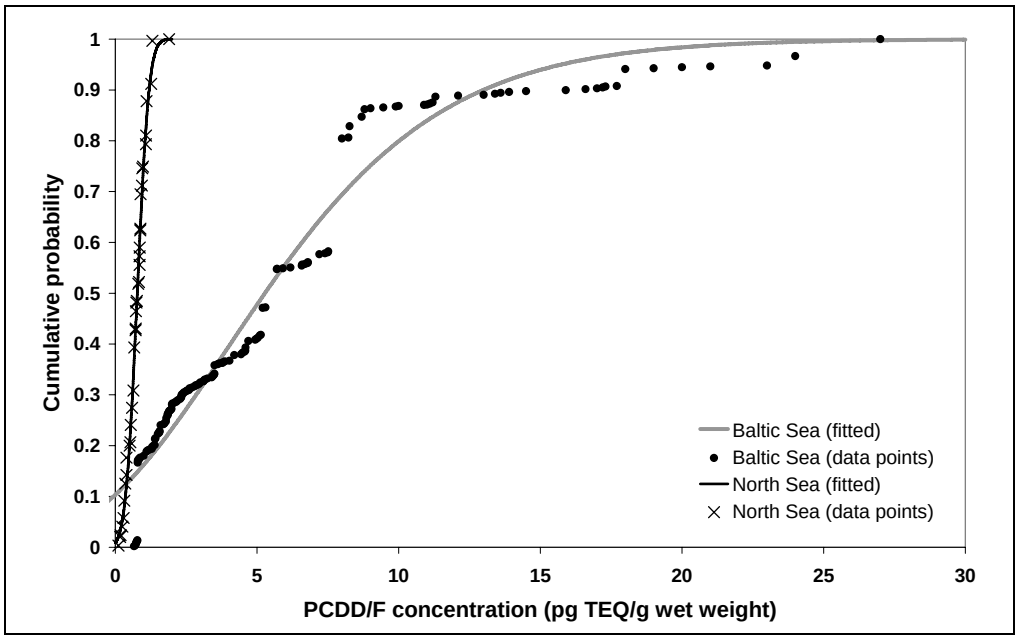


Fig. III.14 Used data points and cumulative probability functions and of the PCDD/F concentration in herring from the Baltic Sea and the North Sea

To assess the effect of the use of the weighing factor, all the distributions were fitted a second time, but without weighing the concentration data. In this second approach, every data point was given the same probability of occurrence, regardless of the number of sample units per sample and the total number of samples behind the considered measured concentration. It is hard to decide which results are better, as no "golden standard" exists. But in general, distributions predicting higher concentrations were determined in the non-weighing approach. This is illustrated by Fig. III.15 and III.16. The most probable explanation is that most of the high concentrations measured are not mean values, but rather the result of an individual extreme measurement or a measurement based on a small number of samples. In the weighing approach, a small probability of occurrence will be attributed to these "hot spot" concentrations. In contrast, in the non-weighing approach, these high concentrations will have the same probability of occurrence than mean concentrations based on a large number of samples, leading to distributions predicting higher concentrations. On the other hand, the same will count for extremely low observations, but the extreme lows are not as extreme as the extreme highs. As a result, using distributions resulting from the non-weighing approach in a probabilistic intake assessment will lead to higher assessed intakes, closer to toxicological thresholds.

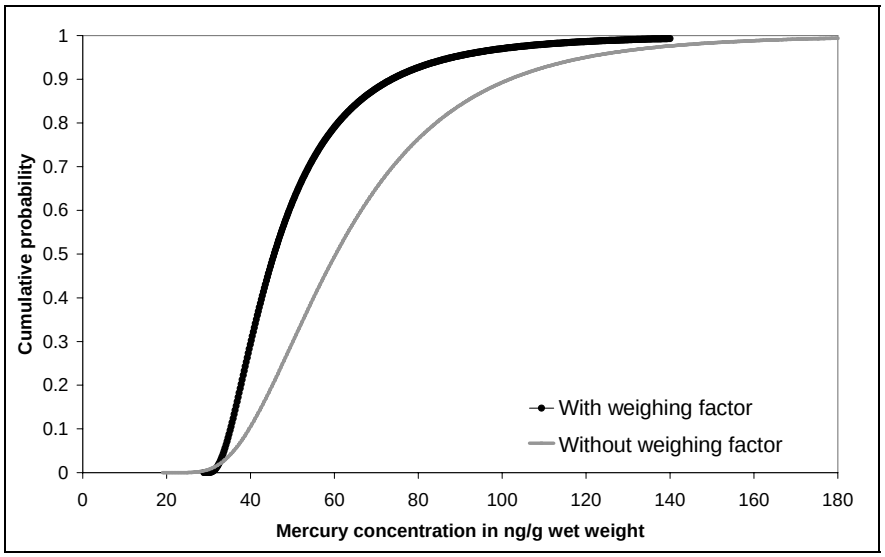


Fig. III.15 Determined distributions for the mercury concentration in trout, with and without using the weighing factor

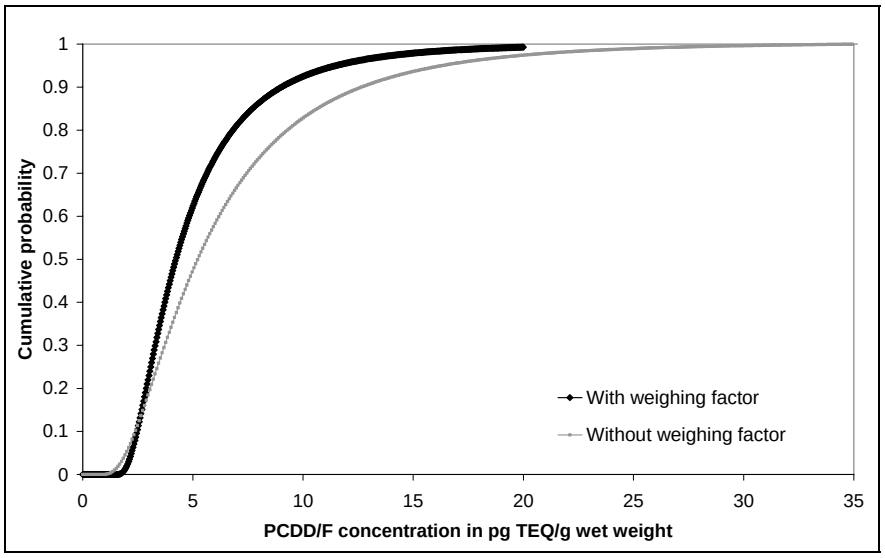


Fig. III.16 Determined distributions for the PCDD/F concentration in trout, with and without use of the weighing factor

4.4. Conclusion

An extended contamination database of seafood was established, covering relevant seafood species from several relevant fishing grounds for human consumption in Belgium. However, several problems were encountered during the establishment of the database, related to a lack of international recommendations to standardize analytical methodologies and the format for reporting the results. Harmonization of sampling plans, sample handling, analytical methodologies, and the format of results reporting would improve the usefulness of published contamination data. Our conclusions confirm those earlier made by the EU

Scientific Steering Committee about the need to improve the comparability of data critical to conduct human intake assessments (EU Scientific Steering Committee, 2000). Because of current lack of established guidance regarding methods dealing with uncertainty in intake assessment and probabilistic approaches, the EFSA will consider further work in this area, by contributing to the development of a European framework for the harmonization of food-related data collection in the EU and making these data publicly accessible (EFSA, 2005a). In order to solve the current encountered problems, a weighing method has been drawn up, applied, and compared in order to determine adequately contamination distributions as close to reality as possible. As such, a probabilistic assessment regarding the intake of contaminants via seafood consumption can be executed on the basis of these distributions.

5. Annexes of chapter III

ANNEX III.1 - Nomenclatural table of 41 seafood species, relevant for Belgian consumption

English name	Dutch name	French name	Scientific name
Anchovy	Ansjovis	Anchois	Engraulis encrasicolus
Anglerfish	Zeeduivel, lotte	Badroie, lotte, Crapaud	Lophius piscatorius
Brill	Griet	Barbue	Scophthalmus rhombus
Cod	Kabeljauw	Cabillaud	Gadus morhua
Common (brown) shrimp	Noordzeegarnaal	Crevette grise	Crangon crangon
Common whelk	Slak/Wulk	Buccin	Buccinidae
Conger	Zeepaling, congeraal	Congre	Conger conger
Crab	Krab	Crabe	Cancer pagurus
Eel	Paling	Anguille	Anguilla anguilla
European catfish	Meerval	Silure, poisson-chat	Clarias gariepinus
European plaice	Schol, pladijs	Plie	Pleuronectes platessa
Haddock	Schelvis	Eglefin	Melanogrammus aeglefinus
Halibut	Heilbot	Flétan	Hippoglossus hippoglossus/stenolepis – Reinhardtius hippoglossoides
Herring	Haring	Hareng	Clupea harengus
John dory	Zonnevis	Saint-pierre	Zeus faber
Ling	Leng	Lingue	Molva molva/dypterygia
Lobster	Zeekreeft	Homard	Homarus gammarus
Mackerel	Markeel	Maquereau	Scomber scombrus
Milkfish	Melkvis, bandeng	Chanos	Chanos chanos
Mussel	Mossel	Moule	Mytilus edulis
Nile perch	Victoriabaars	Perche du Nil	Lates niloticus
Norway lobster	Langoestine	Langoustine	Nephrops norvegicus
Oyster	Oesters	Huître	Ostrea edulis - Crassostrea gigas
Redfish	Roodbaars	Sébaste	Sebastes marinus/mentella
Saithe &Pollack	Alaska koolvis	Lieu de l'Alaska	Theregra chalcogramma
Saithe &Pollack	Koolvis & Pollack	Lieu noir/jeune	Pollachius pollachius/virens
Salmon	Zalm, Atlantische	Saumon	Salmo salar
Salmon	Zalm, Pacifische	Saumon	Oncorhynchus spp
Sardine, pilchard	Sardien	Sardine, pilchard	Sardina pilchardus
Scampi	Scampi, tijgergarnaal, gamba	Crevette géante, tigrée	Penaeus spp
Sea bream	Zeebrasem, dorade	Dorade	Pagellus bogaraveo
Skate, ray	Rog	Raie	Rajidae spp.
Sole (Dover)	Tong	Sole (commune)	Solea solea
Sprat	Sprot	Sprat, amelette	Sprattus sprattus
Squid, octopus	Inktvis, octopus	Poulpe, encornet	Octopus vulgaris
Squid, octopus	Inktvis, pijlinktvis	Calmar	Loligo forbesi/vulgaris
Squid, octopus	Inktvis, zeekat	Sèche	Sepia officinalis
Scallop	Sint-Jakobsschelp	Coquille Saint-Jacques	Pecten maximus/jacobeus
Swordfish	Zwaardvis	Espadon	Xiphias gladius
Tilapia	Tilapia	Tilapia	Oreochromis niloticus/aureaus/mossambica
Trout	Forel	Truite	Salmo trutta
Trout, rainbow	Forel, regenboog-	Truite arc-en-ciele	Oncorhynchus mykiss
Tuna	Tonijn	Thon	Thunnus albacares/alalunga/maccoyii/ obesus/thynnus – Katsuwonus pelamis
Turbot	Tarbot	Turbot	Scophthalmus maximus, Psetta maxima
Whiting	Wijting	Merlan	Merlangius merlangus
Wolf fish	Zeewolf	Loup de mer	Anarhichas lupus

ANNEX III.2 - Distribution and its parameters(Param) for the EPA&DHA concentration in relevant seafood species, as well as the number of data points used (N)

Species	Distribution	Param1	Param2	Param3	Param4	Param5	N
	uniform			min	max		
	betageneral	α1	α2	min	max		
	normal	μ	σ	Tr min	Tr max		
	loglogistic	β	α	Tr min	Tr max	γ (min)	
	lognormal	μ	σ	Tr min	Tr max	shift	
Anchovy	uniform	NA	NA	3.0852	20.6615	NA	7
Anglerfish	normal	1.5832	0.8678	0.5000	5.2200	NA	5
Caviar	normal	15.2756	13.1798	0.0000	NA	NA	9
Cod	normal	3.3168	0.9774	0.5000	8.8000	NA	29
Common shrimp	normal	3.6600	0.9785	1.5000	14.0000	NA	3
Common whelk	betageneral	0.3113	2.9108	0.0692	1.0713	NA	3
Conger	uniform	NA	NA	0.0000	4.0000	NA	2
Crab	betageneral	0.9539	3.6449	2.3222	9.5440	NA	21
Eel	betageneral	0.5414	2.3157	0.0000	40.3848	NA	10
European plaice	normal	2.5188	2.5569	0.5000	10.0200	NA	6
Haddock	betageneral	0.5221	2.7852	1.6850	2.9060	NA	9
Halibut	normal	4.7376	3.6458	0.2450	23.5600	NA	14
Herring	loglogistic	16.1697	10.6812	3.4350	56.0000	-3.5261	37
John dory	uniform	NA	NA	2.5000	7.5000	NA	1
Lobster	normal	1.7130	1.6039	0.0000	NA	NA	6
Mackerel	betageneral	0.3500	2.3370	12.0212	45.1811	NA	18
Mussel	loglogistic	56.5106	42.5258	0.9840	16.2200	-53.1307	11
Nile perch	betageneral	0.2856	2.7272	0.2626	10.5809	NA	11
Norway lobster	normal	1.7130	1.6039	0.0000	NA	NA	6
Saithe and Pollack	betageneral	1.0133	2.4937	1.7505	10.4212	NA	12
Salmon	normal	20.6855	8.0150	2.2500	65.4000	NA	67
Sardine	normal	19.9393	10.9294	1.9730	NA	NA	45
Scallop	loglogistic	0.0484	1.0334	0.9450	7.4000	1.8880	6
Scampi	normal	4.1746	0.8729	1.0000	10.9000	NA	10
Sea bream	normal	4.4740	3.9125	0.0000	17.7000	NA	8
Skate	uniform	NA	NA	1.0000	2.5500	NA	2
Sole	betageneral	0.2856	2.7272	0.2626	10.5809	NA	11
Sprat	loglogistic	22.6204	2.9426	6.5000	71.4600	-9.4869	3
Squid	normal	3.5102	1.7501	0.5250	18.2000	NA	9
Surimi	normal	3.5900	0.2735	1.6000	NA	NA	3
Trout	lognormal	13.9674	2.7366	1.0000	48.2400	-4.2529	33
Tuna	betageneral	0.3667	2.0300	2.1431	34.7867	NA	20
Whiting	normal	2.3234	0.5577	0.5000	10.3600	NA	4
Wolffish	normal	4.4740	3.9125	0.0000	17.7000	NA	8

ANNEX III.3 - Distribution and its parameters (Param) for the vitamin D concentration in relevant seafood species, as well as the number of data points used (N)

Species	Distribution	Param1	Param2	Param3	Param4	Param5	N
	uniform			min	max		
	betageneral	α1	α2	min	max		
	normal	μ	σ	Tr min	Tr max		
	lognormal	μ	σ	Tr min	Tr max	shift	
Anchovy	lognormal	0.0486	0.0757	0.0000	0.2800	-0.0039	12
Anglerfish	uniform	NA	NA	0.0000	0.0010	NA	2
Caviar	betageneral	0.6357	2.2928	0.0191	0.3793	NA	16
Cod	lognormal	0.0300	0.0817	0.0000	0.1560	-0.0022	16
Common shrimp	uniform	NA	NA	0.0000	0.0010	NA	3
Common whelk	uniform	NA	NA	0.0000	0.0010	NA	1
Conger	uniform	NA	NA	0.0000	0.0010	NA	3
Crab	uniform	NA	NA	0.0000	0.0010	NA	2
Eel	lognormal	0.1574	0.3891	NA	1.4000	0.0196	16
European plaice	lognormal	0.0013	0.0121	0.0000	0.0600	0.0000	6
Haddock	lognormal	0.0015	0.0015	0.0000	0.0280	-0.0004	6
Halibut	betageneral	0.3895	0.6429	0.0088	0.1526	NA	11
Herring	normal	0.1477	0.0909	0.0000	0.8140	NA	60
John dory	uniform	NA	NA	0.0000	0.0010	NA	1
Lobster	uniform	NA	NA	0.0000	0.0010	NA	2
Mackerel	lognormal	0.1127	0.0329	0.0005	0.4220	-0.0608	44
Mussel	lognormal	0.0024	0.0084	0.0000	0.0276	-0.0001	8
Nile perch	uniform	NA	NA	0.0000	0.0010	NA	1
Norway lobster	uniform	NA	NA	0.0000	0.0010	NA	1
Saithe and Pollack	normal	0.0091	0.0090	0.0000	0.0500	NA	9
Salmon	lognormal	0.1383	0.0669	0.0000	0.6000	NA	58
Sardine	lognormal	0.2484	0.0629	0.0050	0.6000	-0.1633	34
Scallop	uniform	NA	NA	0.0000	0.0010	NA	1
Scampi	normal	0.0006	0.0003	NA	0.0100	NA	4
Sea bream	normal	0.0070	0.0061	0.0000	0.0460	NA	4
Skate	uniform	NA	NA	0.0000	0.0010	NA	1
Sole	lognormal	0.0522	0.0442	0.0000	0.1813	-0.0352	5
Sprat	normal	0.1477	0.0909	0.0000	0.8140	NA	3
Squid	uniform	NA	NA	0.0000	0.0010	NA	5
Surimi	uniform	NA	NA	0.0000	0.0010	NA	0
Trout	lognormal	3.4966	0.0267	0.0000	0.3800	-3.4261	28
Tuna	lognormal	0.0343	0.0240	0.0000	0.4600	-0.0039	31
Whiting	uniform	NA	NA	0.0000	0.0010	NA	11
Wolffish	betageneral	0.2263	0.4041	0.0049	0.0160	NA	4

ANNEX III.4 - Distribution and its parameters (Param) for the iodine concentration in relevant seafood species, as well as the number of data points used (N)

Species	Distribution	Param1	Param2	Param3	Param4	Param5	N
	uniform			min	max		
	betageneral	α1	α2	min	max		
	normal	μ	σ	Tr min	Tr max		
	logistic	α	β	Tr min	Tr max		
	loglogistic	β	α	Tr min	Tr max	γ (min)	
Anchovy	betageneral	0.4037	1.9862	0.0036	1.8848	NA	15
Anglerfish	loglogistic	5.3796	5.5783	0.002	21.26	-3.1012	44
Caviar	normal	0.4617	0.3209	0.002	2.798	NA	3
Cod	logistic	2.3563	0.3901	0.145	21.26	NA	19
Common shrimp	loglogistic	0.1989	1.1331	NA	14	0.1551	29
Common whelk	loglogistic	0.1989	1.1331	NA	14	0.1551	29
Conger	betageneral	0.4037	1.9862	0.0036	1.8848	NA	15
Crab	loglogistic	5.3796	5.5783	0.002	21.26	-3.1012	44
Eel	loglogistic	0.1138	1.9765	0.005	1.6	-0.0199	5
European plaice	logistic	0.3322	0.0044	0.05	3.8	NA	10
Haddock	logistic	1.449	0.5608	0.12	5.34	NA	12
Halibut	betageneral	0.2566	0.963	0.001	1.2251	NA	5
Herring	logistic	0.3361	0.0528	0.1215	1.33	NA	13
John dory	loglogistic	0.1989	1.1331	NA	14	0.1551	29
Lobster	normal	1.19	0.75	0.5	14	NA	3
Mackerel	logistic	0.7664	0.0362	0.22	3.02	NA	11
Mussel	normal	1.3081	0.0317	0.525	2.8	NA	3
Nile perch	uniform	NA	NA	0.002	0.006	NA	2
Norway lobster	loglogistic	0.1989	1.1331	NA	14	0.1551	29
Saithe and Pollack	loglogistic	1.1553	3.5795	0.002	4.7	-0.4792	10
Salmon	normal	0.3106	0.0637	0.15	1.3	NA	5
Sardine	uniform	NA	NA	0.145	0.48	NA	3
Scallop	loglogistic	5.3796	5.5783	0.002	21.26	-3.1012	44
Scampi	loglogistic	0.1989	1.1331	NA	14	0.1551	29
Sea bream	betageneral	0.4037	1.9862	0.0036	1.8848	NA	15
Skate	loglogistic	5.3796	5.5783	0.002	21.26	-3.1012	44
Sole	normal	0.1773	0.0229	0.002	0.5	NA	3
Sprat	betageneral	0.7072	2.4604	0.2206	1.6409	NA	23
Squid	loglogistic	0.1989	1.1331	NA	14	0.1551	29
Surimi	loglogistic	5.3796	5.5783	0.002	21.26	-3.1012	44
Trout	normal	0.1309	0.0668	0.0752	0.5	NA	8
Tuna	normal	-0.0882	0.4142	0.035	1.12	NA	6
Whiting	loglogistic	5.3796	5.5783	0.002	21.26	-3.1012	44
Wolffish	betageneral	0.4037	1.9862	0.0036	1.8848	NA	15

ANNEX III.5 - Distribution and parameters (Param) for the fat concentration in relevant seafood species, as well as the number of data points used (N)

Species	Distribution	Param1	Param2	Param3	Param4	Param5	N
	uniform			min	max		
	normal	μ	σ	Tr min	Tr max		
	lognormal	μ	σ	Tr min	Tr max	shift	
	logistic	α	β	Tr min	Tr max		
Anchovy	logistic	35.7088	10.1068	11.5000	160.0000	NA	10
Anglerfish	lognormal	4.3921	5.1988	2.0000	39.0000	3.4499	15
Caviar	lognormal	80.4766	73.9635	9.5000	424.0000	8.5785	32
Cod	lognormal	19.2092	2.3810	0.0000	34.0000	-12.3241	60
Common shrimp	uniform	NA	NA	4.1370	24.5890	NA	9
Common whelk	normal	8.6198	2.7826	2.0000	24.0000	NA	4
Conger	lognormal	99.0803	32.8900	3.5000	228.0000	-46.9713	8
Crab	lognormal	28.9413	4.9991	2.5000	110.0000	-20.5289	40
Eel	normal	213.6286	68.2837	35.5000	660.0000	NA	28
European plaice	uniform	NA	NA	4.5910	20.4270	NA	31
Haddock	lognormal	3.3519	2.1557	2.3500	42.0000	3.8266	19
Halibut	logistic	33.1170	16.2834	0.5000	354.8000	NA	28
Herring	logistic	123.7569	20.0719	6.0000	662.0000	NA	82
John dory	normal	7.6881	5.1868	1.5000	28.0000	NA	4
Lobster	normal	12.9476	3.7172	2.9500	38.0000	NA	21
Mackerel	lognormal	272.8077	65.2393	55.0000	618.0000	-89.6701	58
Mussel	lognormal	73.5831	10.1289	3.0000	89.6000	-51.2564	21
Nile perch	normal	13.7534	6.8617	4.0000	48.0000	NA	4
Norway lobster	uniform	NA	NA	3.5000	16.0000	NA	3
Saithe and Pollack	lognormal	22.6623	3.0536	1.5000	40.0000	-13.1067	30
Salmon	normal	98.1080	35.8896	16.0000	361.2000	NA	100
Sardine	logistic	137.6906	25.5402	24.0000	368.0000	NA	56
Scallop	lognormal	12.8756	5.3110	0.5000	28.0000	-5.7020	7
Scampi	normal	12.9792	5.4646	3.0000	50.0000	NA	24
Sea bream	uniform	NA	NA	14.0000	70.0000	NA	4
Skate	normal	7.0500	3.6473	1.0000	25.2000	NA	12
Sole	lognormal	40.7345	6.0389	0.6500	52.0000	-35.0947	22
Sprat	normal	130.1250	41.4813	2.9000	36.8000	NA	8
Squid	lognormal	14.5848	12.6205	2.0000	94.0000	2.2428	29
Surimi	logistic	8.1651	0.7409	2.0000	26.2000	NA	8
Trout	lognormal	57.7602	44.4512	50.2500	366.0000	8.7146	56
Tuna	lognormal	93.5312	34.8306	1.0000	310.0000	-54.9499	34
Whiting	logistic	5.8661	0.5255	1.5000	16.2000	NA	18
Wolffish	logistic	31.1497	6.6996	2.9500	118.0000	NA	19

ANNEX III.6 - Distribution and parameters (Param) for the mercury concentration in relevant seafood species, as well as the number of data points used (N)

Species	%	Distribution	Param1	Param2	Param3	Param4	Param5	N
		uniform			min	max		
		betageneral	α1	α2	min	max		
		normal	μ	σ	Tr min	Tr max		
		lognormal	μ	σ	Tr min	Tr max	shift	
		logistic	α	β	Tr min	Tr max		
		loglogistic	β	α	Tr min	Tr max	γ (min)	
Anchovy	100	normal	53.2489	18.1537	14.0000	208.0000	NA	13
Anglerfish	100	uniform	NA	NA	21.3641	1141.271	NA	14
Caviar	100	no_distribution	NA	NA	NA	NA	NA	0
Cod	100	logistic	66.6564	6.7232	7.0000	8780.000	NA	104
Common shrimp	100	loglogistic	34.6900	3.0850	10.0000	1134.000	2.8172	42
Common whelk	100	uniform	NA	NA	50.5000	151.5000	NA	1
Conger	100	no_distribution	NA	NA	NA	NA	NA	0
Crab	100	loglogistic	65.1614	7.0722	8.5000	300.0000	-1.9272	11
Eel	100	normal	95.8212	71.4331	5.0000	640.0000	NA	27
European plaice	100	lognormal	147.393	22.9030	7.5000	404.0000	-100.71	41
Haddock	100	betageneral	1.2056	2.9272	8.1556	151.1400	NA	11
Halibut	100	normal	82.1316	7.8708	34.5000	308.0000	NA	3
Herring	100	logistic	33.2401	12.7331	1.5000	230.0000	NA	15
John dory	100	uniform	NA	NA	20.0000	75.0000	NA	2
Lobster	100	betageneral	0.3575	0.3728	59.7916	454.5314	NA	7
Mackerel, Mediterranean Sea	1.8	lognormal	262.450	32.3860	63.0000	580.0000	-57.2340	5
Mackerel, Northeast Atlantic Ocean	98.2	logistic	29.8991	8.6537	1.5000	200.0000	NA	12
Mussel	100	betageneral	0.0906	4.3860	8.4665	156.4585	NA	13
Nile perch	100	normal	82.3325	41.0294	4.8500	1181.600	NA	13
Norway lobster	100	betageneral	0.3575	0.3728	59.7916	454.5314	NA	7
Saithe and Pollack	100	logistic	47.6786	7.5200	3.5000	500.0000	NA	15
Salmon, farmed	64.07	betageneral	0.7041	2.8444	16.9038	81.5676	NA	42
Salmon, Pacific Ocean	35.93	logistic	49.4647	13.8422	5.0000	234.0000	NA	32
Sardine, Eastern Central Atlantic Ocean	60.62	lognormal	15.8642	9.5737	NA	208.0000	5.7499	18
Sardine, Mediterranean Sea	39.38	logistic	101.107	11.5022	23.3650	468.0000	NA	8
Scallop	100	loglogistic	0.3039	1.0202	NA	84.0000	19.9561	9
Scampi	100	betageneral	0.0624	2.9870	17.0001	266.2788	NA	25
Sea bream	100	uniform	NA	NA	25.0000	75.0000	NA	1
Skate	100	uniform	NA	NA	4.6500	1650.000	NA	24
Sole	100	betageneral	0.7578	2.3677	4.4894	363.8900	NA	32
Sprat	100	uniform	NA	NA	5.0000	90.0000	NA	4
Squid, Mediterranean Sea	89.82	logistic	11.5608	1.5213	1.5000	166.8000	NA	9
Squid, Southwest Atlantic Ocean	10.18	uniform	NA	NA	35.0000	133.5000	NA	3
Surimi	100	no_distribution	NA	NA	NA	NA	NA	0
Trout	100	lognormal	22.3350	20.2067	NA	298.0000	29.0953	27
Tuna	100	lognormal	658.790	892.639	NA	6460.000	15.6585	153
Whiting	100	lognormal	54.2950	46.8510	NA	588.0000	22.5420	39
Wolffish	100	uniform	NA	NA	25.0000	75.0000	NA	1

ANNEX III.7 - Distribution and parameters (Param) for the calculated methyl mercury concentration in relevant seafood species, as well as the number of data points used (N)

Species	%	Distribution	Param1	Param2	Param3	Param4	Param5	N
		uniform			min	max		
		betageneral	α1	α2	min	max		
		normal	μ	σ	Tr min	Tr max		
		lognormal	μ	σ	Tr min	Tr max	shift	
		logistic	α	β	Tr min	Tr max		
		loglogistic	β	α	Tr min	Tr max	γ (min)	
Anchovy	100	normal	42.5055	14.4910	11.1754	245.8584	NA	13
Anglerfish	100	betageneral	0.3877	0.1551	63.5953	606.7781	NA	14
Caviar	100	no_distribution	NA	NA	NA	NA	NA	0
Cod	100	logistic	53.2079	5.3668	5.5877	7008.5602	NA	104
Common shrimp	100	loglogistic	11.7632	3.0850	NA	384.5291	0.9553	42
Common whelk	100	uniform	NA	NA	34.3575	103.0725	NA	1
Conger	100	no_distribution	NA	NA	NA	NA	NA	0
Crab	100	loglogistic	22.0956	7.0722	2.8823	101.7273	-0.6535	11
Eel	100	normal	76.4884	57.0208	3.9912	510.8745	NA	27
European plaice	100	loglogistic	15.2829	1.6400	NA	322.4896	17.8938	41
Haddock	100	betageneral	1.2056	2.9272	6.5101	120.6478	NA	11
Halibut	100	normal	65.5609	6.2828	27.5393	245.8584	NA	3
Herring	100	logistic	26.5336	10.1641	1.1974	183.5955	NA	15
John dory	100	uniform	NA	NA	15.9648	59.8681	NA	2
Lobster	100	betageneral	0.3575	0.3728	20.2748	154.1275	NA	7
Mackerel, Mediterranean Sea	1.8	lognormal	209.5021	25.8515	50.2892	462.9801	-45.6865	5
Mackerel, Northeast Atlantic Ocean	98.2	logistic	23.8667	6.9077	1.1974	159.6483	NA	12
Mussel	100	betageneral	0.0906	4.3860	2.8709	53.0536	NA	13
Nile perch	100	normal	65.7212	32.7514	3.8715	943.2021	NA	13
Norway lobster	100	betageneral	0.3575	0.3728	20.2748	154.1275	NA	7
Saithe and Pollack	100	logistic	38.0590	6.0028	2.7938	399.1207	NA	15
Salmon, farmed	64.1	betageneral	0.7041	2.8444	13.4934	65.1106	NA	42
Salmon, Pacific Ocean	35.9	lognormal	201.7383	18.1925	3.9912	186.7885	-161.618	32
Sardine, Eastern Central Atlantic Ocean	60.6	loglogistic	20.7045	6.4354	1.9956	208.0000	-5.1385	18
Sardine, Mediterranean Sea	39.4	logistic	80.7084	9.1815	18.6509	373.5770	NA	8
Scallop	100	loglogistic	0.1030	1.0202	NA	28.4836	6.7669	9
Scampi	100	lognormal	34.1832	40640.102	NA	168.8673	5.7644	25
Sea bream	100	uniform	NA	NA	19.9560	59.8681	NA	1
Skate	100	betageneral	0.5848	1.3428	17.2469	1217.7227	NA	24
Sole	100	betageneral	0.7578	2.3677	3.5836	290.4710	NA	32
Sprat	100	uniform	NA	NA	3.9912	71.8417	NA	4
Squid, Mediterranean Sea	10.2	uniform	NA	NA	23.8121	90.8263	NA	3
Squid, Southwest Atlantic Ocean	89.8	logistic	7.8653	1.0350	1.0205	113.4818	NA	9
Surimi	100	no_distribution	NA	NA	NA	NA	NA	0
Trout	100	lognormal	17.8287	16.1298	NA	237.8760	23.2251	27
Tuna	100	betageneral	0.6326	3.3760	80.1298	2628.8888	NA	153
Whiting	100	lognormal	43.3407	37.3987	NA	469.3660	17.9942	39
Wolffish	100	uniform	NA	NA	19.9560	59.8681	NA	1

ANNEX III.8 - Distribution and parameters (Param) for the iPCB concentration in relevant seafood species, as well as the number of data points used (N)

Species	%	Distribution	Param1	Param2	Param3	Param4	Param5	N
		uniform			min	max		
		betageneral	α1	α2	min	max		
		normal	μ	σ	Tr min	Tr max		
		lognormal	μ	σ	Tr min	Tr max	shift	
		logistic	α	β	Tr min	Tr max		
		loglogistic	β	α	Tr min	Tr max	γ (min)	
Anchovy	100	betageneral	0.5762	3.0084	13.2207	92.6398	NA	10
Anglerfish	100	uniform	NA	NA	0.1000	1.8000	NA	3
Caviar	100	loglogistic	11.0376	2.5872	NA	203.0000	9.6586	21
Cod	100	logistic	0.9595	0.2620	0.1000	70.0000	NA	57
Common shrimp	100	loglogistic	0.7017	0.6924	NA	392.0000	0.2917	45
Common whelk	100	lognormal	10.6747	21.5897	0.1500	392.0000	-0.5751	120
Conger	100	loglogistic	11.0376	2.5872	NA	203.0000	9.6586	21
Crab	100	loglogistic	60.5177	1.5296	0.9950	560.0000	-24.0733	13
Eel	100	lognormal	386.1751	2410.4067	NA	11472.6680	9.4147	159
European plaice	100	loglogistic	1.0512	1.8705	0.1500	70.0000	0.1442	31
Haddock	100	loglogistic	0.4932	2.2905	0.1000	4.6763	-0.1356	11
Halibut	100	betageneral	1.9509	0.4589	9.8699	22.1100	NA	6
Herring, Baltic Sea	5.44	betageneral	1.0979	3.7898	4.7632	193.0384	NA	156
Herring, others	94.56	betageneral	0.3269	5.1964	7.0347	68.5782	NA	5
John dory	100	lognormal	10.6747	21.5897	0.1500	392.0000	-0.5751	120
Lobster	100	lognormal	10.6747	21.5897	0.1500	392.0000	-0.5751	120
Mackerel	100	lognormal	8.3498	10.4416	NA	116.0000	3.6131	16
Mussel	100	normal	7.4274	6.0454	0.5800	44.6200	NA	13
Nile perch	100	lognormal	10.6747	21.5897	0.1500	392.0000	-0.5751	120
Norway lobster	100	lognormal	10.6747	21.5897	0.1500	392.0000	-0.5751	120
Saithe and Pollack	100	uniform	NA	NA	0.3691	1.8667	NA	3
Salmon	100	loglogistic	91.3367	21.7584	0.1523	640.7226	-79.8300	90
Sardine	100	logistic	13.6936	3.6041	2.6635	208.0000	NA	10
Scallop	100	logistic	0.8816	0.3054	0.1700	35.0000	NA	6
Scampi	100	uniform	NA	NA	0.0425	1.0990	NA	4
Sea bream	100	loglogistic	11.0376	2.5872	NA	203.0000	9.6586	21
Skate	100	betageneral	0.0682	0.9075	0.3674	38.0536	NA	13
Sole	100	lognormal	11.5521	12.1603	0.1900	70.0000	-4.4486	19
Sprat	100	logistic	9.1463	1.3977	3.5000	65.3874	NA	6
Squid	100	uniform	NA	NA	0.2900	66.2355	NA	6
Surimi	100	logistic	0.9959	0.4176	0.0500	560.0000	NA	120
Trout	100	logistic	13.3240	3.8499	2.6635	208.0000	NA	12
Tuna	100	loglogistic	11.0376	2.5872	NA	203.0000	9.6586	21
Whiting	100	lognormal	5.7108	13.9496	0.0500	70.0000	-0.1728	28
Wolffish	100	loglogistic	11.0376	2.5872	NA	203.0000	9.6586	21

ANNEX III.9 - Distribution and parameters (Param) for the dl PCB concentration in relevant seafood species, as well as the number of data points used (N)

Species	%	Distribution	Param1	Param2	Param3	Param4	Param5	N
		uniform			min	max		
		betageneral	α1	α2	min	max		
		normal	μ	σ	Tr min	Tr max		
		lognormal	μ	σ	Tr min	Tr max	shift	
		logistic	α	β	Tr min	Tr max		
Anchovy	100	uniform	NA	NA	0.25	11.85	NA	4
Anglerfish	100	loglogistic	0.0747	1.2186	0.0014	2.8	0.0002	94
Caviar	100	loglogistic	1.1167	1.598	0.01	46.1106	-0.319	68
Cod	100	lognormal	0.1891	0.3882	NA	1.064	0.0232	43
Common shrimp	100	normal	0.4227	0.3025	0.005	1.86	NA	5
Common whelk	100	lognormal	1.2752	0.3955	0.0008	4.8	-0.7468	65
Conger	100	loglogistic	1.1167	1.598	0.01	46.1106	-0.319	68
Crab	100	loglogistic	0.0747	1.2186	0.0014	2.8	0.0002	94
Eel	100	lognormal	11.4557	20.5263	0.045	230	0.0159	142
European plaice	100	logistic	0.3374	0.0465	0.063	2.078	NA	14
Haddock	100	loglogistic	0.0227	2.5595	NA	0.732	0.0177	31
Halibut	100	normal	1.1586	0.5388	0.215	23.32	NA	10
Herring, Baltic Sea	5.44	lognormal	6.3217	3.0684	0.2403	74.98	-2.1033	182
Herring, North Sea	50.89	betageneral	0.2291	2.0719	0.89	6.5646	NA	34
Herring, others	43.67	betageneral	0.4297	1.2207	4.9682	12.1607	NA	4
John dory	100	lognormal	1.2752	0.3955	0.0008	4.8	-0.7468	65
Lobster	100	normal	0.1695	0.3498	0.0021	1.08	NA	3
Mackerel	100	loglogistic	2.8421	13.5998	0.18	41.18	-1.6156	48
Mussel	100	normal	0.5565	1.2006	0.05	4.06	NA	13
Nile perch	100	lognormal	1.2752	0.3955	0.0008	4.8	-0.7468	65
Norway lobster	100	lognormal	1.2752	0.3955	0.0008	4.8	-0.7468	65
Saithe and Pollack	100	betageneral	0.7251	1.7462	0.0029	0.8	NA	4
Salmon, Baltic Sea	45.08	loglogistic	3.0346	3.3585	NA	31.4	6.1069	46
Salmon, farmed	27.62	lognormal	3.6574	1.517	0.035	16	-1.9129	162
Salmon, Pacific Ocean	27.30	betageneral	0.4565	1.7419	0.0076	4.2119	NA	14
Sardine	100	logistic	2.0999	1.3447	0.001	12.6	NA	8
Scallop	100	loglogistic	0.0747	1.2186	0.0014	2.8	0.0002	94
Scampi	100	betageneral	1.5552	7.3677	0.0011	0.2469	NA	16
Sea bream	100	loglogistic	1.1167	1.598	0.01	46.1106	-0.319	68
Skate	100	loglogistic	0.0747	1.2186	0.0014	2.8	0.0002	94
Sole	100	lognormal	1.2752	0.3955	0.0008	4.8	-0.7468	65
Sprat	100	logistic	3.1729	0.2637	0.4825	7.8	NA	8
Squid	100	betageneral	2.3843	1.2448	0.3454	1.9616	NA	4
Surimi	100	loglogistic	0.0747	1.2186	0.0014	2.8	0.0002	94
Trout	100	loglogistic	0.4389	1.7373	NA	11.8	0.4799	100
Tuna	100	lognormal	16.4224	2603.779	0.01	46.1106	-0.0018	50
Whiting	100	betageneral	0.3179	2.987	0.0301	0.6557	NA	16
Wolffish	100	loglogistic	1.1167	1.598	0.01	46.1106	-0.319	68

ANNEX III.10 - Distribution and parameters (Param) for the PCDD/F concentration in relevant seafood species, as well as the number of data points used (N)

Species	%	Distribution	Param1	Param2	Param3	Param4	Param5	N
		uniform			min	max		
		betageneral	α1	α2	min	max		
		normal	μ	σ	Tr min	Tr max		
		lognormal	μ	σ	Tr min	Tr max	shift	
		logistic	α	β	Tr min	Tr max		
		loglogistic	β	α	Tr min	Tr max	γ (min)	
Anchovy	100	normal	0.1196	0.4232	0.0200	2.6600	NA	7
Anglerfish	100	loglogistic	0.0687	3.3976	0.0009	5.0000	-0.0162	106
Caviar	100	normal	0.5372	0.4404	0.0040	15.1400	NA	92
Cod, Iceland	15.46	betageneral	0.9217	2.5834	0.0018	0.0632	NA	17
Cod, North Sea	38.05	loglogistic	0.4288	69.2198	0.0150	0.6000	-0.3815	10
Cod, others	46.49	uniform	NA	NA	0.0151	0.1890	NA	4
Common shrimp	100	logistic	0.6012	0.2085	0.0500	3.1400	NA	9
Common whelk	100	normal	0.5529	0.4326	0.0095	7.2900	NA	95
Conger	100	normal	0.5372	0.4404	0.0040	15.1400	NA	92
Crab	100	uniform	NA	NA	0.0350	3.7500	NA	5
Eel	100	betageneral	0.8510	2.4160	0.1660	5.9656	NA	82
European plaice	100	loglogistic	0.7626	25.2105	0.0280	2.9600	-0.4768	19
Haddock	100	lognormal	0.0248	0.0350	NA	0.2000	0.0214	33
Halibut, Northeast Atlantic Ocean	42.43	logistic	0.6826	0.1760	0.0900	15.1400	NA	27
Halibut, Northwest Atlantic Ocean	57.57	uniform	NA	NA	0.0262	0.9190	NA	12
Herring, Baltic Sea	5.44	betageneral	0.4462	3.3479	0.7263	37.2970	NA	261
Herring, North Sea	50.89	loglogistic	1.3309	6.7805	0.0555	5.4400	-0.5048	63
Herring, others	43.67	normal	9.3960	6.8145	0.1970	40.0000	NA	4
John dory	100	normal	0.5529	0.4326	0.0095	7.2900	NA	95
Lobster	100	uniform	NA	NA	0.0625	1.1400	NA	3
Mackerel	100	loglogistic	0.3335	4.9583	0.0500	13.8200	-0.0225	63
Mussel	100	normal	0.4420	0.7677	0.0500	7.2900	NA	16
Nile perch	100	normal	0.5529	0.4326	0.0095	7.2900	NA	95
Norway lobster	100	normal	0.5529	0.4326	0.0095	7.2900	NA	95
Saithe and Pollack	100	normal	0.0573	0.0383	0.0009	0.3460	NA	17
Salmon, Baltic Sea	45.08	lognormal	3.7818	3.4750	NA	34.8000	1.4440	51
Salmon, others	54.92	loglogistic	0.4700	1.5254	NA	15.6000	0.1247	152
Sardine	100	lognormal	1.0494	0.4920	0.0100	2.4000	-0.5447	16
Scallop	100	loglogistic	0.0687	3.3976	0.0009	5.0000	-0.0162	106
Scampi	100	lognormal	4.7183	69167.9942	NA	0.5020	0.0320	15
Sea bream	100	normal	0.5372	0.4404	0.0040	15.1400	NA	92
Skate	100	loglogistic	0.0687	3.3976	0.0009	5.0000	-0.0162	106
Sole	100	normal	0.5529	0.4326	0.0095	7.2900	NA	3
Sprat	100	uniform	NA	NA	1.8595	4.1191	NA	23
Squid	100	betageneral	0.3239	1.8157	0.1069	1.7820	NA	9
Surimi	100	loglogistic	0.0687	3.3976	0.0009	5.0000	-0.0162	106
Trout	100	loglogistic	0.2700	1.6604	NA	9.6000	0.1293	99
Tuna	100	logistic	0.0462	0.6612	0.0040	10.0140	NA	42
Whiting	100	uniform	NA	NA	0.0177	0.0755	NA	14
Wolffish	100	logistic	0.5109	0.0190	0.1940	1.7100	NA	5

ANNEX III.11 - Distribution and parameters (Param) for total TEQ concentration in relevant seafood species, as well as the number of data points used (N)

Species	%	Distribution	Param1	Param2	Param3	Param4	Param5	N
		uniform			min	max		
		betageneral	α1	α2	min	max		
		normal	μ	σ	Tr min	Tr max		
		lognormal	μ	σ	Tr min	Tr max	shift	
		logistic	α	β	Tr min	Tr max		
		loglogistic	β	α	Tr min	Tr max	γ (min)	
Anchovy	100	loglogistic	1.6059	1.8653	0.015	49.0126	-0.6089	55
Anglerfish	100	loglogistic	0.1136	1.2063	NA	7.2	0.0232	88
Caviar	100	loglogistic	1.6059	1.8653	0.015	49.0126	-0.6089	55
Cod	100	betageneral	0.4188	1.6878	0.065	1.0239	NA	42
Common shrimp	100	normal	0.9698	0.6299	0.06	5.7	NA	7
Common whelk	100	normal	1.1112	0.8013	0.0139	8.12	NA	76
Conger	100	loglogistic	1.6059	1.8653	0.015	49.0126	-0.6089	55
Crab	100	loglogistic	0.1136	1.2063	NA	7.2	0.0232	88
Eel	100	loglogistic	7.888	2.2451	0.2295	252	-1.5753	106
European plaice	100	logistic	0.5841	0.0818	0.08	2.52	NA	17
Haddock	100	betageneral	0.3402	1.5712	0.0587	0.2269	NA	27
Halibut	100	normal	1.6462	0.8	0.35	38.46	NA	10
Herring, Baltic Sea	5.44	betageneral	0.8608	3.2638	1.3899	54.3135	NA	186
Herring, others	94.56	loglogistic	2.4942	2.2397	NA	59.4	0.6846	67
John dory	100	normal	1.1112	0.8013	0.0139	8.12	NA	76
Lobster	100	uniform	NA	NA	0.0646	1.845	NA	3
Mackerel	100	loglogistic	0.8483	3.1177	NA	55	0.5822	52
Mussel	100	uniform	NA	NA	0.12	6.09	NA	13
Nile perch	100	normal	1.1112	0.8013	0.0139	8.12	NA	76
Norway lobster	100	normal	1.1112	0.8013	0.0139	8.12	NA	76
Saithe and Pollack	100	normal	0.2928	0.1498	0.0036	1.8	NA	4
Salmon, Baltic Sea	31.32	loglogistic	17.8256	7.9011	1.485	65.96	-3.6945	50
Salmon, farmed	51.12	lognormal	6.8722	1.9378	0.05	30.8	-4.4695	176
Salmon, Pacific Ocean	17.56	betageneral	0.1903	1.1646	0.0999	0.5514	NA	8
Sardine	100	lognormal	16.4456	2.4166	0.05	15.8	-13.2736	6
Scallop	100	loglogistic	0.1136	1.2063	NA	7.2	0.0232	88
Scampi	100	betageneral	2.6814	4.9127	0.0381	0.163	NA	14
Sea bream	100	loglogistic	1.6059	1.8653	0.015	49.0126	-0.6089	55
Skate	100	loglogistic	0.1136	1.2063	NA	7.2	0.0232	88
Sole	100	normal	1.1112	0.8013	0.0139	8.12	NA	76
Sprat	100	normal	6.2662	1.1229	0.9233	15	NA	10
Squid	100	uniform	NA	NA	0.065	4.8	NA	4
Surimi	100	loglogistic	0.1136	1.2063	NA	7.2	0.0232	88
Trout	100	loglogistic	0.5654	1.5764	NA	21.2	0.6732	80
Tuna	100	betageneral	0.1073	1.4759	0.0283	33.7238	NA	41
Whiting	100	betageneral	0.1771	1.4633	0.077	0.9459	NA	15
Wolffish	100	loglogistic	1.6059	1.8653	0.015	49.0126	-0.6089	55

Chapter IV.

Probabilistic intake assessment of multiple compounds as a tool to quantify the nutritional-toxicological conflict related to seafood consumption

This chapter is based on the following papers:

1. Sioen I, Van Camp J, Verdonck F, Verbeke W, Vanhonacker F, Willems J, De Henauw S. Probabilistic intake assessment of multiple compounds as a tool to quantify the nutritional-toxicological conflict related to seafood consumption. *Chemosphere*. Submitted.

2. Sioen I, De Henauw S, Verbeke W, Verdonck F, Willems J, Van Camp J. Fish consumption is a safe solution to increase the intake of long chain omega-3 fatty acids. *Public Health Nutrition*. Submitted.

1. Introduction

For the intake assessment of nutrients and contaminants via seafood consumption, it was chosen to use a probabilistic approach, taking into account the variability of seafood consumption data, body weight data, and nutrient and contaminant concentration data. Therefore, a **methodology** and a **software module** were developed to calculate the simultaneous intake of multiple (n=10) nutrients and contaminants via seafood consumption, i.e. mercury (Hg), methyl mercury (MeHg; deduced from the Hg concentration by a conversion factor), indicator PCBs (iPCBs), dioxin-like PCBs (dl PCBs), dioxins plus furans (PCDD/Fs), total dioxin-like compounds (total TEQ), long-chain omega-3 poly-unsaturated fatty acids (LC n-3 PUFAs), vitamin D, iodine, and fat. This software module is applicable both for **real intake assessments** as well as for **scenario analyses**.

The results of both real intake assessments as scenario analyses related to seafood consumption in Belgium are described in this chapter. The intakes were evaluated and seafood consumption recommendations were formulated balancing the associated risks and benefits to maximize public health. Firstly, the results of an intake assessment based on existing seafood consumption data of the Belgian population are described. Within this part, several scenarios related to the contamination data are considered. Secondly, intake assessment results based on different consumption scenarios are described. These scenario analyses were executed in order to find out whether the recommendation of LC n-3 PUFAs can be reached without a contaminant intake of toxicological concern.

2. Intake assessment based on the current seafood consumption pattern in Belgium

In this part of chapter IV, the intake assessment based on the current seafood consumption pattern (i.e. real intake assessment) is described. The results are discussed in part 4 of this chapter.

2.1. Materials and Methods

2.1.1. Nutrient and contaminant data

The following **contaminants** were included: **Hg** and **MeHg**, seven indicator PCBs (**iPCBs**), dioxin-like PCBs (**dl PCBs**), sum of 7 polychlorinated dibenzo-*p*-dioxin congeners (PCDDs) and 10 polychlorinated dibenzofuran congeners (PCDFs) (from here on referred to as **PCDD/Fs**), and total dioxin-like compounds (from here on referred to as **total TEQ** (totTEQ)). Although several other contaminants may accumulate in the marine food chain (e.g. arsenic, polycyclic aromatic hydrocarbons, polybrominated diphenyl ethers), it was decided to limit this study to the above mentioned compounds. The main reason for this limitation is the fact that for these contaminants a huge amount of data concerning different species and different origins relevant for the Belgian market are available in international scientific literature. Additionally, the rationale behind selecting the **dioxin-like compounds** originates from the observation that seafood typically has a higher concentration of dioxin-like compounds compared to other food items (when expressed per g fat) (Kiviranta *et al*, 2004). Moreover, a recent Belgian study showed that seafood is the most important dietary source of dioxin-like compounds (see Chapter V part 1) (Bilau *et al*, 2007). The selection of **MeHg** was motivated by the fact that seafood is the most important dietary source of Hg in the human food chain. In the marine environment, inorganic Hg is to a high extent transformed to MeHg, which further accumulates in the marine food chain. This organic MeHg is for humans the most toxic form of Hg (Clarkson & Magos, 2006).

The following **nutrients** were included: the sum of **EPA and DHA** (EPA plus DHA), **vitamin D**, **iodine**, and total **fat**. The rationale behind selecting those nutrients is that their natural concentration in seafood is high compared to other food items. Fat was included specifically to study the relation with the intake of the other compounds. In contrast to the other

nutrients, it was not relevant to discuss the intake of fat by seafood consumption only, since a lot of other food items in the diet contribute to the total fat intake.

The concentrations and probability distributions used for both the nutrients and contaminants originate from the two extensive, newly compiled databases described in chapter III.

2.1.2. Consumption data

The intake assessment results described in this part of chapter IV were based on **recorded data of seafood consumption** in Belgium. Two different existing food consumption databases were used: one of Flemish **adolescents** and another of Belgian **adults**.

1. The first database contains consumption data of 341 adolescents (129 boys and 212 girls) aged between 13 and 18 years, collected during seven consecutive days, using an estimated food record method (semi-structured diary), in Ghent (Flanders, the Dutch-speaking part of Belgium) between March and May 1997. It is the same database that was used in chapter II to assess the overall PUFA and vitamin D intake of Flemish adolescents.
2. The second database was collected from 821 Belgian adults (202 men and 619 women) aged between 19 and 83 years, representative for the Belgian population with respect to age and region. The data were collected between November and December 2004 as part of the pan-European SEAFOODplus consumer survey (Honkanen & Brunsø, 2007). In fact, a comprehensive questionnaire, including many behavioural and attitudinal aspects relevant to consumer science was distributed to the study population. The questionnaire assessed the frequency and the amount of consumption of the ten most consumed seafood species in Belgium, i.e. cod, salmon, tuna, saithe, sole, European plaice, herring, trout, mackerel, and eel. Methodological details of this study were recently described by Olsen *et al* (2007) and Pieniak *et al* (2007).

2.1.3. Simulation model and probabilistic methodology

The following **simulation model**, combining species-specific seafood consumption data with nutrient and contaminant concentration data, was used for the intake assessment:

$$Y_i = \frac{\sum_a \sum_v \sum_t (\mathrm{b}_{v,a} \cdot X_{v,i,t} \cdot C_{v,a})}{T \cdot bw_i}$$

where Y_i= average daily intake of individual i per kg body weight (bw);

$X_{v,i,t}$= amount (g) of seafood species v consumed by individual i (with body weight bw_i), at day t ($t = 1,\ldots,T$);

$\mathrm{b}_{v,a}$= probability determining whether seafood species v originates from region a;

$C_{v,a}$= concentration of a specific nutrient/contaminant in seafood species v from region a.

As previously indicated, a **probabilistic approach** was applied for simulating, taking into account the observed variability of the consumption, body weight, and concentration data. Marien (2002) previously described the importance of applying species-specific consumption data on individual level as well as individual body weight data when assessing intake of contaminants through seafood. To perform the simulations of this probabilistic intake assessment, **a software module called ProbIntake^{UG}** was developed at the Ghent University (Ghent, Belgium). ProbIntake^{UG} is applicable in the free available software program R® (R Development Core Team, 2006). For the consumption, the variability was taken into account in a non-parametric way, i.e. by using the data as such, because the dataset was large enough. For the concentration data, the variability was taken into account in a parametric way, i.e. by using species and compounds specific probability distributions. For the body weight data, a non-parametric approach was applied in the simulations using the adolescent consumption data, since the body weight of each individual adolescent was known and as such, no assumptions needed to be made. In contrast, for the adults' consumption dataset no individual body weight data were available. Therefore, normal body weight probability distribution were applied per gender and age interval, based on available data for the Belgian population (B.I.R.N.H. study (De Backer, 1984; Kornitzer & Dramaix, 1989)) (Table IV.1).

Table IV.1: Mean and standard deviation (S.D.) of the applied body weight distributions *

Age interval (years)	Men		Women	
	Mean (kg)	S.D.	Mean (kg)	S.D.
30-39	77.2	11.2	62.7	10.9
40-49	78.9	11.5	66.7	11.7
50-59	77.4	11.4	69.5	11.2
60-69	75.3	12.3	69.5	11.9

* From references (De Backer, 1984; Kornitzer & Dramaix, 1989)

The **simulation procedure** for each individual works as follows: each single consumption data point is multiplied with a concentration data point. This combination is conducted for all consumed seafood species and for all different compounds. Next, the assessed intakes per compound were enumerated and this sum was divided by the number of days and the individual's body weight. Finally, this procedure was repeated for all individuals.

Two extensions were applied increasing the number of individuals and the number of recording days per individual:

- First of all, the number of individuals was artificially repeated ten times. This repetition accounted predominantly for the uncertainty arising from a relatively small sample size when compared to the total population.
- Secondly, the 7-day diary was extended to a fictitious 35-day diary per adolescent (by simply repeating the diary five times consecutively) and the adults' consumption data were extended to ten weeks per adult (by repeating the data ten times), accounting for the uncertainty arising from a relatively short period in time.

Repeating the dataset was needed to ensure that the whole concentration range of the nutrients and contaminants was reflected in the intake calculation and that a good convergence of the population intake assessment was reached. A larger extension would increase the simulation processing time, without significantly improving the intake estimations.

2.1.4. Scenario analyses related to the contamination level

The probabilistic intake assessment based on real consumption data was run for three scenarios, differing on the level of contaminant concentrations used:

1. **In a first scenario**, all the concentration data in the contaminant database were used, whether or not the EU maximum levels for seafood products from the European Commission (2006) were exceeded.
2. **In a second scenario**, the intake of dl PCBs, PCDD/Fs, and total TEQ has been estimated excluding concentration data from the herring and salmon originating from the Baltic Sea, hence using only the concentration data from herring and salmon originating from other fishing regions. The rationale behind this scenario is that the Baltic Sea has been contaminated for many years by emissions of PCDD/Fs from paper and metal industry plants and waste incineration plants, as well as from rivers discharging into the Baltic, leading to higher concentrations in seafood (Danish Veterinary and Food Administration, 2004). This can also be seen in the box plots for dioxin-like compounds given in chapter III. In order to reduce human consumption of dioxin-like compounds, in July 2002 the European Commission set a new maximum allowable concentration of PCDD/Fs in edible parts of seafood of 4 pg WHO-TEQ/g fresh weight. Finland and Sweden got an exemption order until the end of 2006 to place seafood from the Baltic region with PCDD/F levels above this maximum allowable concentration on the domestic market.
3. **In a third scenario**, the intake of dioxin-like compounds and total TEQ has been estimated excluding all concentration data exceeding the EU maximum levels, being 4 pg WHO-TEQ/g fresh weight for PCDD/Fs and 8 pg WHO-TEQ/g fresh weight for dl PCBs plus PCDD/Fs (total TEQ), with the exception of eel that may contain 12 pg WHO-TEQ/g fresh weight of dl PCBs plus PCDD/Fs (European Commission, 2006). The rationale behind this third scenario was to assess the intake of dioxin-like compounds under the assumption that the EU regulation is applied under the strictest conditions.

2.1.5. Evaluation of nutrient and contaminant intakes

To evaluate whether the intake of nutrients was adequate, 'ad hoc' reference values were calculated for the two study populations on the basis of the dietary reference intakes (DRI) formulated by the Belgian Health Council (2007).

- For **EPA plus DHA**, this DRI is 0.3% of the total energy intake per day. For the adolescents study population, the reference value for EPA plus DHA was calculated for each individual based on his/her energy intake and body weight; the mean EPA plus DHA reference value for this population was 12.2 mg/kg bw/day (Matthys *et al*, 2003).

For the Belgian adult study population, a mean bw of 70 kg was applied and a mean energy intake of 2046 kcal was used, based on the data of the most recent Belgian Food Consumption Survey (De Vriese *et al*, 2006), leading to a reference value of 9.7 mg/kg bw/day for EPA plus DHA.

- For **vitamin D** and **iodine**, the Belgian DRI is 5 µg/day (applied in this approach as 0.085 µg/kg bw/day for the adolescents (based on a mean bw of 59.1 kg) and 0.071 µg/kg bw/day for the adults (based on a mean bw of 70 kg)) and 150 µg/day (applied in this approach as 2.54 µg/kg bw/day for the adolescents and 2.14 µg/kg bw/day for the adults), respectively. Dividing the reference values for nutrients by the bw was relevant in this study in order to express the reference values for nutrients and contaminants on the same scale.

Tolerable daily/weekly intakes (TDI/TWI) were used to evaluate contaminant intakes. For **MeHg**, a TWI of 1.6 µg/kg of bw/week (i.e. 0.228 µg/kg bw/day) has been proposed (EFSA, 2004) and for **dioxin-like compounds**, the EU proposes 2 pg WHO-TEQ/kg bw/day (14 pg WHO-TEQ/kg bw/week) (Scientific Committee on Food, 2001). For the **non-dioxin like PCBs** (ndl PCBs), no health based guidance value for humans has been established. The health based advice is, therefore, that the intake of ndl PCBs should be as low as possible (EFSA, 2005c). Finally, it is important to note that the thresholds used for nutrient and contaminant intake refer to the intake via the **total diet**, whereas in this study, they were used to evaluate the intake via one single group of food items, namely seafood.

2.2. Results

Of all 341 adolescents in the first consumption database, 123 (36%) did not consume any seafood during the week of the study; hence, their assessed multiple compound intake via seafood was equal to zero. Of all 821 respondents in the database of Belgian adults, 52 (6.3%) claimed not to consume seafood. The results in the tables are provided for both the whole population sample and the consumers-only (Table IV.2 and Table IV.3).

The assessed **contaminant** intake results are given in Table IV.2.

Table IV.2: Summary of assessed intakes of contaminants via seafood consumption for Flemish adolescents and Belgian adults, for the whole population sample and for the seafood consumers only (the intakes exceeding the TDI are indicated in bold)

		Including all concentration data										Excluding concentration data of Baltic herring and salmon			Excluding concentrations data > EU max levels	
		MeHg		iPCB		dl PCB		PCDD/Fs		totTEQ		dl PCB	PCDD/Fs	totTEQ	PCDD/Fs	totTEQ
		ng/kg bw/day				pg WHO-TEQ/kg bw/day						pg WHO-TEQ/kg bw/day			pg WHO-TEQ/kg bw/day	
		All	Cons.	All	Cons.	All	Cons.	All	Cons.	All	Cons.	Cons.	Cons.	Cons.	Cons.	Cons.
	TDI	228		-		2		2		2		2	2	2	2	2
ADOLESCENTS	Mean	16.8	26.3	3.1	4.8	0.4	0.6	0.2	0.4	0.5	0.8	0.4	0.2	0.5	0.2	0.4
	P50	5.3	16.4	0.4	1.5	0.0	0.2	0.0	0.1	0.1	0.3	0.1	0.1	0.2	0.1	0.2
	P90	47.3	64.3	5.9	7.6	1.1	1.6	0.7	1.0	1.5	**2.2**	0.9	0.6	1.4	0.6	1.1
	P95	73.3	90.7	8.5	10.3	1.8	**2.3**	1.3	1.6	**2.6**	**3.2**	1.3	0.9	**2.0**	0.8	1.8
	P97.5	97.5	116.1	11.7	14.3	**2.7**	**3.3**	1.8	**2.2**	**3.4**	**4.0**	1.7	1.4	**2.8**	1.1	**2.4**
	P99	133.1	150.4	18.1	21.7	**3.7**	**4.0**	**2.5**	**2.9**	**4.8**	**5.4**	**3.0**	**2.1**	**3.5**	1.4	**3.1**
ADULTS	Mean	42.7	45.6	6.4	6.8	0.8	0.9	0.5	0.5	0.9	1.0	0.6	0.4	0.7	0.3	0.5
	P50	28.70	31.2	3.1	3.4	0.5	0.6	0.3	0.4	0.6	0.7	0.4	0.2	0.5	0.2	0.3
	P90	91.8	94.7	13.8	14.5	1.9	1.9	1.1	1.1	**2.1**	**2.3**	1.2	0.8	1.5	0.6	1.0
	P95	125.3	128.6	22.6	23.5	**2.5**	**2.6**	1.5	1.5	**2.9**	**3.0**	1.7	1.1	2.0	0.8	1.4
	P97.5	164.9	167.6	35.0	36.5	**3.2**	**3.2**	1.9	1.9	**3.5**	**3.7**	**2.3**	1.4	**2.5**	1.0	1.7
	P99	**229.1**	**232.3**	56.1	58.0	**4.6**	**4.7**	**2.6**	**2.6**	**4.9**	**5.0**	**3.1**	1.9	**3.2**	1.3	**2.2**

Cons.: consumers-only; TDI: tolerable daily intake; for the choice of the TDI see text.

The intake assessment of MeHg via seafood for both populations indicates that only a small percentage of the studied populations (~ 1%) exceeded the MeHg TDI through seafood consumption. A high correlation was found between the amount of tuna consumed and the intake of MeHg (r^2=0.87 for the total group of adult seafood consumers and r^2=0.90 for adult tuna consumers). The iPCBs intake via seafood was characterized by some extreme intakes, as illustrated by the 99th percentiles (Table IV.2). A high correlation was found between the amount of eel consumed and the iPCB intake (r^2=0.82 for the total group of adult seafood consumers and r^2=0.90 for adult eel consumers). The intake of dioxin-like compounds was calculated for the three different scenarios, for both populations. It is important to note that the intake assessment for dl PCBs, PCDD/Fs, and total TEQ all start from independent data sets. There is, hence, no direct link between the TEQ dl PCB, the TEQ PCDD/F, and the total TEQ intake (Table IV.2).

Table IV.3 gives the results of the assessed **nutrient** intakes for the adolescents and the adults, respectively, showing that only a very small percentage of the studied populations achieved the reference values via seafood consumption.

Table IV.3: Summary of the intake assessment for nutrients via seafood consumption for Flemish adolescents and Belgian adults, for the whole population sample and for the seafood consumers only (the figures given in bold are intakes higher than the reference value for that nutrient)

		EPA and DHA (mg/kg bw/day)		Vitamin D (µg/kg bw/day)		Iodine (µg/kg bw/day)	
		All	Cons.	All	Cons.	All	Cons.
ADOLESCENTS	Reference value	12.2		0.085		2.54	
	Mean	1.7	2.73	0.010	0.015	0.28	0.44
	50th percentile	0.6	1.56	0.001	0.005	0.06	0.20
	90th percentile	5.3	7.59	0.030	0.046	0.94	1.19
	95th percentile	8.4	10.03	0.052	0.064	1.32	1.46
	97.5th percentile	10.7	12.05	0.069	0.085	1.52	1.74
	99th percentile	**14.0**	**14.61**	**0.096**	**0.110**	2.07	2.43
ADULTS	Reference value	9.7		0.071		2.14	
	Mean	3.5	3.8	0.023	0.024	0.33	0.36
	50th percentile	2.6	2.8	0.016	0.017	0.25	0.27
	90th percentile	7.7	7.9	0.050	0.051	0.72	0.75
	95th percentile	**10.1**	**10.4**	0.067	0.067	1.00	1.02
	97.5th percentile	**13.1**	**13.6**	**0.086**	**0.087**	1.29	1.31
	99th percentile	**18.0**	**18.2**	**0.119**	**0.122**	1.54	1.55

Cons.: consumers-only; The reference values are based on the dietary reference intakes proposed by the Belgian Health Council (2007), but are expressed in function of body weight, for explanation see text

Fig. IV.1 presents a two-dimensional matrix of results for the nutrients and contaminants under study (after a log-transformation) based on the consumption database of the adults and on contamination scenario 1 (taking into account all concentration data).

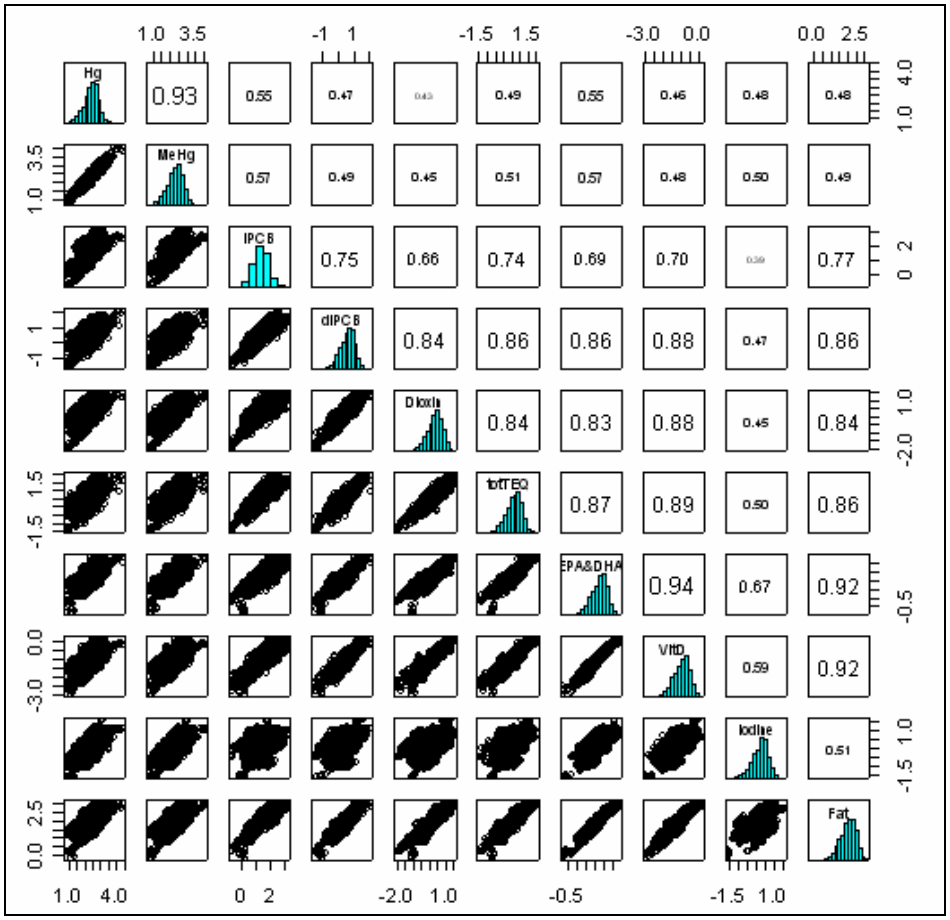


Fig. IV.1 Matrix visualising the log-transformed results of the probabilistic intake assessment via seafood consumption for the ten compounds of interest for Belgian adults; mercury (Hg), methyl mercury (MeHg), sum of 7 indicator PCBs (iPCBs), dioxin-like PCBs (dl PCBs), PCDD/Fs (dioxins), total dioxin-like compounds (totTEQ), EPA and DHA, vitamin D (vitD), iodine, and fat

On the main descending diagonal axis of the matrix, frequency distributions of the intake assessment results of all individual compounds are shown. At the lower left half of the matrix, 45 scatter plots show the combined intake for each possible pair of nutrients and/or contaminants. At the upper right, the correlation coefficients between the intakes of each pair of compounds are given. To facilitate the interpretation, the font size is related to the size of the correlation coefficient. The highest correlations are observed between the assessed intake of several fat-soluble compounds, e.g. r^2(EPA plus DHA and vitamin D) = 0.94, r^2(total TEQ and EPA plus DHA) = 0.87. A negligible correlation exists between the iodine and (Me)Hg intake and all the other compounds, respectively. The figure shows relatively high correlations between (non) dioxin-like PCBs and PCDD/Fs and EPA plus DHA. This

indicates that it is hard to achieve a higher EPA plus DHA intake via seafood consumption without increasing the intake of ndl PCBs and dioxin-like compounds.

Based on the adolescent and adult food consumption databases, an evaluation of the intake of LC n-3 PUFAs (EPA plus DHA) and dioxin-like compounds (total TEQ) can be combined in the output of the ProbIntake^{UG} module. Fig. IV.2 (A1 and A2) provides a scatter plot, focussing on EPA plus DHA and total TEQ, after exclusion of the concentration data of Baltic herring and salmon.

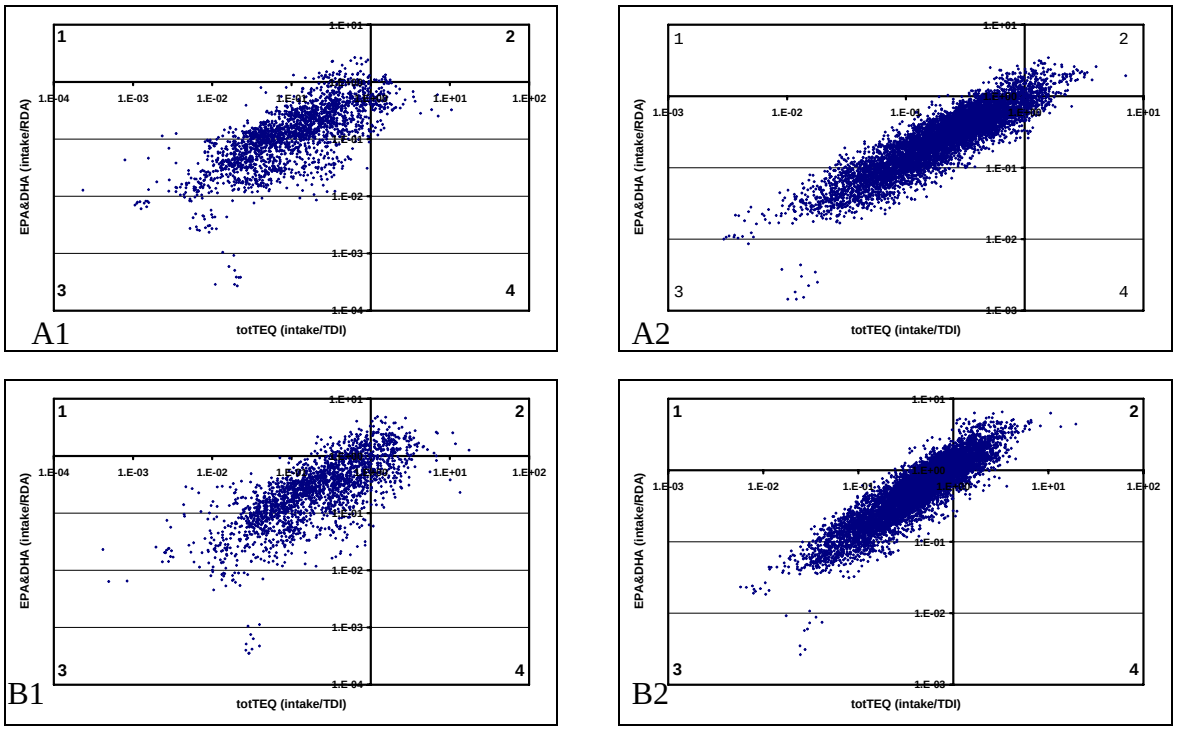


Fig. IV.2 Ratio of intake of total TEQ divided by TDI (2 pg WHO-TEQ/kg bw/day) and intake of EPA plus DHA divided by DRI (0.3% of total energy requirement) as a result of the current seafood consumption of Belgian adolescents (A1) and adults (A2) and as a result of doubling of the current seafood consumption of Belgian adolescents (B1) and adults (B2) with exclusion of concentration data of Baltic seafood (log-scale)

The graphs in Fig. IV.2 show the ratio of the intake of total TEQ (pg WHO-TEQ/kg bw/day) divided by the TDI (2 pg WHO-TEQ/kg bw/day) and the intake of EPA plus DHA (mg/kg bw/day) divided by the reference value. By expressing the intakes relative to the reference values, the discriminative value for being at the 'risk' versus at the 'benefit' side is '1' on both axes. As such, four quadrants (zones) are obtained, all with a relevant interpretation describing whether or not a sufficient amount of seafood was consumed to meet the EPA plus DHA recommendation, and with or without exceeding the 2 pg WHO-TEQ/kg bw/day limit. Four quadrants (zones) are obtained:

- Zone 1: consumption of sufficient amount of seafood to meet the EPA plus DHA DRI, without exceeding the 2 pg WHO-TEQ/kg bw/day limit (most beneficial zone);
- Zone 2: consumption of sufficient amount of seafood to meet the EPA plus DHA DRI, but exceeding the 2 pg WHO-TEQ/kg bw/day limit;
- Zone 3: consumption of too little seafood to meet the EPA plus DHA DRI, and not exceeding the 2 pg WHO-TEQ/kg bw/day limit;
- Zone 4: consumption of too little seafood to meet the EPA plus DHA DRI, but exceeding the 2 pg WHO-TEQ/kg bw/day limit (worst case zone).

Only a few individuals met their EPA plus DHA requirement without exceeding the limit for total dioxin-like compounds. It was useful to assess the effect of doubling the seafood without any other changes to their dietary pattern (no alterations of the species consumed), shown in Fig. IV.2 (B1 and B2). The point cloud representing the EPA plus DHA on total TEQ intake ratio shifted to the upper right corner (towards zone 2), indicating that more individuals met their EPA plus DHA intake, but at the same time increased their intake of dioxin-like compounds proportionally.

3. Intake assessment based on consumption scenarios

In this third part of chapter IV, the intake assessment based on the seafood consumption scenarios is described. The results are discussed in part 4 of this chapter together with the results of the intake assessment based on real seafood consumption data.

3.1. Materials and Methods

The consumption scenario analyses started from the current Belgian seafood species consumption pattern and the recommendation to consume seafood twice a week. Different variations were elaborated on (1) the **fish species** consumed and (2) the **frequency** of fish consumption:

- First, the seafood **species** consumption pattern was artificially changed in two different ways:
    - people increasing their fatty fish consumption up to 50% of their total fish consumption (simulated pattern n°1)
    - people replacing all the lean fish species by fatty fish species (simulated pattern n°2).
- Second, in relation to the **frequency** of seafood consumption, two variations on the consumption recommendation were considered:
    - people consuming seafood only once a week (50% of recommendation)
    - people consuming seafood three times a week (150% of recommendation).

A scheme of the different scenarios is presented in Fig. IV.3. The different aspects are described in detail below.

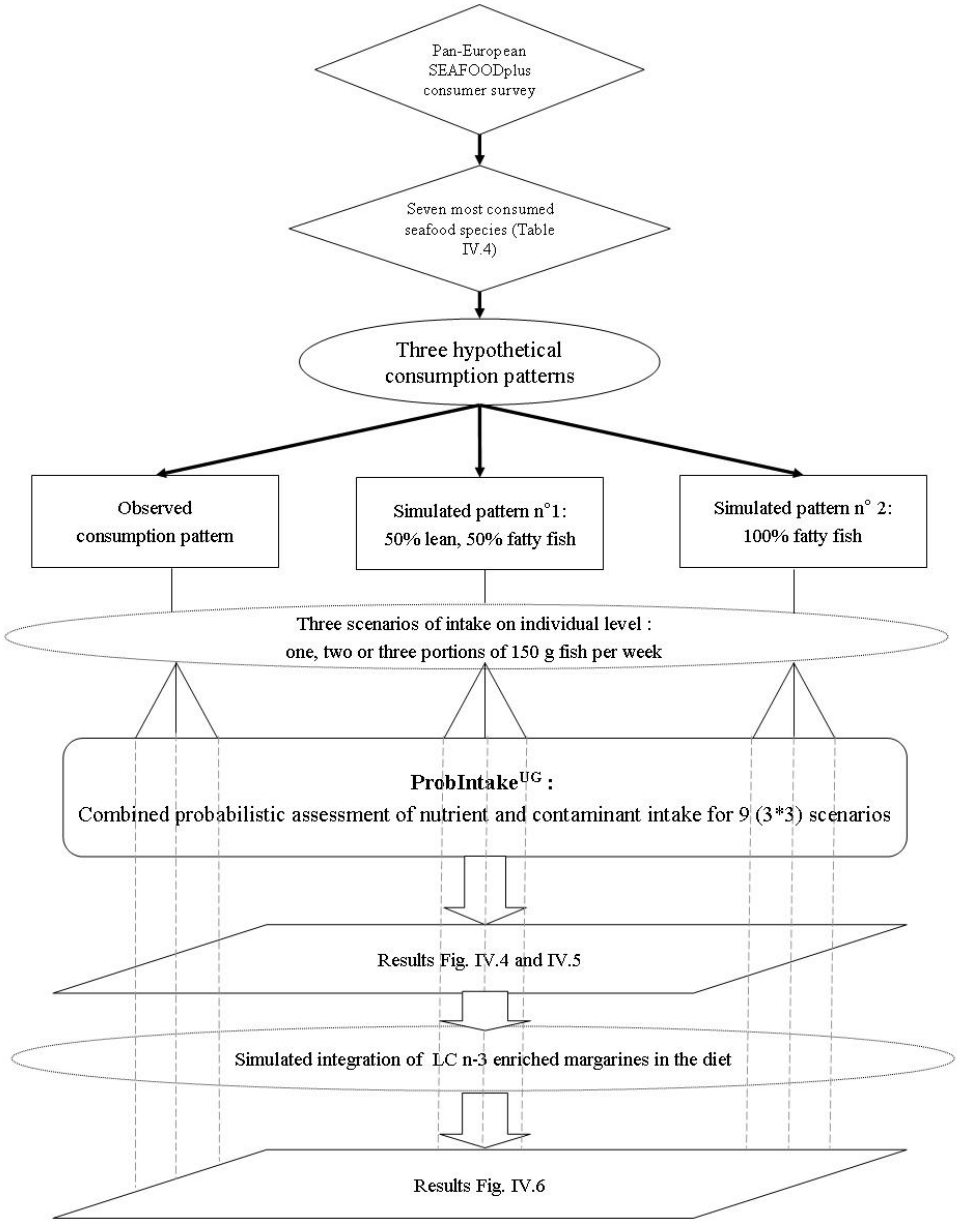


Fig. IV.3 Scheme of the elaboration and implementation of the different scenarios

3.1.1. Nutrient and contaminant data

The same probability distributions of nutrient and contaminant concentrations are used as described in chapter III. However, for this consumption scenario study, only the sum of **EPA and DHA** concentrations was considered; the other nutrients were not taken into account. Reason for this is that the aim of the consumption scenario analyses was to find out whether the recommendation of LC n-3 PUFAs can be reached without a contaminant intake of toxicological concern. Concerning the contaminants, this consumption scenario analyses focused **MeHg**, **dl PCBs**, **PCDD/Fs**, and total dioxin-like compounds (**total TEQ**) because

toxicological concern was most relevant for these four compounds. Considering the concentrations of dioxin-like compounds in salmon and herring, contaminant concentrations measured in Baltic salmon and herring were excluded from the analyses executed in this scenario study, as their presence on the Belgian market is considered as negligible due to a European regulation.

3.1.2. Consumption and body weight data

Three times three different consumption scenarios were investigated, starting from the current seafood species consumption pattern taking into account the seven most consumed seafood species (Table IV.4), determined through the pan-European SEAFOODplus consumer survey (Honkanen & Brunsø, 2007). No crustacean or molluscs species were taken into account in the scenarios, since all of the seven most consumed seafood species were finfish species. Two hypothetical scenarios with an altered pattern of fish species consumption were constructed: (1) assuming that the population increases the contribution of fatty fish (> 5% fat) consumption to 50% of the total seafood consumption, and (2) considering that people replace all lean fish species (≤ 5% fat) by fatty fish species. The contribution of the different species in both scenarios was calculated proportionally to their current contribution. For the three different consumption patterns considered, three sub scenarios were investigated differing in consumption frequency, i.e. once, twice, or three times a portion of 150 g seafood per week.

Table IV.4: Contribution of the seven different seafood species (%) to the total seafood consumption for three consumption scenarios, as well as the ratio of the concentration of EPA plus DHA on methyl mercury (MeHg) and total dioxin-like compounds (totTEQ)

Species	Percentage of total seafood consumption (%)			Concentration ratio (median, [5th percentile, 95th percentile])	
	Current consumption pattern	50% lean & 50 % fatty fish	Only fatty fish	EPA&DHA / MeHg (10^{-3})	EPA&DHA / totTEQ (10^{-6})
Cod	24.7	19.0	0.0	0.06 [0.03-0.11]	18.89 [4.04-59.13]
Tuna	19.2	14.7	0.0	0.02 [0.00-0.12]	2.39 [0.19-42.38]
Alaska Pollack	13.6	10.6	0.0	0.11 [0.05-0.26]	13.75 [5.09-58.74]
Plaice	7.4	5.7	0.0	0.16 [0.04-0.36]	5.57 [1.39-13.98]
Total lean fish	*64.9*	*50.0*	*0.0*		
Atlantic Salmon	19.7	28.0	56.0	0.93 [0.31-1.99]	8.73 [2.35-57.64]
Herring	8.0	11.4	22.8	0.45 [0.20-2.06]	3.92 [1.20-9.86]
Mackerel	7.4	10.6	21.2	0.94 [0.31-2.28]	10.33 [5.03-23.05]
Total fatty fish	*35.1*	*50.0*	*100.0*		

For the intake assessment, a population of 600 individuals was used (300 men - 300 women), equally divided over four different age classes (30-39y; 40-49y; 50-59y; 60-69y). Normal body weight distributions were applied per gender and age interval, based on available data for the Belgian population, being the same as applied for the real intake assessments based on the adults' consumption data described in the previous part of this chapter (B.I.R.N.H study (De Backer, 1984; Kornitzer & Dramaix, 1989)) (Table IV.1, given in the previous part of this chapter). A number of 600 individuals was sufficient ensure that the whole concentration range of the nutrients and contaminants was reflected in the intake calculation and that a good convergence of the population intake estimated was reached.

3.1.3. Simulation model and probabilistic methodology

The simulation model, the approach, and the software module (ProbIntake^{UG}) used for the consumption scenario analyses are equal as described in part 2.1.3. of this chapter. The sole difference is that it was assumed that consumers kept this consumption pattern for a whole year (52 weeks) for the purpose of optimising integration of the inter-species variability in the nutrient and contaminant concentrations during the intake assessment and to calculate at the end the average daily intake over a long term period.

3.1.4. Evaluation of nutrient and contaminant intakes

Similar to what was done for the intake assessment based on real consumption data, reference values were used to evaluate nutrient and contaminant intakes. To evaluate population intakes of **EPA plus DHA**, an 'ad hoc' reference value of 681 mg/day or 9.7 mg/kg bw/day for EPA plus DHA was calculated starting from the existing Belgian DRI equal to 0.3% of the total energy intake (Belgian Health Council, 2007) and assuming a mean body weight of 70 kg and a mean energy intake of 2046 kcal, the latter based on the data of the most recent Belgian Food Consumption Survey (3245 individuals above 15 years; 1623 women, 1622 men) (De Vriese *et al*, 2006). Dividing the EPA plus DHA reference value by the body weight was relevant in this study in order to express the reference values for nutrients and contaminants on the same scale. For **MeHg**, a tolerable weekly intake (TWI) of 1.6 µg/kg of bw/week (i.e. 0.228 µg/kg bw/day) is proposed (EFSA, 2004) and for **dioxin-like compounds**, the EU proposes 2 pg WHO-TEQ/kg bw/day (Scientific Committee on Food, 2001).

3.1.5. Inclusion of LC n-3 PUFA enriched margarine

Currently, EPA and DHA enriched margarine is commonly available on the Belgian market and is therefore considered in this study. The EPA and DHA concentration in enriched margarine varies a lot depending on the brand, but it varies also in time. A first brand of margarine available in Belgium claims that their EPA plus DHA enriched margarine contains 5 mg EPA plus DHA/g margarine. A second brand indicated that 1 g of enriched margarine contains 7.5 mg EPA plus DHA. A third supplier stated that its enriched variant of margarine contains 0.9 mg DHA/g margarine. Belgian dieticians assessed that one slice of bread with a regular layer of margarine contains 5 g of margarine (Belgian Health Council, 2005). Assuming a consumption of 4 to 7 slices of bread a day leads to a consumption of 20 to 35 g of margarine a day and 100 to 262.5 mg EPA plus DHA per day (using the two EPA plus DHA-richest versions of enriched margarine). The results of the most recent Belgian Food Consumption Survey (De Vriese *et al*, 2006) indicated that currently the mean daily consumption of culinary fats and margarines is 21.2 g with an interquartile range of 6.0 g to 28.6 g. In the scenario analyses executed, it was assumed that all consumers would use daily the average amount of enriched margarine containing 7.5 mg EPA plus DHA/g margarine.

3.2. Results

Table IV.4 (given previously in this part of the chapter) shows that currently 65% of the total seafood consumption in Belgium is composed of lean fish species (≤ 5% fat), with cod being the most important species. Salmon is the most consumed fatty fish (> 5% fat) in Belgium. Table IV.4 also shows the median, the 5th and the 95th percentile of the species specific ratio of the EPA plus DHA concentration on MeHg and total TEQ concentration. The higher the ratio, the higher the nutrient concentration compared to the contaminant concentration. The results illustrate that for some species the distribution of the ratio is very wide and skewed to the right, e.g. EPA&DHA/totTEQ for tuna and salmon (Table IV.4).

3.2.1. Seafood as only source of EPA and DHA

Table IV.5, Fig. IV.4, and Fig. IV.5 show the intake assessment results for the different scenarios.

Table IV.5: Mean intake of the different compounds for three different seafood consumption patterns and three different scenarios of consumption frequency

	MeHg	iPCB	dl PCB	PCDD/F	totTEQ	EPAplusDHA
	ng/kg bw/day		pg WHO-TEQ/kg bw/day			mg/kg bw/day
				1x 150 g per week		
Current pattern	36.19	2.63	0.33	0.24	0.40	2.66
50% lean and 50% fatty	30.40	2.88	0.39	0.30	0.41	3.22
Only fatty fish	8.28	3.44	0.54	0.51	0.58	5.14
				2x 150 g per week		
Current pattern	70.01	5.26	0.68	0.52	0.79	5.32
50% lean and 50% fatty	57.93	5.70	0.77	0.64	0.82	6.43
Only fatty fish	16.52	6.86	1.08	1.08	1.14	10.28
				3x 150 g per week		
Current pattern	106.19	7.98	1.02	0.73	1.20	8.01
50% lean and 50% fatty	86.45	8.50	1.14	0.94	1.23	9.64
Only fatty fish	24.79	10.27	1.60	1.56	1.74	15.41

MeHg = methyl mercury; iPCB = seven indicator PCBs; dl PCB = dioxin-like PCBs; totTEQ = total dioxin-like compounds

The results indicate that changing the current seafood consumption pattern by increasing the contribution of fatty fish will reduce the intake of MeHg (Table IV.5). This could already be concluded based on the comparison between the EPA plus DHA over MeHg ratio of lean and fatty seafood species (Table IV.4). Nevertheless, in none of the scenarios considered, consumers exceed the TDI for MeHg on a long term base. In contrast to MeHg, the intake of the other contaminants increases when replacing lean fish species by fatty fishes. This was expected given the lipophilic character of these contaminants. Simultaneously, increasing the contribution of fatty fish species increases the intake of beneficial EPA plus DHA. Some lean species also have a relative high EPA plus DHA over total TEQ ratio compared to other species, e.g. cod and Pollack (Table IV.4), but the absolute EPA plus DHA concentration in these species is so low that an unrealistically large amount of these species should be eaten to achieve the recommended EPA plus DHA intake.

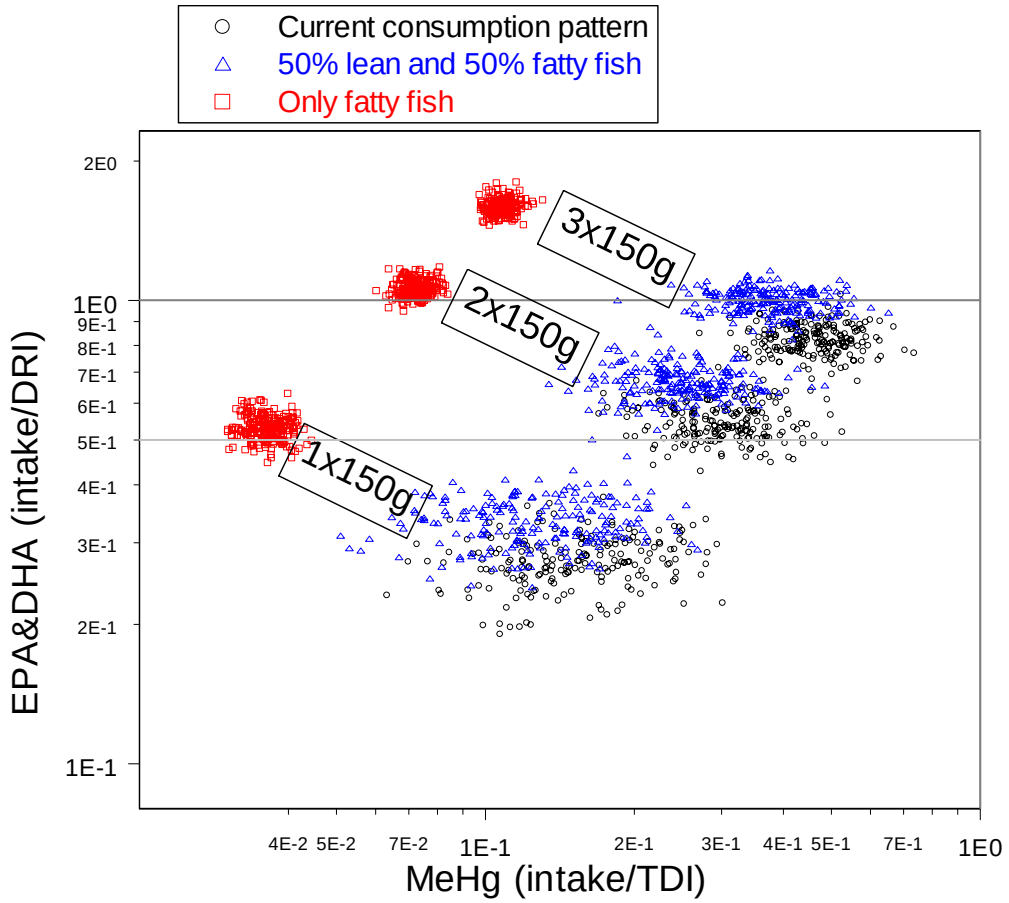


Fig. IV.4 Methyl mercury (MeHg) intake divided by the TDI (228 ng/kg bw/day) in relation to the EPA plus DHA intake divided by the DRI (9.7 mg/kg bw/day) for three different seafood consumption patterns and three different scenarios of consumption frequency (logarithmic scales); 1x, 2x, 3x seafood consumption per week: clouds from lower left to upper right; the limit value for being at risk due to a too high MeHg intake or inadequate EPA plus DHA intake is '1' on both axes; an extra reference line was added at half of the reference value for EPA plus DHA

Two scatter plots are provided, focussing on EPA plus DHA and MeHg (Fig. IV.4) and total TEQ (Fig. IV.5), based on the results of the different consumption scenarios. The plots show the intake of MeHg and total TEQ, respectively, divided by their TDI in relation to the intake of EPA plus DHA divided by the reference value (9.7 mg/kg bw/day). Consequently, the limit value for being at risk due to a too high contaminant intake or inadequate EPA plus DHA intake is '1' on both axes. On both scatter plots, extra reference lines were added: (1) at half of the TDI for total TEQ, to take into account that the human diet contains other sources of dioxin-like compounds besides seafood; (2) at half of the reference value for EPA plus DHA, since the Belgian DRI for EPA plus DHA is high compared to other countries (see discussion part of this chapter). By adding these reference lines, different zones are obtained, all with a relevant interpretation describing whether or not a sufficient amount of seafood was consumed to meet the DRI for EPA plus DHA, with or without exceeding the contaminant TDI.

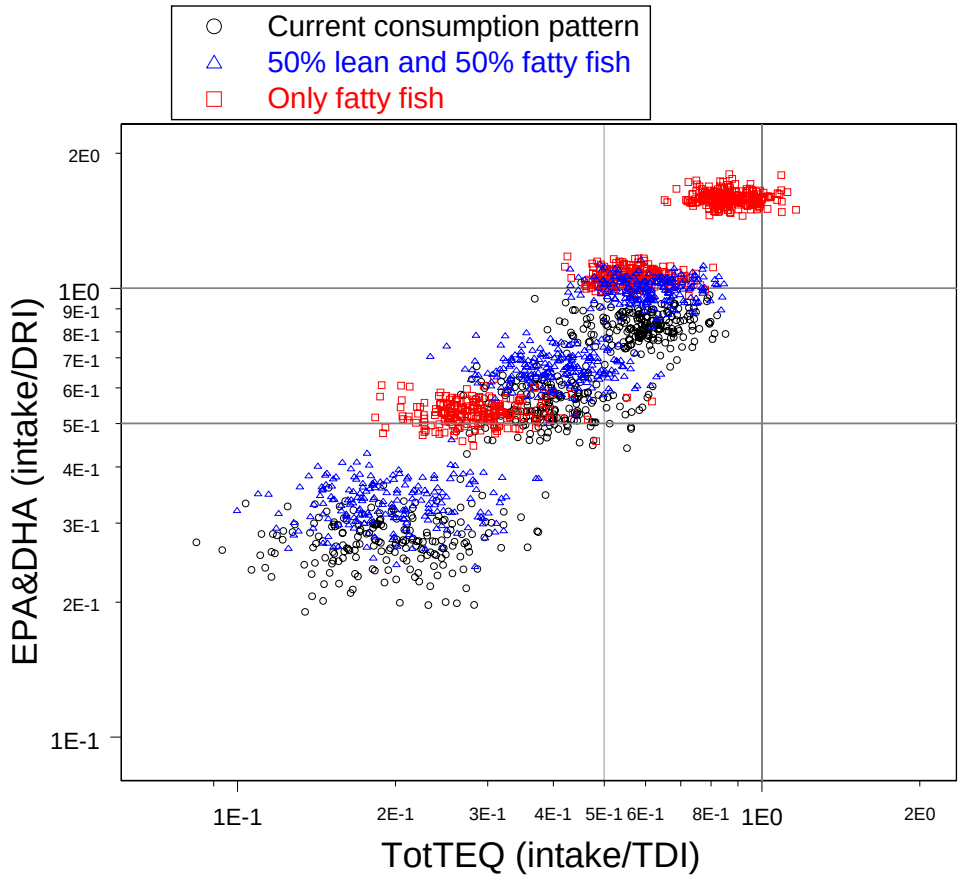


Fig. IV.5 Intake of total dioxin-like compounds (totTEQ) divided by the TDI (2 pg WHO-TEQ/kg bw/day) in relation to the EPA plus DHA intake divided by the DRI (9.7 mg/kg bw/day) for three different seafood consumption patterns and three different scenarios of consumption frequency (logarithmic scales); 1x, 2x, 3x seafood consumption per week: clouds from lower left to upper right; the limit value for being at risk due to a too high totTEQ intake or inadequate EPA plus DHA intake is '1' on both axes; two extra reference lines were added (1) at half of the TDI for total TEQ, to take into account that the human diet contains other sources of dioxin-like compounds besides seafood; (2) at half of the reference value for EPA plus DHA

Considering the EPA plus DHA intake, the results show that only a seafood consumption pattern consisting for 50% lean fish species and 50% fatty fish species with a minimum consumption frequency of three times a week, or a seafood consumption pattern consisting only of fatty species with a frequency of minimum twice a week, will lead to an adequate EPA plus DHA intake using the Belgian DRI and not taking into account other sources of these fatty acids. Fig. IV.4 shows that none of the considered consumption scenarios will lead to exceeding of the TDI for MeHg, indicating that the mercury contamination of seafood available on the Belgian market is not an issue of major concern. In contrast, when consuming three times a week a portion of fatty fish, the intake of dioxin-like compounds will approach the TDI value (Fig. IV.5). Knowing that the human diet contains also other important sources of dioxin-like compounds, an intake of three portions of fatty fish per week may be of toxicological concern.

3.2.2. Enriched margarine as extra dietary sources of EPA and DHA

Assuming that all consumers would use daily 21.2 g enriched margarine containing 7.5 mg EPA plus DHA/g margarine, this would lead to a mean daily intake of 159 mg EPA plus DHA, being 23.3% of the Belgian DRI (681 mg/day). In Fig. IV.6, scatter plots are shown for the different seafood consumption scenarios with and without adding enriched margarine as an LC n-3 PUFA source (mean daily intake of 21.1 g), assuming that the margarine consumption will not contribute to the intake of contaminants. Consuming enriched margarine will help to increase the EPA plus DHA intake. Nevertheless, the contribution is rather limited and margarine as such is not sufficient to reach the DRI. A consumption scenario of weekly 150 g lean fish and 150 g fatty fish combined with a daily consumption of LC n-3 PUFA enriched margarine leads to an EPA plus DHA intake close to the DRI with a mean total TEQ intake below half of the TDI.

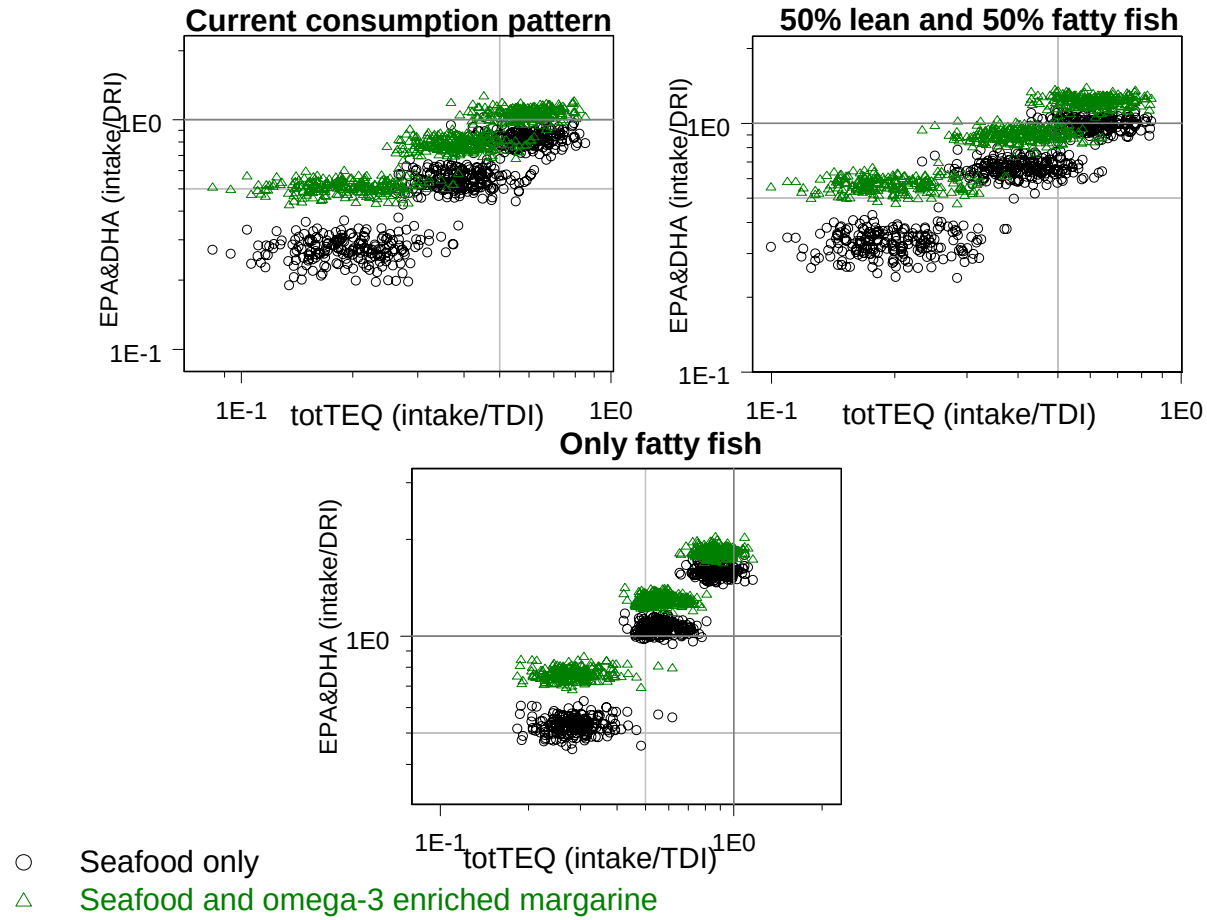


Fig. IV.6 Intake of total dioxin-like compounds (totTEQ) divided by the TDI (2 pg WHO-TEQ/kg bw/day) in relation to the EPA plus DHA intake divided by the DRI (9.7 mg/kg bw/day) for three different seafood consumption patterns and three different scenarios of consumption frequency, with and without taking omega-3 enriched margarine into account (logarithmic scales); 1x, 2x, 3x seafood consumption per week: clouds from lower left to upper right

4. Discussion

4.1. Intake assessment of multiple compounds via seafood consumption

*4.1.1. Results of the intake assessment based on **real** seafood consumption data*

Related to consumption datasets used in the first part of this chapter, some limitations have to be mentioned. First, it is relevant to reiterate that the two different seafood consumption databases have been collected by two different methodologies, i.e. a food record indicating all consumed seafood products versus a food frequency questionnaire asking consumption information on the ten most consumed seafood species, and in two different periods (1997 and 2004). In both food consumption databases, seasonal variation in seafood consumption was not taken into account. Nevertheless, these were the only consumption data available for the Belgian population giving sufficiently detailed information on the weekly intake of seafood consumption.

Another shortcoming is that the consumption data of the adolescents describe a short-term intake, whereas it would be more appropriate to end up with long term intake data (i.e. usual intake) to compare with a TDI. Consequently, the assessed variance of the intakes includes not only between-person variability, but also within-person variability, while the latter is of less interest for long term intake assessments. In addition, it was assumed in this paper that non-seafood consumers in the consumption studies were people who did never consume any seafood. Both assumptions lead to an overestimation of the assessed variance of the nutrient and contaminant intake distributions. The overestimation due to within-person variability is smaller when dietary records are used during a longer period in time (in this study, a seven day dietary record was used for the adolescent food consumption database). Statistical modelling techniques exist filtering within-person variability to calculate usual intake starting from short term consumption data and taking into account that non-consumers can be consumers on other moments in time and vice versa (Dodd *et al*, 2006; Nusser *et al*, 1996; Slob, 1993; Tooze *et al*, 2006). These methods were not used here as the improvement in accuracy was considered to be small relative to other sources of uncertainty in this assessment, e.g. uncertainty related to the contamination data due to different analytical methods, different sampling plans, etc.

The results of the intake assessment based on **real seafood consumption data** show that the seafood consumption of the considered populations is **not sufficient to reach the DRI for EPA plus DHA, vitamin D, and iodine**. The current seafood consumption does **not lead to a MeHg intake of major toxicological concern**. In contrast, for **dioxin-like compounds**, **the TDI is reached by people with high seafood consumption**. Additionally, the results of the third contamination scenario analysis confirmed that heavy seafood consumers can exceed the TDI for dioxin-like compounds on the basis of seafood consumption only, even when all seafood complies with the EU limits. This result has also been found in other studies (Baars *et al*, 2004; Sirot *et al*, 2006).

The assessed contaminant intakes of the intake assessment based on real seafood consumption data can also be compared with the results of other calculations:

- A **French** study, executed in 2006 and focussing on French high seafood consumers gave the following results: an average iPCB intake of 57.14 ng/kg bw/day, an average intake of dl PCB, PCDD/Fs, and total TEQ intake of 2.04, 0.62, and 2.67 pg WHO-TEQ/kg bw/day, respectively (Sirot *et al*, 2006). These results are of the same order of magnitude as the assessed intakes for the higher percentiles of the Belgian adult population.
- The British Scientific Advisory Committee on Nutrition (SACN) and Committee on Toxicity (COT) in the **UK** calculated a total TEQ intake via seafood of 0.6 and 3.9 pg WHO-TEQ/kg bw/day for an average and a heavy seafood UK adult consumer, respectively (SACN/COT, 2004).
- A comparison can also be made with data of a recent **Spanish** study focussing on contaminant intake via seafood consumption only (Bocio *et al*, 2007). The average contaminant intake assessed for an adult man (70 kg) amounts to 0.46 pg WHO-TEQ/kg bw/day for dl PCBs, 0.086 pg WHO-TEQ/kg bw/day for PCDD/Fs, and 0.54 pg WHO-TEQ/kg bw/day for total TEQ (Bocio *et al*, 2007). The intake of total TEQ is quite similar to the one assessed in our study when excluding the Baltic herring and salmon (Table IV.2). In contrast, the assessed intake of PCDD/Fs in our study is high compared to the Spanish study. This can partly be caused by a different seafood consumption pattern and a different level of contamination.

*4.1.2. Results of the intake assessment based on seafood consumption **scenarios***

The results of the consumption scenario analyses show that the Belgian **DRI for EPA plus DHA can be reached** through regular consumption of seafood, more specifically:

1. a combination of **lean and fatty fish species** (on average 50% of each) minimum **three times a week**; or
2. **fatty fish species two times a week**.

A consumption of three times a week fatty fish, however, leads to an intake of dioxin-like compounds close to the TDI, which is of potential toxicological concern since also other food items, mainly of animal origin, contribute to the daily intake of dioxin-like compounds. Again, MeHg contamination does not seem to be an issue of toxicological concern, even in scenarios with elevated fish consumption frequencies. Hence, the **consumption limits** for fish determined in this PhD-study are **driven by the presence of dioxin-like contaminants**, which was also concluded by Foran *et al* (2005) performing an analysis of the risks and benefits related to salmon consumption.

Despite the fact that it is possible to meet the EPA plus DHA recommendation by consuming twice a week fatty fish without exceeding the TDI for dioxin-like compounds, many obstacles at the consumer level exist to convince people to consume seafood twice a week. Low perceived convenience, high price perception, and low liking of fish taste by one of the family members act as major barriers to increase seafood consumption in Belgium (Olsen *et al*, 2007). In chapter V of this PhD-thesis, some more attention is given to the perception of Belgian consumers concerning seafood.

Due to the existing barriers to increase seafood consumption, it was worth investigating what can be the role of EPA plus DHA enriched food items as a dietary source of LC n-3 PUFAs. The results showed that regular seafood consumption (twice a week), including fatty fish species, in combination with regular consumption of **EPA plus DHA enriched margarine** can be advised to safely increase the LC n-3 PUFA intake. Apart from margarines, **LC n-3 enriched eggs** are supplied by two different brands on the Belgian market. The first brand stated that an enriched egg contains 110 mg of EPA plus DHA. The second reported a concentration of 125 mg DHA per egg. The mean weight of a normal egg is assumed to be 60 g (Belgian Health Council, 2005). On the basis of the most recent Belgian Food Consumption

Survey (De Vriese *et al*, 2006), it is known that the Belgian adults consume on average 10.0 g egg/day, i.e. one egg a week, with the 97.5th percentile equal to 31.9 g/day, i.e. 3 to 4 eggs a week (De Vriese *et al*, 2006). Assuming that consumers would all eat EPA and DHA enriched eggs (110 mg EPA plus DHA/egg), this would lead to an average daily intake of 18.3 mg EPA plus DHA, being 2.7 % of the DRI. To reach the DRI of 681 mg EPA plus DHA a day, consumers should eat six eggs a day, increasing the cholesterol intake to 1483.2 mg/day (412 mg cholesterol/egg), whereas the Belgian recommendation states to reduce the cholesterol intake to a maximum of 300 mg/day (Belgian Health Council, 2007). This indicates that the contribution of EPA plus DHA enriched eggs to the total intake is low, due to the rather low EPA and DHA concentration and the limited consumption of eggs. Enriched eggs can help to increase the LC n-3 PUFA intake, but they can not be advised as only or major source to achieve the EPA plus DHA DRI. Nevertheless, it must be admitted that the use of eggs in prepared food items as cakes and pastries are not taken into consideration in this estimation, which leads to an underestimation. Moreover, apart from margarines and eggs the supply of omega-3 enriched food items and supplements is currently increasing. However, currently there is still a lack of information about the EPA and DHA concentration of these food items and supplements as well as about the current consumption rate of these products. Further research is needed to take also this new fortified food items into account.

4.2. Risk and benefit – reference values

At this moment, no common currency exists to evaluate benefits and risks in one single step. In other words, no common scale of measurement exists to compare human health risks and benefits, hampering a complete and in depth risk-benefit analysis. Attempts have been made to combine both assessments in terms of QALY's, i.e. quality-adjusted life years (Cohen *et al*, 2005; Ponce *et al*, 2000), but many uncertainties remain to be solved in order to make a broad application of this procedure possible. The largest uncertainties are associated with the dose-response relationships (Cohen *et al*, 2005). Moreover, both published QALY-investigations related to seafood consumption did not take into account dioxin-like contaminants, but focused on MeHg only (Cohen *et al*, 2005; Ponce *et al*, 2000). In our approach, DRIs and TDIs are used for the evaluation of human health benefits and human health risks, respectively, but these values were determined taking into consideration different end points and they refer to the intake via the total diet, while in this chapter they were used to evaluate the

intake via seafood consumption only. In the future, a more common methodology should be developed to go beyond these boundary conditions. Yet, in the meantime an attempt was made to describe the situation as accurately as possible, by means of an intake assessment of multiple nutrients and contaminants. This answered the need recently indicated by Domingo *et al* (2007a; 2007b), stating that it is of high public health interest to calculate seafood consumption limits taking into account multiple contaminants and multiple seafood species on the level of overall diets.

It is clear that the executed evaluation heavily depends on the TDI and DRI values applied. Differences in the actual choice of the TDI and DRI will lead to differences in seafood consumption recommendations independent from differences in the overall data used. For MeHg, we applied a TDI of 0.229 µg/kg bw/day (TWI of 1.6µg/kg bw/day) as proposed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) taking into account the latest epidemiological results with regard to developmental toxicity (EFSA, 2004; World Health Organization, 2007). Also the SACN/COT adapted the JECFA reference value of 1.6 µg/kg bw/week but only when assessing the dietary exposure of pregnant women and women who may become pregnant within the following year (SACN/COT, 2004). Within the context of a risk-benefit analysis concerning seafood consumption, SACN/COT proposed to apply an intake limit of 3.3 µg MeHg/kg bw/week to the rest of the population as a TWI for the rest of the population to protect against non-development adverse effects (SACN/COT, 2004). Since MeHg intake via seafood did not seem to be of toxicological concern when using the most stringent limit, it will of course be evaluated in the same way when using a higher intake limit.

For dioxin-like compounds, a TDI of 2 pg WHO-TEQ/kg bw/day is applied, as proposed by the EU (Scientific Committee on Food, 2001). This TDI is mainly based on developmental toxicity, e.g. effects on the developing male reproductive system resulting from maternal exposure to dioxin-like compounds and is also considered adequate to protect against other possible effects of dioxin-like compounds, such as cancer (non-genotoxic mechanism) and cardiovascular effects (Scientific Committee on Food, 2001). SACN/COT also proposed a TDI of 2 pg WHO-TEQ/kg bw/day to protect against developmental toxicity but proposed a different guideline of 8 pg WHO-TEQ/kg bw/day (SACN/COT, 2004), which should be appropriate when considered in relation to the most sensitive and relevant non-development effects of dioxin-like compounds (increased cancer risk). According to SACN/COT, this

guideline level should be used for older women and males when considering risk-benefit aspects of seafood consumption. The reason for this is that developmental toxicity is of less concern in the older population, whereas the positive effects of seafood consumption on cardiovascular disease become more important. The application of the latter TDI would lead to a lower percentage of people being at risk to exceed the TDI and to a higher consumption limit for fatty fish.

A similar discussion is relevant for the DRI used to evaluate the EPA plus DHA intake. The EPA plus DHA recommendation in Belgium seems to be quite high when compared to other countries, i.e. 0.3 % of the total energy intake per day (Belgian Health Council, 2007). For the adolescent and adult population considered in this intake assessment study, this yields a reference value for EPA plus DHA of 720.5 mg/day and 682 mg/day, respectively. In comparison:

- in the Netherlands, 200 mg EPA plus DHA per day is recommended (Kromhout, 2001);
- in France, the DRI for EPA and DHA is 0.2 % of the total energy intake, with a minimum of 0.05 % contributed by DHA (Legrand *et al*, 2001), estimated to be equal to 500 mg/day for French men and 400 mg/day for French women;
- in Germany, a daily intake of 350 mg LC n-3 PUFA is recommended (Bauch *et al*, 2006);
- in the UK, SACN/COT recommends a LC n-3 PUFA intake of minimal 450 mg/d (SACN/COT, 2004);
- in the United States, the American Heart Association (AHA) formulated a dietary recommendation of 500 mg/day of EPA plus DHA for cardiovascular disease risk reduction. For patients with documented coronary heart disease, the AHA recommends 1 g of EPA plus DHA per day (Gebauer *et al*, 2006; Kris-Etherton *et al*, 2003).
- the International Society for the Study of Fatty Acids and Lipids (ISSFAL) recommends to healthy adults a minimum intake of 500 mg per day for EPA and DHA for cardiovascular health (ISSFAL, 2007).

Application of a lower reference value to evaluate the EPA plus DHA intake as assessed in this PhD-study would increase the percentage of individuals having an intake above this level and would lead to the conclusion that (1) a consumption of seafood twice a week, varying between lean and fatty species (Fig. IV.4 and IV.5), and (2) combination of once a week seafood with regular use of LC n-3 enriched margarine (Fig. IV.6) would be sufficient to reach the EPA plus DHA intake recommendation.

5. Conclusions

The probabilistic intake assessments executed with the ProbIntake^{UG}-module gives **better insight in the problematic and complex nature of seafood consumption including health benefits as well as risks**. The results make clear that the intake of fat-soluble nutrients and contaminants is highly correlated, which is an important fact that has to be taken into consideration when formulating public food and health recommendations.

The current recommendation of the Belgian Health Council is to consume one or two portions of seafood per week, corresponding to 150 to 300 g of seafood per week (Belgian Health Council, 2004b). The AHA recommends that adults should eat fish (particularly fatty fish) at least two times a week (Kris-Etherton *et al*, 2003).

On the basis of both consumption databases (adolescents and adults), the simulation results predicted that both populations currently do not reach an adequate intake for the three nutrients considered, at least when only seafood consumption is accounted for. This is mainly due to low frequency of seafood consumption. Regarding the contaminants, (Me)Hg contamination of seafood assumed to be available on the Belgian market is not a major issue. In contrast, exceeding the TDI for dioxin-like compounds was noticed for heavy seafood consumers.

Combination of regular seafood consumption (twice a week), with important contribution of fatty fish species (at least 50%), in combination with regular consumption of EPA plus DHA enriched margarine can be advised to maximize LC n-3 PUFA intake without exceeding the TDI for dioxin-like compounds. Meanwhile, vitamin D and iodine intake will increase as well. It is important to add that no other dietary sources of dioxin-like compounds were taken into account in this assessment. Some information about the contribution of seafood to the total dietary intake of dioxin-like compounds is given in chapter V, part 1.

It should, however, be kept in mind that the conclusion "seafood consumption twice a week would not lead to contaminant intakes of major toxicological concern" is essentially conditional upon compliance of existing structural and rather strict rules and regulations, and that extensive control programs aiming to prevent that highly contaminated food items

(above the EU limits) become available for consumers. In other words, the positive conclusion from this study should in no way be interpreted as an argument in favour of any weakening or downplaying of current regulations and monitoring programmes.

Chapter V.

General discussion

This last chapter starts with summarizing the main findings of this PhD-thesis. Briefly, the current intake of long chain omega-3 fatty acids (LC n-3 PUFAs) by the Belgian population is too low compared to the recommendations and compared to the levels necessary to lead to cardiovascular benefits (Chapter II). The most direct approach to correct this deficiency is to increase consumption of foods rich in LC n-3 PUFAs, mainly seafood (Harris, 2007). Indeed, the American Heart Association recommends adults to consume at least two preferably fatty fish meals per week (Kris-Etherton *et al*, 2003). Similar recommendations have been made by health authorities in other countries, e.g. the United Kingdom (SACN/COT, 2004).

Before formulating similar recommendations for the Belgian population, it was investigated whether the LC n-3 PUFA recommendations could be reached without increasing the intake of contaminants via seafood to levels of toxicological concern. It was concluded in chapter IV that the Belgian population can consume fatty fish up to twice a week without exceeding tolerable daily intake (TDI) levels for mercury and dioxin-like compounds. However, this evaluation did not consider intake of dioxin-like compounds from other dietary sources, i.e. mainly other foods from animal origin. In this chapter, the importance of other food items to the total intake of dioxin-like compounds is briefly described.

Considering the current low consumption of seafood by a large part of the Belgian population, major dietary shifts will be needed in the future to achieve increased seafood consumption. Additionally, different barriers exist related to increased seafood consumption and these can not be neglected in this PhD-thesis. Two main barriers are discussed: consumer perception about seafood and sustainability issues. Related to the sustainability question, aquaculture and non-seafood sources of LC n-3 PUFAs are briefly introduced and discussed. Next, some aspects, opportunities, and disadvantages of omega-3 supplements and fortified foods are discussed. The chapter concludes with some thoughts for future research.

1. Main findings

Chapter II of this PhD-thesis presented the results of an intake assessment of individual PUFAs via the total diet for three different subgroups of the Flemish population and an intake assessment of vitamin D via the total diet for Flemish adolescents. The following PUFAs were considered: linoleic acid (LA), α-linolenic acid (LNA), arachidonic acid (AA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA). First, the results made clear that a **lower intake of LA** and a **higher intake of LNA** are necessary to decrease the LA/LNA ratio, since a lower LA/LNA ratio can help in the prevention of some chronic diseases. This can be reached by a higher consumption of LNA rich foods e.g. by replacement of omega-6 (n-6) rich oils by omega-3 (n-3) rich oils such as linseed and rapeseed in food formulations. Second, **dietary shifts** are necessary to **bridge the gap between the intakes and the recommendations of LC n-6 and n-3 PUFAs**. Regular replacement of meat products rich in saturated fatty acids (SFAs) by poultry meat is a possible solution to increase the AA intake, which was evaluated to be very low for Flemish pre-school children. Moreover, this study suggests that **seafood and particularly fatty fish consumption should be stimulated** in all subgroups of the population since it is a rich source of LC n-3 PUFAs of which the current intakes were evaluated to be far below the recommendations. Moreover, increased seafood consumption can lead to higher vitamin D intake and can replace SFA-rich food items. In spite of the fact that iodine was considered as a nutrient in the probabilistic intake assessment of nutrients and contaminants via seafood consumption only, no assessment of iodine intake via the total diet was performed, as a lot of the available food consumption databases lack good information about the iodine concentration in food items.

However, when recommending higher seafood consumption, one may not neglect the **nutritional-toxicological conflict** related to seafood consumption. This conflict arises from the fact that increased consumption of seafood will at the same time increase the intake of environmental contaminants like methyl mercury (MeHg), PCBs, and dioxin-like (dl) compounds (dl PCBs and PCDD/Fs). Therefore, a methodology was developed in this PhD-thesis, including the elaboration of databases and the development of a software module in order to execute a probabilistic assessment of the simultaneous intake of multiple compounds (LC n-3 PUFAs, vitamin D, iodine, (Me)Hg, PCBs, and dioxin-like substances) via seafood consumption.

Subsequently, the assessed intakes were evaluated to determine:

1. if **nutrient** intakes reached the **recommendations**, and
2. if **contaminant** intakes did not exceed a level of **toxicological** concern.

Two different situations were studied:

1. *Starting form the current seafood consumption data of two different subgroups of the Belgian population (adolescents and adults).*

Based on these consumption data, the simulation results predicted that the studied populations did not reach a sufficiently high intake for the three nutrients under consideration (LC n-3 PUFAs, vitamin D, iodine) taking into account only seafood consumption. Regarding the contaminants, MeHg contamination of seafood on the Belgian market did not seem to be an issue of toxicological concern. In contrast, the TDI for dioxin-like compounds was exceeded in the case of high seafood consumers on the basis of their seafood consumption only.

2. *It was investigated whether the Belgian recommendation for LC n-3 PUFAs (i.e. 0.3 % of total energy intake) could be reached through seafood consumption, without exceeding TDIs of MeHg and dioxin-like compounds. Also the contribution of LC n-3 enriched margarines was assessed.*

The results indicated that the Belgian recommendation for EPA plus DHA can be reached by consuming **fatty fish twice a week**, or by **varying between lean and fatty fish minimally three times a week.** It was found that **MeHg intake** was not an issue of toxicological concern for the Belgian population at this level of seafood consumption. The intake of **dioxin-like compounds** from seafood approximated the TDI when consuming fatty fish three times a week or more, being a potential toxicological risk since also other food items contribute to the daily intake of dioxin-like compounds. Use of LC n-3 enriched margarine can further help to increase the LC n-3 PUFA intake, on average by 159 mg/day. To conclude, combination of regular seafood consumption (twice a week), with important contribution of fatty fish species, in combination with regular consumption of LC n-3 enriched margarine can be advised to maximize LC n-3 intake. This quite positive message related to seafood consumption is in line with the conclusion of a recent review on the risks and benefits of seafood consumption (Mozaffarian & Rimm, 2006). The conclusion of this review was that for major health outcomes among adults, the benefits of seafood consumption exceed the potential risks. They made a separate conclusion for women of childbearing age, namely that

also for these group benefits of modest seafood consumption, excepting a few selected species, also outweigh risks (Mozaffarian & Rimm, 2006).

There is, however, an **important limitation** related to this study, namely that it was focused on seafood. As a result contaminant intake from other food sources was not taken into account in this PhD-thesis. For MeHg this is not a problem since exposure to MeHg occurs almost exclusively through consumption of seafood (Clarkson & Magos, 2006). In contrast, consumption of other food items, mainly from animal origin, contributes to the intake of dioxin-like compounds. Recent research executed at the Department of Public Health (Ghent University) assessed the dietary exposure to dioxin-like compounds **via the total diet** in three age groups on the basis of data from the Flemish Environment and Health study. The intake of dioxin-like compounds via animal fat of various sources was assessed. In total, 1636 adolescents (14-15 years), 1186 mothers (18-44 years), and 1586 adults (50-65 years) participated in the study and completed a semi-quantitative food frequency questionnaire (FFQ). Individual consumption data were combined, via a simple distribution approach, with recent data on PCDD/Fs and dl PCBs in food items, available on the Flemish market. It was found that **seafood was the most important contributor**, counting for 25.0, 29.4, and 43.3% in the group adolescents, mothers, and adults, respectively (Bilau *et al*, 2007). It is important to note that these percentages are averages per age group. On individual level, there is a high influence of the overall dietary pattern. For example, people regularly replacing meat by seafood will have a higher contribution of seafood, whereas heavy meat consumers who never or seldom eat seafood will have a higher contribution of meat and a low contribution of seafood. The other main contributors were in order of importance added fats, dairy products, and meat and meat products (Bilau *et al*, 2007).

2. Consumer issues

When aiming to increase seafood consumption in a population, it is important that the consumers are convinced of the importance to do so. I participated in different research studies of the Department of Agricultural Economics (Ghent University), investigating the perception of Belgian consumers about benefits and risks of seafood, farmed and wild fish, and sustainable and ethical issues. The following papers describe the results of these studies:

1. Verbeke W, Sioen I, Pieniak Z, Van Camp J, De Henauw S. Consumer perception versus scientific evidence about health benefits and safety risks from fish consumption. *Public Health Nutrition* 2005; **8**(4): 422-429.
2. Verbeke W, Sioen I, Brunsø K, De Henauw S, Van Camp J. Consumer perception versus scientific evidence of farmed and wild fish: exploratory insights from Belgium. *Aquaculture International* 2007; **15**(2): 121-136.
3. Verbeke W, Vanhonacker F, Sioen I, Van Camp J, De Henauw S. Perceived importance of fish ethics and sustainability: a consumer behavior perspective. *Ambio* 2007; In Press.

The main findings of these studies being important in the light of this PhD-thesis are summarized below.

2.1. Consumer perception about benefits and risks from seafood consumption

Several studies indicate that fish and other seafood is strongly perceived as a healthy food by consumers, particularly as compared with meat as its main substitute protein source (Brunsø, 2003; Gross, 2003). However, one of the potential barriers to eat fish more frequently may pertain to safety risks (Leek *et al*, 2000; Trondsen *et al*, 2003; Verbeke & Vackier, 2005).

This first mentioned Belgian study of Verbeke *et al* (2005) aimed

1. to investigate consumer attitude and perception towards fish, and
2. to explore the potential gap between scientific evidence versus consumer perception related to fish consumption benefits and risks.

A self-administered questionnaire was used and the study sample consisted of 429 adults being the main person responsible for food purchasing in the household (284 women and 145 men, aged 18-83 years).

The results of this study showed that consumers believe with reason that fish is healthy and nutritious, being an interesting result when aiming to convince consumers to eat regularly seafood. Nevertheless, 43% of the respondents of this study reported not to eat fish at least once a week. The consumers' knowledge of the vitamin D content in fish was good, but the results with respect to n-3 PUFAs showed that most people are not fully aware of the specific

nutrient content of seafood. The latter was a quite surprising result and showed that there is a definite need to inform people about the nutritional value and benefit of seafood. A majority of consumers scored neutral on the belief that fish is safe, probably being a result of the messages in the media about contamination of seafood. Attitude towards fish was found to include positive versus negative perceived attributes. In general, the taste and the healthy image of fish were two well-appreciated characteristics which can be used when promoting fish consumption. However, the bones in fish and the price are identified as the most likely attitudinal barriers to more frequent fish consumption. In general, the healthy image of fish prevails over its image of being potentially unsafe. This study exemplified **the need for nutritional education and more effective communication about seafood** to the broader public (Verbeke *et al*, 2005).

2.2. Consumer perception of farmed and wild fish

Data resulting from the questionnaire mentioned in 2.1. of this chapter were also used by Verbeke *et al* (2007b) to explore the perception of Belgian consumers regarding farmed versus wild fish. In addition, three consumer focus groups were conducted with six to eight participants in each session with the aim to gain further insights into consumer perception of farmed versus wild fish.

The majority of the consumer sample reported no perceived differences between farmed and wild fish. However, mean perception scores were slightly in favour of wild fish on the attributes taste, health, and nutritious value, in particular amongst consumers aged 55 years and older. The availability of farmed fish was perceived to be better than that of wild fish, while the consumers' perception of safety did not differ between farmed and wild fish. The focus group discussions indicated that consumers' opinions and beliefs about farmed fish are mainly based on emotion and image transfer from intensive terrestrial livestock production rather than on awareness and factual knowledge of aquaculture (Verbeke *et al*, 2007b). These results indicate that a **good indication about the production method** of the seafood product as well as some **information about the advantages and disadvantages** of the production method would help consumers to get a good opinion about farmed seafood. This can be crucial when promoting farmed seafood in the future, since the contribution of aquaculture

to the total food supply is increasing year by year. A lack of good information can result in a wrong opinion of consumers being negative for their consumption attitude.

2.3. Consumer perception about ethical and sustainability issues of fish

Although sustainability and ethics are of increasing public importance, little research has been conducted to reveal its association with fish consumer behaviour. In a third study, Verbeke *et al* (2007a) collected data through a postal self-administered survey from a sample of 381 Flemish women aged 20 to 50 years, with the objective to verify if ethical matters contribute to choosing or rejecting either farmed or wild fish. In addition, characterisation was made of persons who show more interest in ethical matters and sustainability, based on socio-demographics, behaviour, and attitude towards fish and involvement in fish consumption.

The results indicated that ethical and sustainability issues were indicated as being important by the consumer in relation to fish consumption. This claimed importance, however, was neither translated in a significant correlation with total fish consumption frequency nor with general attitude towards eating fish. The choice not to eat wild fish seemed to have part of its origin in ethical and sustainability issues, given a significantly higher importance attached to these issues by consumers who claim to refuse wild fish, and a negative correlation between interest in sustainability and cod consumption. This was not the case with respect to rejecting farmed fish, which was not associated with importance attached to ethical or sustainability issues. Finally, respondents with the highest importance attached to ethical and sustainability issues are the ones with the highest interest in information in general, with the highest belief in a potential benefit of receiving more information, and the highest perceived efficacy of their own deliberate choice (Verbeke *et al*, 2007a).

2.4. Conclusion related to consumer perception about seafood

The above summarized studies performed amongst Belgian consumers indicated that **a gap exists between consumers' knowledge and scientific facts about seafood**. This is mainly caused by a lack of information. Moreover, confusion among consumers is probably explainable by the conflicting messages about seafood in the media last years. Efforts to inform consumers about the composition and the origin of seafood products in an understandable and univocal way are therefore crucial when aiming to convince them to eat seafood on a more regularly basis. In contrast, other consumer barriers as the presence of bones and the price of seafood are more difficult to eliminate.

3. Sustainability

3.1. Depletion of natural fish stocks

There are concerns that the increased consumption of seafood and the increased use of fish oils for enriched foods are not a sustainable solution from an ecological point of view. The worldwide increase in consumption of seafood and derived seafood products during recent decades are mainly due to recommendations that seafood is part of a healthy human diet (World Health Organization, 2003), the increasing world population, higher living standards, and the overall image of seafood among consumers (Brunsø, 2003; Cahu *et al*, 2004; Verbeke *et al*, 2005). In 2004, total capture fisheries and aquaculture supplied the world with 106 million tonnes of seafood for human consumption, providing an apparent per capita of 16.6 kg (FAO, 2007). The increase in demand and supply has led to an expansion of the fishing fleet. Together with higher fish capture efficiency, this has contributed to overfishing and the risk of depletion of some natural fish stocks. As such, seafood species like Alaska Pollack, blue whiting, tuna, and mackerel became globally limited food sources (Bauch *et al*, 2006; European Commission, 2007). Moreover, the FAO (2007) reported that total catches in 2004 decreased by over 10% in comparison with 2002 in the Northeast Atlantic area, which was estimated to be the most important natural source for seafood on the Belgian market (Chapter III).

3.2. Aquaculture: a valuable alternative?

As an alternative to wild caught seafood, consumers are offered now farmed seafood. In 2004, aquaculture accounted for 43% of the total world seafood supply and the contribution of aquaculture will continue to expand, while marine capture fisheries seem to have reached a ceiling (FAO, 2007). It is predicted that in 2030 aquaculture will provide half of the total amount of seafood consumed worldwide (European Commission, 2007; Tidwell & Allan, 2001). Therefore, it is a challenge to develop sustainable aquaculture, which in the same time minimizes bioaccumulation of contaminants and other food safety risks, as the risk of contamination by chemical and biological agents is greater in freshwater and coastal ecosystems than in open seas (McMichael & Butler, 2005; World Health Organization, 1999).

3.2.1. Wild versus farmed seafood

To set both ways of seafood supply side by side, a limited comparison of wild and farmed seafood is given, considering nutritional as well as food safety aspects. The **nutrient content** may differ between wild and farmed seafood species, viewed the difference in their diet. Wild seafood eats plankton, small algae, small fishes, and other seafood species. Their diet is affected by environmental and seasonal changes. This will affect the proximate composition of the muscle. In aquaculture, farmed seafood is provided with a supply of nutrient-dense formulated feed, which is constant throughout the year and which enables them to deposit large reserves of lipids. Moreover, the content of the formulated feed can be changed in function of the wishes of the producer and the consumer. Different studies indicated that the lipid content of farmed fish is generally larger than that of their free-living counterparts and the levels of EPA and DHA are generally lower, when expressed relatively to total fatty acids (Bandarra *et al*, 1997; Haard, 1992; Nettleton & Exler, 1992; Olsson *et al*, 2003; Sérot *et al*, 1998). But viewed their higher lipid content, the amounts of EPA and DHA provided by a given quantity of farmed fish may be higher than in the same amount of wild fish. On the other hand, cholesterol and protein levels are similar in farmed and in wild fish. Nettleton & Exler (1992) showed that levels of different vitamins (vitamin A, C, B12, B2, B3, B5, B8, and folic acid) were similar or higher in farmed species. Cahu et al (2004) concluded that the nutritional content of farmed fish is at least as beneficial as that of wild fish and farmed fish

also has advantages of freshness because the storage conditions between slaughtering and sale are more verifiable.

Next, **food safety** problems are linked to aquaculture. Regarding the presence of environmental contaminants, the most important contaminant source for farmed seafood is their formulated feed (Bell *et al*, 2005; Karl *et al*, 2003). Some recent studies showed that concentrations of organochlorine contaminants are significantly higher in farmed salmon than in wild and that farmed salmon of Europe are significantly more contaminated than farmed salmon from South and North America (Easton *et al*, 2002; Hites *et al*, 2004a). In contrast, the European Food Safety Authority (EFSA) conducted a scientific assessment of the health risks related to human consumption of wild and farmed fish and concluded that with respect to their safety for the consumer there is no difference between wild and farmed fish (EFSA, 2005b). Nevertheless, aquaculture industries should be able to take steps to reduce contaminants in fish feed by applying purification processes and as such be able to provide the population with naturally rich dietary LC n-3 sources, being safe from a toxicological point of view. Another food safety issue related to aquaculture is the use of additives like colorants and the use of drugs (Alderman & Hastings, 1998). However, due to the large expansion of global aquaculture, countries worldwide have implemented a large number of aquaculture regulations to control inadequate developments for example related to drug use (FAO, 2007).

Nevertheless, these current aquaculture regulations cannot guarantee sustainability, especially as most of them focus on the individual farmer and do not consider the additive or synergetic effect of multiple farms on a particular area. At the same time, farmers' economic appraisals tend to have a narrow view, which do not include the medium- and long-term revenues and costs that may be imposed on the farming activity itself and on the rest of the society in the form of a reduced supply of ecosystem goods and services (FAO, 2007).

3.2.2. Feeding farmed seafood

A final aspect related to aquaculture is the widespread practice of feeding wild-caught seafood to farmed piscivorous seafood species, e.g. salmon. This practice decreases the availability of seafood for direct human consumption on a per capita basis (McMichael &

Butler, 2005; Naylor *et al*, 2000). This leads to a paradox: aquaculture is a possible solution, but also a contributing factor to the collapse of fisheries stocks worldwide (Naylor *et al*, 2000). It must be said that up to now, aquaculture is still the largest consumer of fish derived oils, and so is clearly not capable of operating in a sustainable manner (Napier & Sayanova, 2005). So, to meet consumer demand for piscivorous fish and at the same time reduce the pressure on wild fish stocks, land-based plants - such as grains, soybeans, legumes, and blended vegetable oils - are increasingly being used as staple fish food. The disadvantage is that using this feed will lower the LC n-3 PUFA concentration compared to the wild-caught species, affecting one of the important nutritional advantages of seafood consumption (McMichael & Butler, 2005). However, actually different studies are running that investigate whether a combination of a diet high in vegetable oil with a diet high in fish oil only during the last weeks of the production cycle can lead to a restoration of EPA and DHA concentration to equal levels when compared to fish oil fed counterparts (Bell *et al*, 2005; Napier & Sayanova, 2005). More research is needed and probably it will take time before such customs are used in practice. An alternative to this kind of combined diets can be the use of single cell oils and genetically modified plants (and animals) as sustainable sources of LC n-3 PUFAs. An introduction to these rather new developments is given in the paragraph below.

4. Potential sustainable sources of LC n-3 PUFAs

4.1. Single cell oils

As a more sustainable source of LC n-3 PUFAs compared to fish and fish oils, commercial companies started with the production of microbial oils, otherwise referred to as single cell oils (SCO), based on a DHA producing organism that can grow in large-scale industrial fermentors. Today, there are at least three different fermentation processes commercially available, each using a different microorganism for the production of DHA. For example, *Crypthecodinium cohnii* produces a triacylglycerol oil in which DHA can reach levels between 40 and 50% of the total fatty acids and, moreover, occurs as the sole PUFA. Currently, the major application of these SCOs is in the form of a supplement for infant nutrition (Ratledge, 2004).

4.2. Metabolic engineering in plants (and animals)

A second alternative way to increase the supply of LC n-3 PUFAs is through plant technology, in other words metabolic engineering in plants which manipulates the lipid metabolism and leads to the production of novel plant oils (Kinney, 2006; Voelker & Kinney, 2001). Advances in gene expression and genomics have recently led to the engineering of multigene pathways in plants, such as microbial LC PUFAs biosynthetic pathways reconstituted in the seeds of crop plants (Domergue *et al*, 2005). In other words, identification of a gene cluster coding the entire pathways of PUFA biosynthesis in the microorganisms used in the SCO applications opened the way to clone this contiguous gene sequence into various plants in order to achieve PUFA production in agronomically important crops such as sunflower, soybean, or rapeseed (Ratledge, 2004). Application of this technology on a larger scale would provide a sustainable source of LC n-3 PUFA for use in human food and animal feed (for terrestrial as well as aquatic animals). A lot of research is done. Nevertheless, more efficient techniques are still required to optimize the LC n-3 PUFA synthesis in plants (Domergue *et al*, 2005; Napier & Sayanova, 2005). It is also worth to mention that very recent studies report on the development of transgenic pigs able to convert n-6 PUFAs to n-3 PUFAs (Prather, 2006). However, a long research and regulatory way will be needed before they will enter the food chain, if ever they will enter, because lot of controversy still exists about the presence of transgenic foods on the market. Discussion of the latter is beyond the scope of this PhD-thesis.

5. Omega-3 supplements and omega-3 fortification

Apart from seafood, fish oil supplements and food items fortified with LC n-3 PUFA are currently available as alternative LC n-3 PUFA dietary source. Supplements are usually taken additionally to the normal diet. In contrast, fortified foods will normally replace certain unfortified food items in the normal diet. Fortification of food items can be done in different ways: direct and indirect fortification. A short discussion with respect to these alternative LC n-3 sources is given in this paragraph.

5.1. Fish oil supplements

In addition to being used in the food and animal feed industry, fish oils have also been used traditionally as dietary supplements. The dietary supplement market expanded significantly and includes these oils in a variety of different formulations (Jacobs *et al*, 2004). Most known are the encapsulated fish oils sold as dietary supplements for their high content of EPA and DHA. The supply of these supplements on the Belgian market is increasing and, thus, consumption of these supplements is increasing as well, mainly due to good promotion campaigns and the 'commercial hype' that is currently related to omega-3 fatty acids. Fish oils are derived from fish liver (e.g. cod liver oil) or from muscle tissue of fatty fish species. However, fish oils represent a large accumulation potential for lipophilic persistent organic pollutants present in the marine ecosystem (Jacobs *et al*, 2004). Therefore, it is important to monitor closely the levels of organic pollutants that might be present (Covaci *et al*, 2006; Fernandes *et al*, 2006; Jacobs *et al*, 2004; Storelli *et al*, 2004). In an effort to remove these contaminants, the industry employs a number of refining processes. Molecular distillation is very effective in removing halogenated contaminants as PCBs, but it removes at the same time beneficial components like EPA and DHA. Thus, some manufactures have invested in newer purification methods that are able to achieve this balance and are claiming success (Fernandes *et al*, 2006).

Several recent papers are available reporting the contamination of fish oil dietary supplements available on the European market (Covaci *et al*, 2006; Fernandes *et al*, 2006; Jacobs *et al*, 2004; Storelli *et al*, 2004). Storelli *et al* (2004) analysed dietary supplements based on cod-liver oil from the Italian market on PCBs, hexachlorobenzene, and chlorinated pesticides. Jacobs *et al* (2004) reported analytical results of selected contaminants, including PCBs, organochlorine pesticides, and polybrominated diphenyl ethers (PBDEs) for a range of commercially available cod liver oil and other fish oil dietary supplements on the market in London, UK, sampled in December 2001 and January 2002. Fernandes *et al* (2006) analysed dioxins and PCBs in fish oil supplements sourced from retail outlets in the UK sampled during 2001-2002. Covaci *et al* (2006) analysed fish oil dietary supplements collected in 2006 and reported the levels of PCBs and (methoxy)polybrominated diphenyl ethers. They choose the samples as such that they covered the products available for sale in the Belgian market; and, additionally, fish oil dietary supplements bought in the Netherlands, Ireland, United Kingdom, and South-Africa were also analyzed. Only a summary of the concentration of

PCBs, dl PCBs, and PCDD/Fs are given here, since the other contaminants reported in the articles were not considered in this PhD-thesis.

In the Italian cod-liver oil supplements, PCB concentrations ranging from 25 to 201 ng/g lipid were found (Storelli *et al*, 2004). Jacobs *et al* (2004) reported a concentration range for the sum of the seven indicator PCBs (iPCBs) equal to 86.7 to 133.3 ng/g oil and not detected (ND) to 49.0 ng/g oil for cod liver oils (n=7) and other fish oils (n=6), respectively. Fernandes *et al* (2006) having analysed a total of 33 dietary supplements, compromising mostly cod liver oils but also other fish oils sampled during the same period and from the same market found iPCB concentrations ranging from 8.3 to 266.6 ng/g oil, so being more extreme than what Jacobs *et al* (2004) found. Also Covaci *et al* (2006) sampled 12 fish oil dietary supplements from the UK market, but later in time, i.e. 2006. They reported a PCB concentration (sum of 20 congeners) range for these samples from <0.3 to 22 ng/g oil, being much lower than the older samples reporting only the sum of seven iPCBs. This can confirm the downward trend, previously reported (Fernandes *et al*, 2006; Jacobs *et al*, 2004). Further reasons for the low contaminant levels include improved sourcing and nutritional profile development to create specific 'optimum' essential fatty acid balances (Covaci *et al*, 2006). Furthermore, Covaci *et al* (2006) found PCB concentration ranges equal to <0.3-57, <0.3-60, <0.3-95 ng/g oil respectively for samples on the market in Belgium (n=27), The Netherlands (n=17), and other countries (n=13).

Only Fernandes *et al* (2006) reported concentration ranges for PCDD/Fs and dl PCBs equal from 0.18 to 8.4 pg TEQ and from 1.1 to 41 ng/g oil in 33 supplements collected on the UK market between 2001 and 2002. When manufacture-recommended doses were applied to the observed levels, the estimated upper bound human exposure to PCDD/Fs and dl PCBs ranged from 0.02 to 7.1 pg TEQ/kg bw/day (Fernandes *et al*, 2006). Currently, the European Commission legislation has set a maximum limit of 2.0 pg TEQ/g fat for PCDD/Fs and 10.0 pg TEQ/g fat for the sum of PCDD/Fs and dl PCBs for marine oils (fish body oil, fish liver oil and oils of other marine organisms intended for human consumption) (European Commission, 2006). Much of the samples analysed by Fernandes *et al* (2006) exceed these maximum limits. But, the decreasing trend in contamination load seen previously and better purification processes will probably cause lower concentrations in the samples currently available on the market.

However, some controversy exists related to the use of supplements. First, there are indications that dietary patterns rather than individual nutrients might account for more important beneficial effects, taking into account the possibility of diverse interactions between different food components (Reinert *et al*, 2007). In particular, opponents of supplements and functional foods respond that it is the total diet that is important for health, not the so-called 'magic bullets' (Lawrence & Rayner, 1998). Second, using supplements can be seen as adding something extra on top of the usual diet, whereas it would be – in a lot of cases – more beneficial to replace less healthy food items by healthy food items instead of adding supplements to a rather unhealthy diet or - in general - unhealthy way of life. As fish and other seafood is a good source of high quality proteins and fatty acids, as well as of vitamin D and micronutrients, it can serve as an excellent substitute for protein sources high in saturated fats. So, in absence of seafood allergy or dislike, seafood consumption can be advised rather than supplement use.

5.2. Direct fortification

Several functional foods, including margarines and dressings, to which LC n-3 PUFAs are added, have been developed in the last years (Patch *et al*, 2005b; Patch *et al*, 2005a). These food items belong to the group of direct fortified foods since an extra nutrient, in this case EPA and DHA, is added to the product at the end of the production process. This became possible since technology made it achievable to produce high quality, deodorized, and stabilized oils enriched in LC n-3 PUFAs (Harris, 2007). Currently, processed fish oils are used as a source of LC n-3 PUFA, generally in microencapsuled form to fortify the foods in a tasteless way. Nevertheless, in the future SCOs and genetically modified plants will probably supply the necessary fatty acids to enrich all kind of food products. Different research groups already investigated the impact of foods enriched with LC n-3 PUFA on cardiovascular risk factors (Baro *et al*, 2003; Harrison *et al*, 2004; Liu *et al*, 2001; Metcalf *et al*, 2003; Murphy *et al*, 2007; Visioli *et al*, 2000). All of them concluded that increased consumption of LC n-3 PUFA enriched foods may be associated with reduced risk in cardiovascular diseases. As for supplements, no recent information is available neither on the consumption of such foods by the Belgian population nor on their contribution to the overall intake of LC n-3 PUFAs.

5.3. Indirect fortification: modifying animals' diet

In the last decades, a lot of research has been devoted to the potential of modifying the fatty acid composition of animal food products to better meet human nutritional guidelines. Additionally, there is also growing interest in the animal feed industry to implement strategies fulfilling these objectives. Modifying animal's diet, i.e. indirect fortification, offers by far the largest opportunities (Raes *et al*, 2004). In this respect, research efforts to increase the (LC) n-3 PUFA content of terrestrial animal products by fortifying the animals' feed could contribute to improving the overall intake of (LC) n-3 PUFAs (Sontrop & Campbell, 2006). To improve the n-3 PUFA content and the n-6/n-3 ratio, the use of vegetable oils or whole seeds rich in LNA and grass or grass products (the latter for ruminants) is a worthwhile strategy. This approach mainly increases the LNA content but also offers benefits in terms of increasing the LC n-3 PUFAs, although the latter response strongly depends on the type of product. Adding fish oil or fishmeal to the diets of farm animals like pigs considerably increases the deposition of both EPA and DHA. However, there are major obstacles for using fish oil or fishmeal as feed ingredients (Raes *et al*, 2004; Sontrop & Campbell, 2006). The use of single cell oils (SCO) or genetically modified crops might be an alternative. Nevertheless, the potential human health benefits of specific enrichment strategies also remain unknown. In a preliminary simulation study using Belgian food consumption data, the impact of alterations in the n-6/n-3 PUFA ratio of farm animal foods on the pattern of fatty acid intakes has been estimated (De Henauw *et al*, 2007). Although the outcome was largely affected by the assumptions that were made, considerable shifts in the intake of LNA could be achieved. Nevertheless, it was difficult to obtain shifts in the intake of LC n-3 PUFA. More data and alternative strategies need to be considered in this respect.

6. Risks related to excessive intakes of LC n-3 PUFAs, vitamin D, and iodine

In this PhD-thesis, one of the major goals was to explore pathways to bridge the gap between the current low intakes and the recommendation of certain nutrients, particularly LC n-3 PUFAs, vitamin D, and iodine. However, data, information, and special attention in the media about the benefits of those nutrients can lead to an increased supply and use of

supplements and fortified foods in some parts of the population, with an excessive intake of those nutrients as a result. Therefore, it is worth to describe shortly the possible risks and safety considerations related to very high intakes of LC n-3 PUFAs, vitamin D, and iodine.

6.1. LC n-3 PUFAs

The American IOM stated that there is up to now insufficient evidence to set a tolerable upper intake level (UL) for LC n-3 PUFAs (Institute of Medicine, 2005). However, a working group of the French Food Safety Agency (AFSSA) considers that the maximum permitted intake for LC n-3 PUFAs should be 2.0 g/d, a level close to the mean values observed in populations which consume large amounts of marine products on a daily basis. Rather than seeing this value of 2 g/d as a safety limit for LC n-3 PUFAs beyond which there would be a risk, it should be emphasised that there is no proven nutritional benefit in recommending a daily intake near or above it (AFSSA, 2003). Two different safety aspects have to be kept in mind. A **first** safety consideration related to the intake of LC n-3 PUFAs is the question whether it leads to a higher risk of bleeding. LC n-3 PUFAs affect thrombosis by decreasing platelet aggregation (Kris-Etherton *et al*, 2002). It is due to this antithrombotic effect that the suggestion was made that LC n-3 PUFAs could maybe increase the risk for bleeding (Carroll & Roth, 2002). However, clinical trial evidence suggest that if such a risk exists, the risk is very small and not of clinical significance and that high-dose fish oil LC n-3 PUFA consumption is safe (Bays, 2007). A **second** safety factor is that LC n-3 PUFAs are naturally highly unstable and susceptible to oxidation, which may increase the risk for toxicity. One of the most common ways to reduce oxidation is to add the antioxidant vitamin E to supplements. This can overcome the oxidative stress to the body that is observed when fish oils without vitamin E are consumed (Bays, 2007). Consequently, LC n-3 PUFA supplements leading to an intake achieving the recommendations and containing an anti-oxidant can generally be considered as safe.

6.2. Vitamin D

Hypervitaminosis D is characterised by a considerable increased plasma concentration of 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$), the biologically active hormone formed from vitamin D. As $1,25(OH)_2D_3$ increases to toxic concentrations, hypercalciuria, hypercalcemia, and, finally, extraskeletal calcification becomes evident (Hollis & Wagner, 2004; Institute of Medicine, 1997). Moreover, reduced renal function and a severe depression illness have been associated with hypervitaminosis D.

Based on toxicological data, an **UL** equal to **50 µg vitamin D per day** was derived for adults and children between 1 and 18 years old and is supported by the American IOM and the European Scientific Committee on Food (Institute of Medicine, 1997; Scientific Committee on Food, 2002). For infants up to 1 year of age, the UL is set at 25 µg vitamin D per day (Institute of Medicine, 1997). The IOM stated that for most people, vitamin D intake from food and supplements is unlikely to exceed this UL. However, people who are at the upper end of the ranges of intake of both sources (food and supplements) and those with high intakes of fish or fortified foods may be at risk for vitamin D toxicity (Institute of Medicine, 1997). Conversely, recent data suggest that the current UL is far more restricted than needed to avoid adverse effects of vitamin D. Based on a new risk assessment, Hathcock et al (2007) suggest that 250 µg/d can be selected as new UL. This should safely permit increased intakes of vitamin D at higher levels than previously recognized (Hathcock *et al*, 2007).

6.3. Iodine

Concerning iodine, it has been reported that a wide range of iodine intakes is tolerated by most individuals, owing to the ability of the thyroid to regulate total body intake. Over **2 mg/day** for long periods should be regarded as excessive or potentially harmful to most people, but such high intakes are unlikely to arise from natural foods. However, the IOM set an UL for iodine at 1100 µg/day for adults (Institute of Medicine, 2001). Hyperthyroidism (result from excess iodine exposure) is largely confined to those over 40 years of age. Symptoms of hyperthyroidism are rapid hearth rate, trembling, lack of sleep, and loss of weight and strength (Strain & Cashman, 2002).

7. Thoughts for future research

This PhD-thesis helped to find out which recommendations about seafood consumption can be formulated for the Belgian population in order to increase the intake of LC n-3 PUFAs to levels that can help in the prevention of chronic Western diseases without leading to contaminant intakes at levels of toxicological concern. However, as described in this final chapter, a lot of other aspects have to be considered when advising increased seafood consumption, as there are consumer perception and barriers, the aspect of sustainability, and the contribution of other food items to the intake of dioxin-like compounds.

In the future, research will need to find out which is the most sustainable and most beneficial way to provide enough LC n-3 PUFAs for the human population, without creating new risks for man or for the environment. These new strategies can be based on the use of direct fortified food items, like bread, yoghurts, meat products, … fortified with LC n-3 PUFAs. Another possibility is to investigate in the production chain of animal food items, e.g. milk, cheese, meat of terrestrial animals, seafood. By modifying the diet of these animals during the production process, a modified composition of the final food item can be achieved as was described in 5.3. of this chapter. However, such strategies still need some optimisation before they can be used on broad scale. Moreover, it is not clear how consumers will react on an increasing supply of such products.

To conclude, it remains a continuing challenge to find a good way to guide consumers to a healthy eating behaviour in a world where large multinational companies control the global food market. Only then, successful and sustainable public health nutrition programs will be raised.

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Summary

Pioneer research in the nineteen sixties and seventies indicated that the consumption of fish was associated with a reduced risk for coronary heart disease in Greenland Eskimos. This Eskimo population experienced a low mortality from coronary heart disease despite a diet rich in fat and cholesterol. It was soon suggested that this could be related to the high content of omega-3 fatty acids, typically present in marine foods. These Eskimo studies have triggered a much broader and intensified research on the importance of omega-3 fatty acids and seafood in the human diet.

This PhD-study is embedded in that research area and examines in the first place the intake of omega-3 and other fatty acids by the Flemish and Belgian population. In a next step, the question is raised whether seafood is a safe dietary source of these fatty acids and whether the consumption conform with physiological needs induces any toxicological concern. The latter is of importance since the favourable health perception is troubled by information regarding the potential adverse health impact of chemical contaminants in marine foods, occuring naturally or resulting from man-made processes. These conflicting facts form a potential base for an important public health conflict between dietary recommendations on the one hand and toxicological safety assurance on the other hand.

A first part of the PhD-thesis presents the results of an intake assessment of individual poly-unsaturated fatty acids (PUFAs) in Flemish pre-school children, adolescents, and young women and an intake assessment of vitamin D in Flemish adolescents. The following PUFAs were considered: linoleic acid (LA), α-linolenic acid (LNA), arachidonic acid (AA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA). First, the results make clear that a **lower intake of LA** and a **higher intake of LNA** are necessary to decrease the LA/LNA ratio, since a lower LA/LNA ratio can help in the prevention of some chronic diseases. This can be reached by a higher consumption of LNA rich foods e.g. by replacement of omega-6 (n-6) rich oils by omega-3 (n-3) rich oils such as linseed and rapeseed in food formulations. Second, **dietary shifts** are necessary to **bridge the gap between the intakes and the recommendations of long chain (LC) n-6 and n-3 PUFAs**. Regular replacement of meat products rich in saturated fatty acids (SFAs) by poultry meat is a possible solution to increase the AA intake, which was evaluated to be very low for

Flemish pre-school children. Moreover, this study suggests that **seafood and particularly fatty fish consumption should be stimulated** in all subgroups of the population since it is a rich source of LC n-3 PUFAs of which the current intakes are evaluated to be far below the recommendations. Furthermore, increased seafood consumption can lead to higher vitamin D intake and can replace SFA-rich food items.

A next part of the thesis focuses on the **nutritional-toxicological conflict** related to seafood consumption. This conflict arises from the fact that increased consumption of seafood will at the same time increase the intake of environmental contaminants like methyl mercury (MeHg), PCBs, and dioxin-like (dl) compounds (dl PCBs and PCDD/Fs). Therefore, a methodology was developed, including the elaboration of databases and the development of a software module in order to perform a probabilistic assessment of the simultaneous intake of multiple compounds (LC n-3 PUFAs, vitamin D, iodine, (Me)Hg, PCBs, and dioxin-like substances) via seafood consumption. Subsequently, the assessed intakes were evaluated to determine: (1) if **nutrient** intakes reached the **recommendations** and (2) if **contaminant** intakes did not exceed a level of **toxicological** concern.

Two different situations were studied:

1. *Starting from the current seafood consumption data of two different subgroups of the Belgian population (adolescents and adults).*

Based on these consumption data, the simulation results predicted that the studied populations did not reach a sufficiently high intake for the three nutrients under consideration (LC n-3 PUFAs, vitamin D, iodine) taking into account only seafood consumption. Regarding the contaminants, MeHg contamination of seafood on the Belgian market did not seem to be an issue of toxicological concern. In contrast, the tolerable weekly intake for dioxin-like compounds was exceeded in the case of high seafood consumers on the basis of their seafood consumption only.

2. *It was investigated whether the Belgian recommendation for LC n-3 PUFAs (i.e. 0.3 % of total energy intake) could be reached through seafood consumption, without exceeding tolerable weekly intakes of MeHg and dioxin-like compounds. Also the contribution of LC n-3 enriched margarines was assessed.*

The results indicated that the Belgian recommendation for EPA plus DHA can be reached by consuming **fatty fish twice a week**, or by **varying between lean and fatty fish minimally**

three times a week. It was found that **MeHg intake** was not an issue of toxicological concern for the Belgian population at this level of seafood consumption. The intake of **dioxin-like compounds** from seafood only approximated the tolerable weekly intake when consuming fatty fish three times a week or more, being a potential toxicological risk since also other food items contribute to the daily intake of dioxin-like compounds.
Use of LC n-3 enriched margarine can further help to increase the LC n-3 PUFA intake, on average by 159 mg/day. To conclude, combination of regular seafood consumption (twice a week), with important contribution of fatty fish species, in combination with regular consumption of LC n-3 enriched margarine can be advised to maximize LC n-3 intake.

There is, however, an important limitation related to this study, namely that contaminant intake from other food sources was not taken into account. For MeHg this is not a problem since exposure to MeHg occurs almost exclusively through consumption of seafood. In contrast, consumption of other food items, mainly from animal origin, contributes to the intake of dioxin-like compounds. This is an important fact that has to be taken into account in further research.

Samenvatting

Baanbrekend onderzoek in de jaren zestig en zeventig van de vorige eeuw bracht aan het licht dat de consumptie van vis het risico op hart- en vaatziekten deed dalen bij Eskimo's in Groenland. Deze Eskimobevolking vertoonde namelijk een lage mortaliteit ten gevolge van hart- en vaatziekten ondanks een voedingspatroon rijk aan vet en cholesterol. Er werd vermoed dat het hoge gehalte aan omega-3 vetzuren in mariene levensmiddelen hier een rol in speelde. De studies bij deze Eskimobevolking gaven aanleiding tot verder intensief onderzoek naar het belang van omega-3 vetzuren en vis en zeevruchten in het humane voedingspatroon.

Ook dit doctoraatsonderzoek kadert binnen dat onderzoeksdomein en behandelt de inname van omega-3 en andere vetzuren bij de Vlaamse en Belgische bevolking. Daarnaast werd ook onderzocht of vis en zeevruchten een veilige voedingsbron zijn van deze omega-3 vetzuren en welke toxicologische risico's verbonden zijn aan visconsumptie die beantwoordt aan de fysiologische noden voor deze vetzuren. Dit laatste is van belang omdat het gezonde imago van vis en zeevruchten de laatste jaren verstoord wordt door negatieve boodschappen over de aanwezigheid van chemische contaminanten in vis en zeevruchten. Deze tegenstrijdige feiten vormen de basis voor een belangrijk conflict tussen voedingsaanbevelingen enerzijds en toxicologische voorzorgsmaatregelen anderzijds.

Het eerste deel van deze thesis beschrijft de inname van de verschillende onverzadigde vetzuren via het totale voedingspatroon voor drie Vlaamse bevolkingsgroepen: kleuters, adolescenten en jonge vrouwen. De volgende vetzuren werden in rekening genomen: linolzuur (LA), α-linoleenzuur (LNA), arachidonzuur (AA), eicosapentaeenzuur (EPA), docosapentaeenzuur (DPA) en docosahexaeenzuur (DHA). Bovendien wordt de vitamine D inname van adolescenten besproken. De resultaten maken duidelijk dat een **lagere LA en een hogere LNA inname** nodig is om de verhouding van LA over LNA te doen dalen. Een voedingspatroon met een lagere LA/LNA verhouding kan immers helpen bij de preventie van sommige chronische ziekten. Praktisch kan dit worden bereikt door bijvoorbeeld omega-6 rijke olieën te vervangen door omega-3 rijke olieën zoals lijnzaad- en koolzaadolie in bepaalde samengestelde levensmiddelen zoals koekjes. Daarnaast zijn nog andere **verschuivingen in het voedingspatroon** noodzakelijk om het verschil te herstellen tussen de

inname en de aanbevelingen van **langketen omega-6 en omega-3 vetzuren**. Om de AA-inname die erg laag was bij de kleuters te verhogen, zou het goed zijn regelmatig vleesproducten rijk aan verzadigde vetzuren te vervangen door gevogelte. Bovendien zou voor alle bevolkingsgroepen de **consumptie van vis en zeevruchten, en in het bijzonder van vette vis, gestimuleerd** moeten worden omdat deze voedingsmiddelengroep een uitzonderlijk rijke bron is van langketen omega-3 vetzuren (EPA, DPA en DHA). De inname van deze vetzuren ligt momenteel ver beneden de aanbevelingen. Daarenboven zal een regelmatigere consumptie van vis en zeevruchten de inname van vitamine D verhogen en zijn deze voedingsmiddelen een gezond alternatief voor voedingsmiddelen rijk aan verzadigde vetzuren.

Het tweede deel van de thesis gaat over het nutritioneel-toxicologisch conflict verbonden aan de consumptie van vis en zeevruchten. Dit conflict ontstaat door het feit dat een regelmatigere visconsumptie tegelijkertijd de inname van chemische contaminanten zoals methylkwik, PCBs en dioxines zal verhogen. Om dit van naderbij te kunnen bestuderen werd een methodologie ontwikkeld waarbij databanken werden opgebouwd en waarbij een software module werd ontwikkeld. Deze instrumenten lieten toe op probabilistische wijze de gelijktijdige inname te berekenen van verschillende nutriënten en contaminanten (langketen omega-3 vetzuren, vitamine D, jodium, methylkwik, PCBs en dioxineachtige componenten) bij de consumptie van vis. Vervolgens werden de resultaten van deze innameschatting gebruikt om te bepalen: (1) of de **nutriëntinnames** voldeden aan de **aanbevelingen** en (2) of de **contaminantinnames** de toxicologische **grenswaarden** niet overschreden.

Binnen het doctoraatsonderzoek werden twee verschillende situaties bestudeerd:

1. *Startend van het huidige patroon van vis- en zeevruchtenconsumptie van twee verschillende Belgische bevolkingsgroepen (adolescenten en volwassenen).*

Op basis van deze consumptiedata en de ontwikkelde methodologie werd voorspeld dat beide populaties de aanbevelingen voor de verschillende **nutriënten** niet halen wanneer enkel vis en zeevruchten als bron in rekening worden gebracht. Uit de evaluatie van de inname van **contaminanten** kan worden besloten dat kwikcontaminatie van vis en zeevruchten niet tot toxicologische risico's leidt voor de Belgische bevolking. Daarentegen werd de grenswaarde voor dioxineachtige componenten wel overschreden door mensen die heel vaak vis consumeren (bv. drie tot vier porties vette vis per week). Bovendien werd deze

overschrijding reeds vastgesteld zonder het in rekening brengen van andere voedingsmiddelen die ook dioxineachtige contaminanten bevatten.

2. *Er werd onderzocht of het mogelijk is de Belgische aanbeveling voor langketen omega-3 vetzuren (EPA en DHA) te halen louter op basis van vis- en zeevruchtenconsumptie, zonder dat de grenswaarden voor methylkwik en dioxineachtige componenten worden overschreden. Ook werd het mogelijke belang van omega-3 verrijkte margarines onderzocht.*

De resultaten van dit onderzoeksluik tonen aan dat de Belgische aanbeveling voor EPA en DHA gehaald kan worden wanneer **twee keer per week vette vis** geconsumeerd wordt. De aanbeveling kan ook worden gehaald door te variëren tussen **vette en magere vis**, maar dan moet vis **drie keer per week** op het menu staan. Hierbij is het wel belangrijk te vermelden dat de inname van dioxineachtige componenten de grenswaarde bereikt wanneer vette vis drie keer of meer per week op het menu staat. Wetende dat er in ons dagelijks voedingspatroon nog andere bronnen zijn van deze dioxineachtige contaminanten, is dit dus een belangrijk aandachtspunt. Het gebruik van margarine verrijkt met langketen omega-3 vetzuren kan verder helpen de inname van omega-3 vetzuren te verhogen.

Tot slot is het belangrijk nog eens te herhalen dat in deze doctoraatsthesis de contaminantinname enkel werd beschouwd via de consumptie van vis en zeevruchten. Andere mogelijke bronnen werden dus buiten beschouwing gelaten. Deze beperking heeft geen grote invloed op de innameschatting van methylkwik, aangezien er weinig andere bronnen van methylkwik in ons voedingspatroon aanwezig zijn. Het humane voedingspatroon bevat echter wel nog andere bronnen van dioxineachtige contaminanten, voornamelijk andere levensmiddelen van dierlijke oorsprong. Bij verder onderzoek is het dan ook erg belangrijk deze mee in rekening te brengen.

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Isabelle Sioen
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About the author

Isabelle Sioen was born on July 15, 1980 in Ghent (Belgium). She finished secondary school in Latin and mathematics at the 'Sint-Bavohumaniora' in Ghent in 1998. The same year, she started the study of bioscience engineering at the Ghent University. In 2001, she studied during one semester at the Department of Science and Technologies of the Food Industry at the ISIM (*Institut des Sciences de l'Ingénieur de Montpellier II*) in France with an Erasmus program. During the last year of her master studies, she conducted three months of fieldwork in the Houndé District in Burkina Faso for her dissertation entitled 'Evaluation of the nutritional status and dietary habits of pregnant women in rural Burkina Faso'. She obtained her MSc degree in Bioscience Engineering, option chemistry, in 2003.

From November 2003 until February 2004, she worked as a scientific staff member at the Research and Information Centre for Consumer Organizations in Brussels.

In March 2004, she started working at the Ghent University as a PhD student on a project financed by the Belgian federal government, half of the time at the Department of Public Health of the Faculty of Medicine and Health Sciences and half of the time at the Department of Food Safety and Food Quality at the Faculty of Bioscience Engineering. In January 2005, she obtained an IWT-scholarship and she continued her PhD-work. During this period, she followed different international courses, for instance the 'EuroFIR Course on Production of Food Composition Data in Nutrition' in Bratislava (Slovak Republic) and the 'Summer School EU Basics in Public Health Nutrition' in Giessen (Germany). From February 10, 2007 to May 10, 2007 she was hosted at the French Food Safety Agency (AFSSA, *Agence Française de Sécurité Sanitaire des Aliments*) in Paris (France) for a scientific mission in relation to her PhD-work.

She participated in many international congresses and workshops where she presented her work by means of posters or oral communications. At the 1st International Congress on Food Safety with the topic 'Nutrition and Food Safety: Evaluation of Benefits and Risks' held in June 2006, in Budapest (Hungary), she won the Award for Best Student Poster with her poster 'Probabilistic risk-benefit analysis regarding seafood consumption'. At the 10th European Nutrition Conference held in July 2007 in Paris (France), she won the Young

Scientist FENS Award for Oral Communication, with her presentation 'Dietary intake and food source of omega-3 and omega-6 poly-unsaturated fatty acids in the Belgian population'. In the summer of 2007, she won the Prof. Dr. G. Verdonk Award for Dietetics presented by the Belgian Royal Academy for Medicine (period 2003-2006) with her work entitled 'Evaluation of benefits and risks related to seafood consumption'.

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