Fluorinated, Chlorinated and Brominated Contaminants in Fish for Human Consumption

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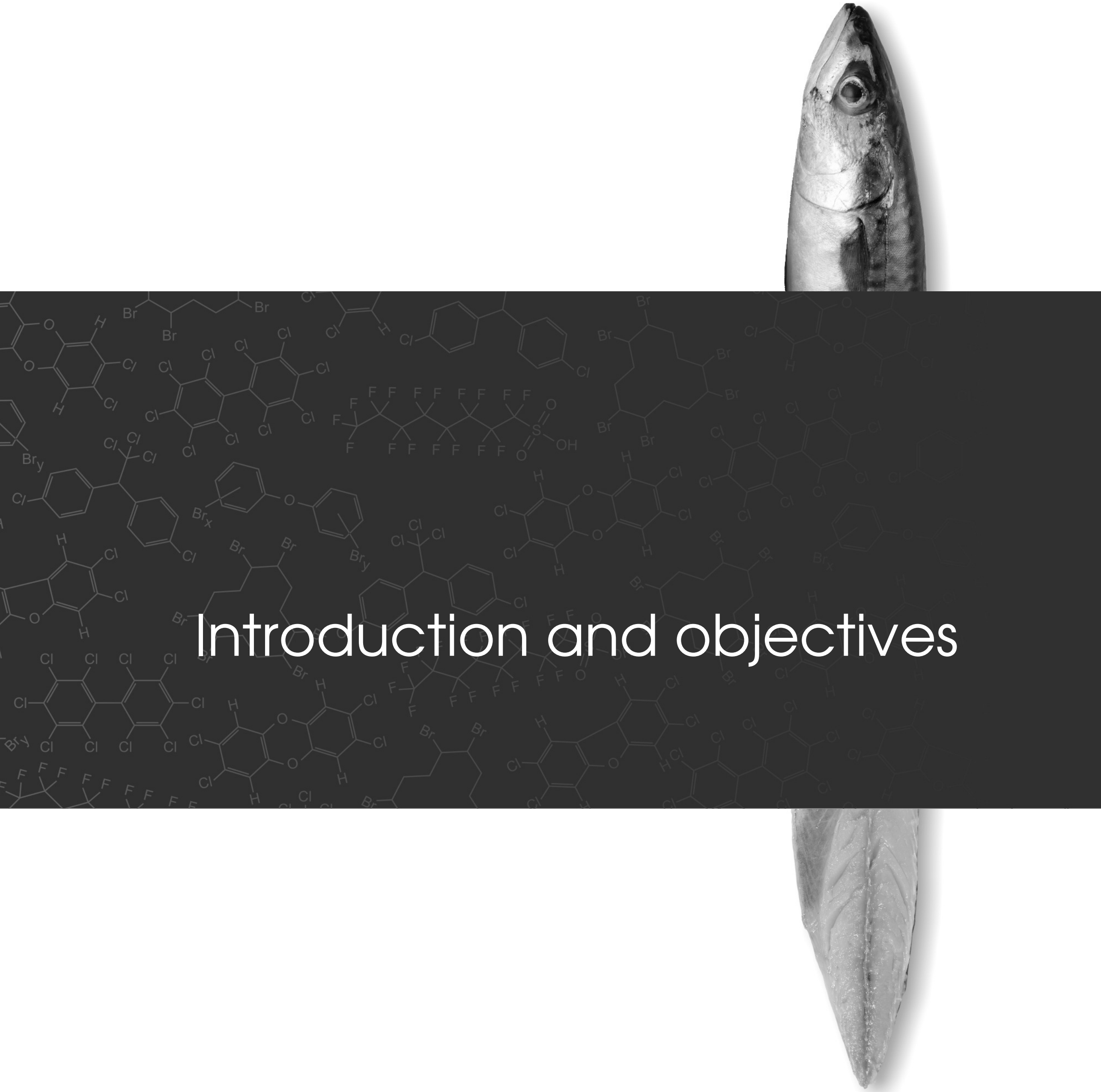
Chapter 1



Introduction and objectives

Introduction

Since the industrial revolution in the 19th century, the knowledge on chemistry has developed rapidly. Together with the strong development of several industries (i.e. petrochemical, pharmaceutical, etc.), the number of chemicals being applied grew to large numbers. In the framework of the EU REACH programme, over 100,000 compounds have been pre-registered[1] for use within the European Union (*1*). Chemicals find their way in all kinds of industrial applications and consumer products. They play an important role in the convenience, safety and wealth we enjoy everyday. Chemistry is everywhere around.

Most of the compounds and products are harmless. However, several compounds can do harm to wildlife and humans, some already at very low concentrations. Well known examples are polychlorinated biphenyls (PCBs), lindane, aldrin, dieldrin, endrin and DDT (*2-6*). These were produced and applied in the past, because of their useful properties as e.g. flame retardant or insecticide. However, their presence in the environment and humans is undesired because of specific toxic effects at very low concentrations. Therefore, and because of their persistent character, these compounds classified as "persistent organic pollutants" (POPs).

Persistent organic pollutants (POPs)

POPs were defined under the Stockholm Convention that entered into force in 2004 (*7*). POPs are compounds that are persistent, bioaccumulate, show long-range transportation and are toxic. The definitions are mentioned in the grey box below. POPs are resistant to degradation processes such as photolytic, chemical and biological degradation (their structure is not easy accessible for micro-organisms). Consequently, they remain intact and do not degrade under environmental conditions. Most POPs are lipophilic and their uptake rates in organisms are higher than the rate of depuration. This results in an accumulation in aquatic and terrestrial organisms and in humans (bioaccumulation). Further transfer up in the food chain can lead to elevated levels in top predators (biomagnification). These properties lead to continuous exposure to POPs. Their toxic properties can cause serious health effects such as certain cancers, birth defects, dysfunctional immune and reproductive systems, greater susceptibility to diseases and even diminished intelligence (*2,3,5,6,8-11*). Because their widespread use and aerial transport, the contamination with POPs has become a world-wide problem.

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[1] Pre-registration in the framework of the EU Reach program (Registration, Evaluation, Authorisation and restriction of Chemicals) is required for chemicals that are *substances* on their own, in preparations and those which are intentionally released from articles. Pre-registration stopped at 1 December 2008.

The group of POPs currently consist of the following 12 compounds: (i) eight chlorinated pesticides (dieldrin, endrin, aldrin, chlordane, heptachlor, DDT, mirex and toxaphene), (ii) two industrial chemicals (hexachlorobenzene (HCB) and polychlorinated biphenyls (PCBs)) and (iii) two unintentionally produced compounds (polychlorinated dibenzo-p-dioxins (PCDDs)), also abbreviated as dioxins, and polychlorinated dibenzofurans (PCDFs), also abbreviated as furans) (*12*). The structures are shown in Figure 1.1. These compounds (except dioxins and furans) were produced intentionally between the 1930s to the 1980s for use as insecticide or fungicide and as flame retardants in heat capacitors (see chapter 2.1 for more information on applications). POPs entered the environment during their production, use and after disposal. Dioxins and furans have never been produced intentionally, but result from incomplete combustions and are by-products of the production of certain pesticides and some other specific chemicals.

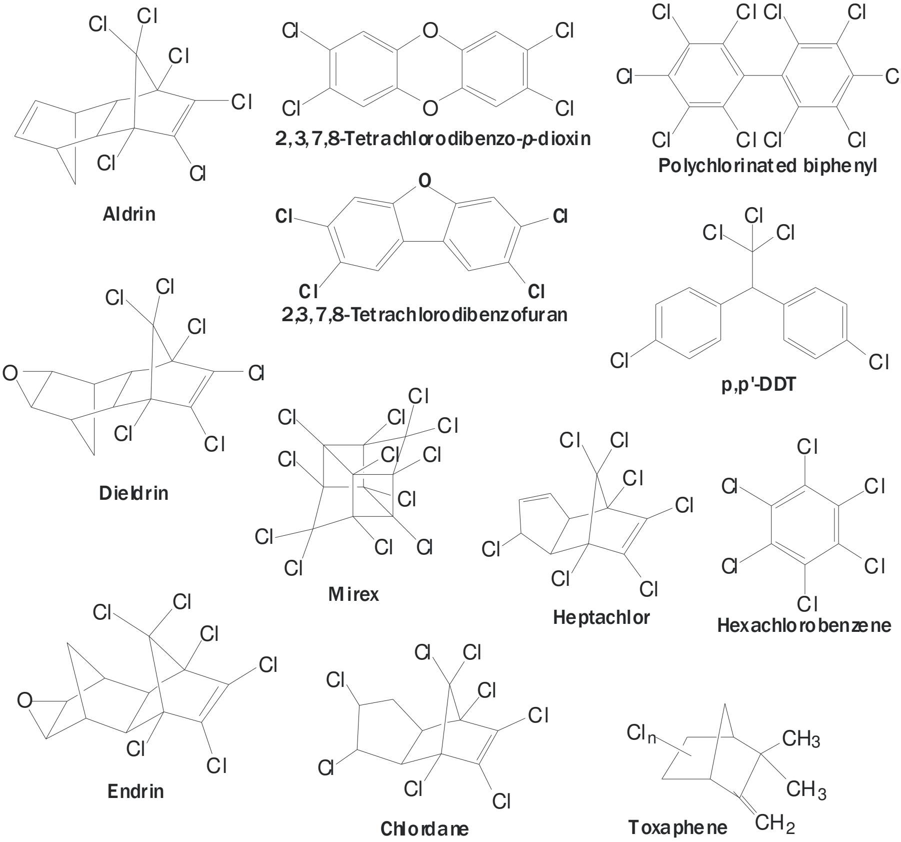


Figure 1.1 Chemical structures of the 12 persistent organic pollutants (POPs).

Stockholm Convention criteria for POPs (Annex D of (*7*)).

Persistence:

(i) Evidence that the half-life of the chemical in water is greater than two months, or that its half-life in soil is greater than six months, or that its half-life in sediment is greater than six months; or

(ii) Evidence that the chemical is otherwise sufficiently persistent to justify its consideration within the scope of this Convention;

Bio-accumulation:

(i) Evidence that the bio-concentration factor or bio-accumulation factor in aquatic species for the chemical is greater than 5,000 or, in the absence of such data, that the log Kow is greater than 5;

(ii) Evidence that a chemical presents other reasons for concern, such as high bio-accumulation in other species, high toxicity or ecotoxicity; or

(iii) Monitoring data in biota indicating that the bio-accumulation potential of the chemical is sufficient to justify its consideration within the scope of this Convention;

Potential for long-range environmental transport:

(i) Measured levels of the chemical in locations distant from the sources of its release that are of potential concern;

(ii) Monitoring data showing that long-range environmental transport of the chemical, with the potential for transfer to a receiving environment, may have occurred via air, water or migratory species; or

(iii) Environmental fate properties and/or model results that demonstrate that the chemical has a potential for long-range environmental transport through air, water or migratory species, with the potential for transfer to a receiving environment in locations distant from the sources of its release. For a chemical that migrates significantly through the air, its half-life in air should be greater than two days; and

Adverse effects:

(i) Evidence of adverse effects to human health or to the environment that justifies consideration of the chemical within the scope of this Convention; or

(ii) Toxicity or ecotoxicity data that indicate the potential for damage to human health or to the environment.

Countries and organisations that are bound to the Stockholm Convention take measures to reduce the environmental presence of these POPs. The reduction of emissions of these contaminants is achieved by two means: (i) reducing the emissions of *intentionally produced compounds* (aldrin, dieldrin, endrin, chlordane, DDT, heptachlor, hexachlorobenzene (HCB), mirex, PCBs and toxaphene), by terminating their production and use and (ii) by reducing the emissions of *unintentionally produced contaminants* (dioxins and furans) (*7*).

POPs in the environment and human food chain

POPs enter the environment through different routes. Figure 1.2 shows an example of the contamination of the environment with contaminants from point sources (e.g. production) and diffuse sources, and subsequent aerial, terrestrial and aquatic distribution.

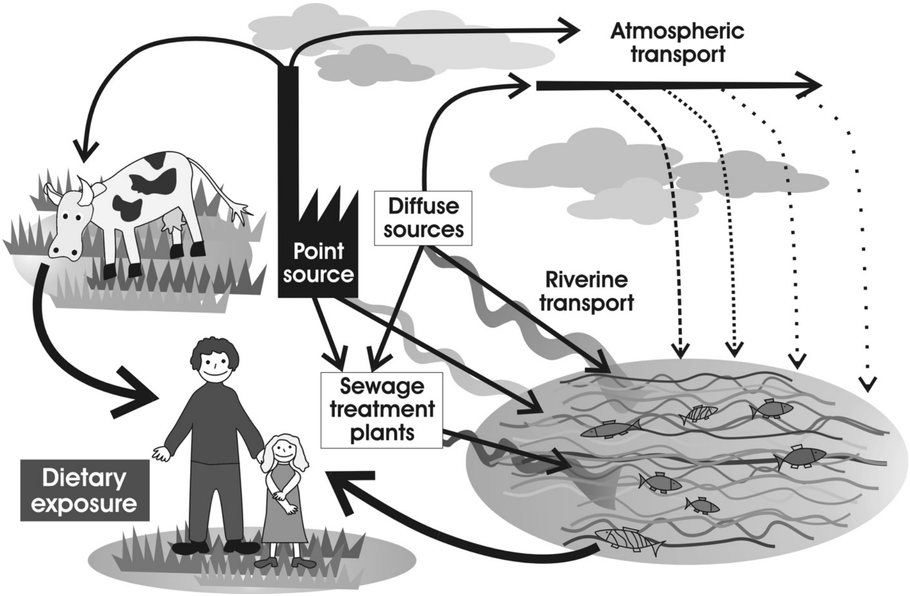


Figure 1.2 Contaminant emissions and typical aerial, terrestrial and aquatic distribution routes. The arrows pointing towards the humans indicate their dietary exposure through food from animal origin. Copyright A. Jahnke.

The emission routes that can be distinguished are:

- *Production or synthesis:* during the production (i.e. synthesis), POPs were emitted from the POP manufacturing plant through spillage or evaporation.

- *Application or product formulation:* POPs were never applied in the pure form, but were applied as an active ingredient in a formulated product. For example, PCBs were added to transformer oil to provide heat resistance to the oil (*2*). DDTs Technical DDT has been formulated in very diverse forms (e.g. emulsifiable concentrates, granules, smoke candles, charges for vaporizers, and lotions) (*6*). During this product formulation stage, POPs may have leached to the environment.
- *In-service life:* during the life-time of a product small amounts may have leached from the material to which they were applied. For example, small amounts of PCB containing transformer oil may have leaked from transformers (*2*). Because of snowfall and rainfall, DDT leached from the crops (and leafs) into surface water, soil and ground water (*6*).
- *Disposal:* after the in-service life has finished, products are disposed to waste incinerations and landfills. In some cases, PCBs have emitted from landfills through evaporation and leaching into soil and groundwater (*2*).

New POPs

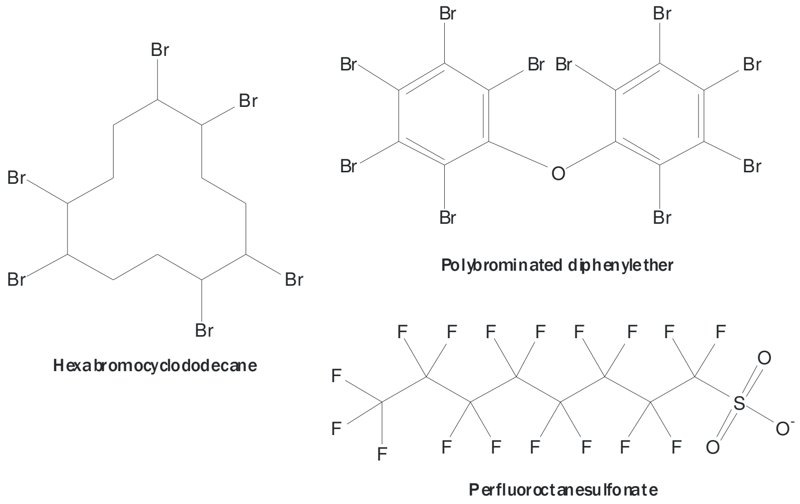
Following the ratification of the Stockholm Convention, parties took action in order to reduce the emissions of the 12 POPs. The production and use of POPs have substantially decreased (DDTs) or even completely stopped (most other POPs) in most countries. Also, the emission of dioxins was reduced in several countries e.g. by removing them from the flue gasses emitted from waste incinerators (*13*).

Unfortunately, several compounds have been produced in the last decades that also meet (some of) the persistency, bioaccumulative, long range transportation and toxicity criteria. In some cases, these have been produced as an alternative for a phased-out POP. Examples of these potential new POPs are brominated flame retardants (BFRs) such as polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD). They are produced because there is a need (and legal obligation) to make materials flame retardant. Another example is the surfactant perfluorooctane sulfonate (PFOS), which was applied in e.g. aqueous fire fighting foams (AFFF).

Because the Stockholm Convention aims at the decrease of the environmental and human exposure to POPs, new substances that fulfil the POP criteria can be proposed for inclusion in the POP list. After a process of evidence gathering and recommendation, the parties decide by voting on the inclusion of these "candidate POPs" in the official POP list. Other international organisations such as the United States Environmental Protection Agency (USEPA), Environment Canada and the European Chemicals Agency (ECHA) are also actively evaluating chemical substances. Some examples of compounds currently being evaluated are:

- *Polybrominated diphenyl ethers (PBDEs)* – This is an additive flame retardant that was applied in e.g polyurethane foams for cars and furniture, textiles, building materials and packaging (*14,15*). In the framework of the Stockholm Convention, the commercial *octabromo diphenyl ether* mixture was recently proposed for the risk management evaluation process (RMEP). This will result in a positive or negative recommendation for inclusion in the POP list (*16*). The commercial *pentabromo diphenyl ether* mixture is recommended for inclusion in the POP list aiming at elimination of its use (*17*).
- *Hexabromocyclododecane (HBCD)* – HBCD is an additive flame retardant applied (mostly) in polystyrene, but also in textile and upholstery (*18,19*). The European Chemicals Agency (ECHA) is evaluating HBCD as a substance of very high concern (SVHC). It was recently concluded that HBCD is a PBT substance (*20*). HBCD was recently proposed by Norway for inclusion in Annex A (elimination of production and use) under the Stockholm Convention (*21,22*).
- *Perfluorooctane sulfonate (PFOS)* – PFOS has excellent surfactant properties and was applied in aqueous fire fighting foams (AFFFs), as mist suppressant and as a water oil and stain repellent (*23*). In the early 2000's, a major PFOS producer voluntarily phased out the production of PFOS (*24*). Recently, PFOS is recommended for inclusion in the POP list of the Stockholm Convention, aiming at elimination (Annex A) or restriction of the use (Annex B) (*25*). The application of PFOS is restricted by 2008 in major applications, effectively resulting in a ban of its use in most applications (although in some cases (e.g. AFFFs) the use of PFOS is still allowed until 2011) (*26*).

Structures of PFOS, HBCD and PBDEs are shown in Figure 1.3.


Hexabromocyclododecane

Polybrominated diphenylether

Perfluoroctanesulfonate

Figure 1.3 Chemical structures of HBCD, PBDE and PFOS.

Dilemmas

The evaluation of POPs and candidate POPs and the restriction of their use are difficult processes. All pro's and con's of a substance should be carefully balanced. Clearly, on the one hand, emissions should be reduced as much as possible and production may have to be terminated. On the other hand, some of these chemicals help to save lives or have other important functionalities that cannot at once be replaced. For example, some BFRs that enter the environment result in environmental and human exposure and accumulation. This is an undesirable side-effect of the use of these compounds and could on the long term lead to health effects on organisms and humans (*27*). On the other hand, BFRs save over hundreds of lives world-wide each year by preventing products from rapidly catching fire (*28*). Another example is the dilemma of DDT. Every 30 seconds a child dies of malaria (*29*). DDT very effectively kills the malaria carrying mosquitoes and saves thousands of lives in Africa where malaria still isn't under control (*30*). On the other hand, DDT is found in every hidden corner of the world and has caused e.g. egg shell thinning of birds of prey (*31*). Furthermore, DDT showed to have *in vivo* and *in vitro* effects on the female reproductive tract of mammals, and was associated with e.g. pancreatic cancer and neuropsychological dysfunction (*32,33*).

As a final example, PFOS was applied as a surfactant in e.g. AFFFs (*23*). Due to the perfluorination of the molecule, PFOS has outstanding surface tension lowering properties. This makes the AFFF spread very rapidly over a fuel, thereby rapidly diminishing a fire by disclosing oxygen from the system. Alternatives have been developed, but these less effectively extinct liquid fires. PFOS enters the environment from fire events where the foam/water mixture leaches to the surface waters or sewage systems. Furthermore accidental and uncontrolled releases may result in environmental exposure. At Schiphol airport, PFOS was recently (summer 2008) released into the environment due to an accidental initiation of a fire sprinkler system (*34*). As a result, consumption of certain fish species that were caught in the receiving waters had to be restricted (*35*). Unfortunately, PFOS has several adverse effects, such as developmental effects (*36*), changes in thyroid hormone system (*37*) and high density lipoprotein levels (*36*). The liver is the major target organ for most effects (*36,38*).

The cases of PFOS, HBCD and PBDEs show that although a simple ban of the compounds would result in a rapid decrease of emissions to the environment, the needs for effective flame retardants, fire fighting foams and malaria insecticides remain. It is therefore very important that industries, policy makers, academia and research institutes jointly search for alternatives that are less harmful to the environment and humans, but offer an equal safety level as the original use substances. This dilemma was recognised by the European Commission. Recently, the ENFIRO project was launched in which industry, academia and research institutes together look for non-halogenated alternatives for BFRs. These alternatives will be evaluated in terms of flame retardency effectiveness and persistency, bioaccumulative and toxicological properties. In addition, (economic) feasibility of production will be regarded.

Human risk assessment of (candidate) POPs

POPs and candidate POPs are found in humans around the world. They have been detected in blood, organs, lipid depots, cord blood and mothers milk (*36,39-45*). A major route of exposure for POPs discussed above is through food (*38,46-52*). Other pathways are exposure from air, through dust ingestion and drinking water (*53-63*). For the traditional lipophilic POPs such as PCBs, organochlorinated pesticides and dioxins, fish is a dominant contributor to dietary exposure in most parts of Europe (*64,65*). In the Netherlands, because of the high consumption, dairy products are the predominant source. Fish is also in important source of dietary exposure (*47,51,52*). Because of the POP emission restriction measures, the emission of most POPs has been reduced substantially, which in turn led to a decrease of the levels in food, and decreased exposures (*51,52*). However, more than 30 years after the ban on PCB production in Europe, eel from the rivers Meuse and Rhine still contain PCB concentrations (*66,67*) that exceed Dutch maximum levels (MLs) (*68*). This shows the enormous impact of these POPs, once they have reached the environment.

The risks of exposure to POPs are evaluated in risk assessment processes. These typically consist of a hazard identification followed by hazard characterisation, exposure assessment and, finally, a risk characterisation (*69*). The *hazard identification* typically involves the identification of the contaminant and of the effects that are considered as adverse (*69*). The identification traditionally follows from in vivo (animal) experiments. However, with the aim of reducing animal experiments, other approaches gain more importance such as computational toxicology and in-vitro toxicology evaluation (*70,71*). The *hazard characterisation* describes the process of quantification of the relevant adverse effects. This is often referred to as the dose-response relationship. This results in benchmarks such as the no-observed-adverse-effect-level (NOAEL), which is the level of exposure of which the effects in the treated animals do not differ significantly from those in the untreated (control) animals. The *exposure assessment* aims at characterisation of the nature and size of the human population exposed to an emission source and the magnitude, frequency and duration of that exposure (*69*). Finally, *risk characterisation* relates to the estimation of the probability of the occurrence and the severity of adverse effects in a certain human population, based on the previous three stages by comparing the estimated exposure and the hazard characterisation (*69*).

Analytical challenges for the analysis of (candidate) POPs

For a reliable risk assessment, accurate exposure data are needed. The quality of an exposure assessment is determined by the quality of two groups of experimental input data: (i) the food item consumption data and (ii) the contaminant concentrations in these food items. In both cases, care should be taken that the data is representative. As regards food consumption, several Dutch National Food Consumption Surveys (DNFCS) have been carried out in which consumers were asked to record in detail their food and beverages consumed at two consecutive days. The DNFCS-3 (1997/1998) focussed on the population of 7-69 years, the DNFCS-Young adults (2003) focussed on the age of 19 to 30 years and the DNFCS-Young children (2005/2006) focussed on the age of 2-6 years (*72*).

For some POPs, accurate methods are available and various quality assurance tools are in place. This is the case for e.g. dioxins and dl-PCBs. Accurate methods are available as well as annually organised interlaboratory studies (*73-75*). Some certified reference materials (CRMs) are available, although there is a need for matrices with relevant concentration ranges (*76*). Because of the need for monitoring dioxins and dl-PCBs in food, the EU has established criteria for accurate analysis (*77*). The drawback of the analysis of dioxins and dl-PCBs is the extensive sample extraction and clean-up and the use of expensive equipment for analysis such as gas chromatography – high resolution mass spectrometry (GC-HRMS) (*78*). The major challenge for the analysis of these compounds is to reduce costs per analysis by improving the speed of extraction and clean-up and by introducing less expensive alternatives to GC-HRMS, while maintaining the same level of performance. The EU projects DIFFERENCE and DIAC (*79*) have shown that alternative methods (e.g. GC-ion trap MS/MS; comprehensive multidimensional GCxGC and CALUX bioassay) are available and can produce reliable results.

For PBDEs, many methods for analysis of fish have become available since the early 2000's (*78*). Methods for PBDEs in other food items are also available (*80,81*). Although the analysis of PBDEs may seem as 'straightforward' as that of PCBs, there are several issues may complicate the determination of some of these compounds. This includes problems with blanks, contamination of samples and degradation of higher brominated BDE congeners (*80*).

The analysis of HBCD is also challenging. HBCD consists of 3 (major) diastereomers (α-, β- and γ-HBCD). Initially, HBCD was analysed by GC, but the accuracy of the results was limited by HBCD degradation in the injector and column, different response factors of each diastereomer and the inability to separate the diastereomers on any GC column (*46,80*).

The class of poly- or perfluorinated compounds (PFCs) only received attention as a food contaminant during the last 3-4 years. PFCs are surfactants and accumulation is not lipid driven. Because they are not stored in lipids, they require different analytical techniques than the lipophilic compounds like dioxins, PCBs, PBDEs and HBCD. Further complicating factors are the diversity of this group of compounds due to different chain lengths of the apolar tail, different degrees of fluorination of the tail and different (polar) functional heads of the molecules (*82*). These differences result in a broad range of aqueous solubilities, which should preferably all be covered by a single method. Complicating factors are the absence of good quality (well-defined) standards, the absence of suitable internal standards, the presence of interferences, matrix effects and the lack of CRMs and interlaboratory studies (*83*). Although these issues have been solved partly in recent years (*84,85*) many analytical issues remain.

Scope and outline of this thesis

The work in this thesis focuses on human exposure assessment aspects, i.e. the assessment of the levels of environmental contaminants in foods. The focus is placed on fish, as in the past fish proved to an important contributor to the exposure to lipophilic compounds (*51,52,64,86-89*). Assessment of contaminant levels in fish requires the following steps to be taken:
- Development of specific, robust, precise and accurate methods of analysis;
- In-house and between laboratory validation of the analytical method;
- Sampling relevant fish species for chemical analysis;
- Determination of the contaminant levels in the fish samples.

In addition to the development of sound methods and the assessment of the contaminant levels in fish, the final part of this thesis deals with an estimation of human exposure to a broad suite of contaminants from wild fish and farmed fish in order to determine the relative importance of specific contaminants and fish species.

In other words, we have tried to answer the following questions:

(i) Can we develop methods for a suite of candidate POPs, which are reliable and sufficiently accurate to produce data for human exposure assessment?

(ii) Which contaminant (group) contributes predominantly to the exposure of the general Dutch population?

(iii) Which fish species contributes most to the exposure and which alternatives are available in order to reduce exposure?

This may provide answers to risk managers on where to put their focus on.

When breaking down to chapters, the reader will find the following information: In chapter 2, an overview is presented on *the current state-of-the-art of methods* for chemical analysis of contaminants. This includes both the traditional lipophilic contaminants (generally analysed by GC) as well as candidate POPs such as several BFRs and the more recently discovered surfactant type of contaminants (analysed by liquid chromatography). In chapter 3, *methods are described that were developed and validated* for the determination of PCDD/Fs, (dl-)PCBs, PBDEs, HBCD diastereomers and

PFCs in fish. In chapter 4, *contaminant levels in a wide range of wild fish, farmed fish, crustacea and shellfish* samples are presented. In addition, the *relevance of these contaminants for human exposure* is discussed. In chapter 5, *concluding remarks* are presented.

References

(1) ECHA. *List of pre-registered substances*. 2008, available at http://echa.europa.eu/

(2) WHO Environmental health criteria 140, Polychlorinated biphenyls and terphenyls (2nd ed.), 1993.

(3) WHO Environmental health criteria 130, Endrin, 1992.

(4) WHO Environmental health criteria 124, Lindane, 1991.

(5) WHO Environmental health criteria 91, Andrin and Dieldrin, 1989.

(6) WHO Environmental health criteria 9, DDT, 1979.

(7) Stockholm convention on Persistent Organic Pollutants, 2001, available at http://chm.pops.int.

(8) WHO Environmental health criteria 195, Hexachlorobenzene, 1997.

(9) WHO Environmental health criteria 88, Polychlorinated dibenzo-para-dioxins and dibenzofurans, 1989.

(10) WHO Environmental health criteria 34, Chlordane, 1984.

(11) WHO Environmental health criteria 45, Camphechlor, 1984.

(12) Fiedler, H. Persistent organic pollutants - chemical identity and properties *European Journal of Lipid Science and Technology*. 2000, *102*, 45-49.

(13) Quass, U.; Fermann, M.; Broker, G. The European dioxin air emission inventory project - Final results *Chemosphere*. 2004, *54*, 1319-1327.

(14) Prevedouros, K.; Jones, K. C.; Sweetman, A. J. Estimation of the production, consumption, and atmospheric emissions of pentabrominated diphenyl ether in Europe between 1970 and 2000 *Environmental Science & Technology*. 2004, *38*, 3224-3231.

(15) Law, R. J.; Allchin, C. R.; de Boer, J.; Covaci, A.; Herzke, D.; Lepom, P.; Morris, S.; Tronczynski, J.; De Wit, C. A. Levels and trends of brominated flame retardants in the European environment *Chemosphere*. 2006, *64,* 187-208.

(16) UNEP, Consideration of chemicals newly proposed for inclusion in Annexes A, B or C of the Convention: octabromodiphenyl ether, 2006, available at http://chm.pops.int.

(17) UNEP. *Commercial Pentabromodiphenyl Ether - Risk Management Evaluation*, 2007, available at http://chm.pops.int.

(18) Covaci, A.; Gerecke, A. C.; Law, R. J.; Voorspoels, S.; Kohler, M.; Heeb, N. V.; Leslie, H.; Allchin, C. R.; de Boer, J. Hexabromocyclododecanes (HBCDs) in the environment and humans: A review *Environmental Science & Technology*. 2006, *40,* 3679-3688.

(19) Law, R. J.; Kohler, M.; Heeb, N. V.; Gerecke, A. C.; Schmid, P.; Voorspoels, S.; Covaci, A.; Becher, G.; Janak, K.; Thomsen, C. Hexabromocyclododecane challenges scientists and regulators *Environmental Science & Technology*. 2005, *39,* 281A-287A.

(20) ECHA Member state committee support document for identification of hexabromocyclododecane and all major diastereoisomers identified as a substance of very high concern; 2008. available at http://echa.europa.eu/.

(21) Royal Ministry of the Environment of Norway. *Proposal to add a new substance to the Stockholm Convention on Persistent Organic Pollutants (POPs)*, 2008, available at http://chm.pops.int.

(22) Themanord. *Hexabromocyclododecane as a possible global POP*, report nr. 520, 2008, available at http://chm.pops.int.

(23) Kissa, E. *Fluorinated Surfactants and Repellents*, 2 ed., Marcel Dekker, New York, 2001

(24) Wood, A.; Clarin, W. 3M to phase out PFOS *Chemical week*. 2000, *162,* 9.

(25) UNEP. *Perfluorooctane sulfonate- Risk Management Evaluation.* 2007, available at http://chm.pops.int.

(26) EC Directive 2006/122/EC of the European Parliament and of the Council of 12 December 2006 amending for the 30th time Council Directive 76/769/EEC on the approximation of the laws, regulations and administrative provisions of the Member States relating to restrictions on the marketing and use of certain dangerous substances and preparations (perfluorooctane sulfonates) *Official Journal of the European Union*. 2006, L372, 32-34.

(27) Vos, J. G.; Becher, G.; Van den Berg, M.; de Boer, J.; Leonards, P. E. G. Brominated flame retardants and endocrine disruption *Pure and Applied Chemistry*. 2003, *75,* 2039-2046.

(28) Bromine Science and Environmental Forum. *Fire safety Benefits.* Information obtained from http://www.bsef.com/. 2008.

(29) WHO, *Global Malaria Program*. 2006. Geneva. http://apps.who.int/malaria/.

(30) WHO, Indoor residual spraying - Use of indoor residual spraying for scaling up global malaria control and elimination -WHO Position Statement; Geneva, 2006. Available at http://apps.who.int/malaria/.

(31) Vos, J. G.; Dybing, E.; Greim, H. A.; Ladefoged, O.; Lambre, C.; Tarazona, J. V.; Brandt, I.; Vethaak, A. D. Health effects of endocrine-disrupting chemicals on wildlife, with special reference to the European situation *Critical Reviews in Toxicology*. 2000, *30,* 71-133.

(32) Tiemann, U. In vivo and in vitro effects of the organochlorine pesticides DDT, TCPM, methoxychlor, and lindane on the female reproductive tract of mammals: A review *Reproductive Toxicology*. 2008, *25,* 316-326.

(33) Beard, J. DDT and human health *Science of the Total Environment*. 2006, *355,* 78-89.

(34) 100 miljoen liter vervuild water Schiphol opgeslagen. Haarlems Dagblad. 23-7-2008, in Dutch.

(35) Voedsel en Waren Autoriteit. Advies inzake PFOS in vis afkomstig uit Rijnland, 2008, in Dutch.

(36) Lau, C.; Anitole, K.; Hodes, C.; Lai, D.; Pfahles-Hutchens, A.; Seed, J. Perfluoroalkyl acids: A review of monitoring and toxicological findings *Toxicological Sciences*. 2007, *99,* 366-394.

(37) Weiss, M. J.; Andersson, P. L.; Lamoree, M. H.; Leonards, P. E. G.; van Leeuwen, S. P. J.; Hamers, T. Competitive Binding of Poly- and Perfluorinated Compounds to the Thyroid Hormone Transport Protein Transthyretin *Toxicological Sciences*. 2009, *109*, 206-216.

(38) EFSA Perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and their salts - Scientific opinion of the panel on contaminants in the food chain *The EFSA journal*. 2008, *653,* 1-131.

(39) Karrman, A.; Van Bavel, B.; Jarnberg, U.; Hardell, L.; Lindstrom, G. Perfluorinated chemicals in relation to other persistent organic pollutants in human blood *Chemosphere*. 2006, *64,* 1582-1591.

(40) Srogi, K. Levels and congener distributions of PCDDs, PCDFs and dioxin-like PCBs in environmental and human samples: a review *Environmental Chemistry Letters*. 2008, *6,* 1-28.

(41) De Felip, E.; Abballe, A.; Casalino, F.; di Domenico, A.; Domenici, P.; Iacovella, N.; Ingelido, A. M.; Pretolani, E.; Spagnesi, M. Serum levels of PCDDs, PCDFs and PCBs in non-occupationally exposed population groups living near two incineration plants in Tuscany, Italy *Chemosphere*. 2008, *72,* 25-33.

(42) Stapleton, H. M.; Sjodin, A.; Jones, R. S.; Niehoser, S.; Zhang, Y.; Patterson, D. G. Serum levels of polyhrominated diphenyl ethers (PBDEs) in foam recyclers and carpet installers working in the united states *Environmental Science & Technology*. 2008, *42,* 3453-3458.

(43) Spliethoff, H. M.; Tao, L.; Shaver, S. M.; Aldous, K. M.; Pass, K. A.; Kannan, K.; Eadon, G. A. Use of Newborn Screening Program blood spots for exposure assessment: Declining levels of perfluorinated compounds in New York State infants *Environmental Science & Technology*. 2008, *42,* 5361-5367.

(44) Leijs, M. M.; van Teunenbroek, J.; Olie, K.; Koppe, J. G.; ten Tusscher, G. W.; van Aalderen, W. M. C.; de Voogt, P. Assessment of current serum levels of PCDD/Fs, dl-PCBs and PBDEs in a Dutch cohort with known perinatal PCDD/F exposure *Chemosphere*. 2008, *73,* 176-181.

(45) Monroy, R.; Morrison, K.; Teo, K.; Atkinson, S.; Kubwabo, C.; Stewart, B.; Foster, W. G. Serum levels of perfluoroalkyl compounds in human maternal and umbilical cord blood samples *Environmental Research*. 2008, *108,* 56-62.

(46) van Leeuwen, S. P. J.; de Boer, J. Brominated flame retardants in fish and shellfish - levels and contribution of fish consumption to dietary exposure of Dutch citizens to HBCD *Molecular Nutrition & Food Research*. 2008, *52,* 194-203.

(47) Bakker, M. I.; De Winter-Sorkina, R.; De Mul, A.; Boon, P. E.; van Donkersgoed, G.; van Klaveren, J. D.; Baumann, B. A.; Hijman, W. C.; van Leeuwen, S. P. J.; de Boer, J.; Zeilmaker, M. J. Dietary intake and risk evaluation of polybrominated diphenyl ethers in The Netherlands *Molecular Nutrition & Food Research*. 2008, *52,* 204-216.

(48) Lorber, M. Exposure of Americans to polybrominated diphenyl ethers *Journal of Exposure Science and Environmental Epidemiology*. 2008, *18,* 2-19.

(49) Midasch, O.; Schettgen, T.; Angerer, J. Pilot study on the perfluorooctanesulfonate and perfluorooctanoate exposure of the German general population *International Journal of Hygiene and Environmental Health*. 2006, *209,* 489-496.

(50) Trudel, D.; Horowitz, L.; Wormuth, M.; Scheringer, M.; Cousins, I. T.; Hungerbuhler, K. Estimating consumer exposure to PFOS and PFOA *Risk Analysis*. 2008, *28,* 251-269.

(51) Baars, A. J.; Bakker, M. I.; Baumann, R. A.; Boon, P. E.; Freijer, J. I.; Hoogenboom, L. A. P.; Hoogerbrugge, R.; van Klaveren, J. D.; Liem, A. K. D.; Traag, W. A.; de Vries, J. Dioxins, dioxin-like PCBs and non-dioxin-like PCBs in foodstuffs: occurrence and dietary intake in The Netherlands *Toxicology Letters*. 2004, *151,* 51-61.

(52) De Mul, A.; Bakker, M. I.; Zeilmaker, M. J.; Traag, W. A.; van Leeuwen, S. P. J.; Hoogenboom, R. L. A. P.; Boon, P. E.; van Klaveren, J. D. Dietary exposure to dioxins and dioxin-like PCBs in The Netherlands anno 2004 *Regulatory toxicology and pharmacology*. 2008, *51,* 278-287.

(53) Moriwaki, H.; Takata, Y.; Arakawa, R. Concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) in vacuum cleaner dust collected in Japanese homes *Journal of Environmental Monitoring*. 2003, *5,* 753-757.

(54) Shoeib, M.; Harner, T.; Wilford, B. H.; Jones, K. C.; Zhu, J. P. Perfluorinated sulfonamides in indoor and outdoor air and indoor dust: Occurrence, partitioning, and human exposure *Environmental Science & Technology*. 2005, *39,* 6599-6606.

(55) Skutlarek, D.; Exner, M.; Farber, H. Perfluorinated surfactants in surface and drinking water *Environmental Science and Pollution Research*. 2006, *13,* 299-307.

(56) Abdallah, M. A. E.; Harrad, S.; Ibarra, C.; Diamond, M.; Melymuk, L.; Robson, M.; Covaci, A. Hexabromocyclododecanes in indoor dust from Canada, the United Kingdom, and-the United States *Environmental Science & Technology*. 2008, *42,* 459-464.

(57) Wanner, A.; Heitmann, D.; Fromme, H.; Korner, W. Polybrominated diphenyl ether (PBDE) in indoor air and house dust from homes in Southern Bavaria *Gefahrstoffe Reinhaltung der Luft*. 2008, *68,* 71-77.

(58) Takagi, S.; Adachi, F.; Miyano, K.; Koizumi, Y.; Tanaka, H.; Mimura, M.; Watanabe, I.; Tanabe, S.; Kannan, K. Perfluorooctanesulfonate and perfluorooctanoate in raw and treated tap water from Osaka, Japan *Chemosphere*. 2008, *72,* 1409-1412.

(59) Allen, J. G.; McClean, M. D.; Stapleton, H. M.; Webster, T. F. Critical factors in assessing exposure to PBDEs via house dust *Environment International*. 2008, *34,* 1085-1091.

(60) Barber, J. L.; Berger, U.; Chaemfa, C.; Huber, S.; Jahnke, A.; Temme, C.; Jones, K. C. Analysis of per- and polyfluorinated alkyl substances in air samples from Northwest Europe *Journal of Environmental Monitoring*. 2007, *9,* 530-541.

(61) Heinzow, B.; Mohr, S.; Ostendorp, G.; Kerst, M.; Korner, W. PCB and dioxin-like PCB in indoor air of public buildings contaminated with different PCB sources - deriving toxicity equivalent concentrations from standard PCB congeners *Chemosphere*. 2007, *67,* 1746-1753.

(62) Wu, N.; Herrmann, T.; Paepke, O.; Tickner, J.; Hale, R.; Harvey, E.; La Guardia, M.; McClean, M. D.; Webster, T. F. Human exposure to PBDEs: Associations of PBDE body burdens with food consumption and house dust concentrations *Environmental Science & Technology*. 2007, *41,* 1584-1589.

(63) Harrad, S.; Hazrati, S.; Ibarra, C. Concentrations of polychlorinated biphenyls in indoor air and polybrominated diphenyl ethers in indoor air and dust in Birmingham, United Kingdom: Implications for human exposure *Environmental Science & Technology*. 2006, *40,* 4633-4638.

(64) Domingo, J. L.; Bocio, A. Levels of PCDD/PCDFs and PCBs in edible marine species and human intake: A literature review *Environment International*. 2007, *33,* 397-405.

(65) Gomara, B.; Bordajandi, L. R.; Fernandez, M. A.; Herrero, L.; Abad, E.; Abalos, M.; Rivera, J.; Gonzalez, M. J. Levels and trends of polychlorinated dibenzo-p-dioxins/furans (PCDD/Fs) and dioxin-like polychlorinated biphenyls (PCBs) in Spanish commercial fish and shellfish products, 1995-2003 *Journal of Agricultural and Food Chemistry*. 2005, *53,* 8406-8413.

(66) van Leeuwen, S. P. J.; Pieters, H.; de Boer, J. *Monitoring zware metalen en organische microverontreinigingen in Nederlandse visserijproducten*; Report nr C032/06; Netherlands Institute for Fisheries Research (RIVO): IJmuiden, The Netherlands, 2006, in Dutch.

(67) van Leeuwen, S. P. J.; Leonards, P. E. G.; Traag, W. A.; Hoogenboom, L. A. P.; de Boer, J. Polychlorinated dibenzo-p-dioxins, dibenzofurans and biphenyls in fish from the Netherlands: concentrations, profiles and comparison with DR CALUX (R) bioassay results *Analytical and Bioanalytical Chemistry*. 2007, *389,* 321-333.

(68) Regeling normen PCBs (Warenwet) *Staatscourant, 239.* 1984. In Dutch.

(69) EC- Health & Consumer Protection Directorate-General. First report on the harmonisation of risk assessment procedures. 2000.

(70) Harju, M.; Hamers, T.; Kamstra, J. H.; Sonneveld, E.; Boon, J. P.; Tysklind, M.; Andersson, P. L. Quantitative structure-activity relationship modeling on in vitro endocrine effects and metabolic stability involving 26 selected brominated flame retardants *Environmental Toxicology and Chemistry*. 2007, *26,* 816-826.

(71) Hamers, T.; Kamstra, J. H.; Sonneveld, E.; Murk, A. J.; Kester, M. H. A.; Andersson, P. L.; Legler, J.; Brouwer, A. In vitro profiling of the endocrine-disrupting potency of brominated flame retardants *Toxicological Sciences*. 2006, *92,* 157-173.

(72) The Dutch National Food Consumption Survey 2005/2006. Available at http://www.rivm.nl/vcp/.

(73) Haug, L. S.; Becher, G. *Interlaboratory Comparison on Dioxins in Food 2005 Sixth Round of an International Study*; Report nr. 2005:7; Norwegian Institute of Public Health, Oslo, Sweden. 2005.

(74) http://www.quasimeme.org.

(75) Liem, A. K. D.; Theelen, R. M. C. University of Utrecht, *Dioxins: chemical analysis, exposure and risk assessment*, PhD Thesis, University of Utrecht, Utrecht, The Netherlands, 1997.

(76) van Leeuwen, S. P. J.; Van Cleuvenbergen, R.; Abalos, M.; Pasini, A. L.; Eriksson, U.; Cleemann, M.; Hajslova, J.; de Boer, J. New certified and candidate certified reference materials for the analysis of PCBs, PCDD/Fs, OCPs and BFRs in the environment and food *Trac-Trends in Analytical Chemistry*. 2006, *25,* 397-409.

(77) EC Commission Regulation (EC) No 1883/2006 of 19 December 2006 laying down methods of sampling and analysis for the official control of levels of dioxins and dioxin-like PCBs in certain foodstuffs *Official Journal of the European Communities*. 2009, *49, L364,* 32-43.

(78) van Leeuwen, S. P. J.; de Boer, J. Advances in the gas chromatographic determination of persistent organic pollutants in the aquatic environment *Journal of Chromatography A*. 2008, *1186,* 161-182.

(79) http://www.dioxins.nl/.

(80) de Boer, J.; Wells, D. E. Pitfalls in the analysis of brominated flame retardants in environmental, human and food samples - including results of three international interlaboratory studies *Trac-Trends in Analytical Chemistry*. 2006, *25,* 364-372.

(81) de Boer, J. Contaminants in food - Brominated flame retardants *Molecular Nutrition & Food Research*. 2008, *52,* 185-186.

(82) de Voogt, P.; Saez, M. Analytical chemistry of perfluoroalkylated substances *Trac-Trends in Analytical Chemistry*. 2006, *25,* 326-342.

(83) Martin, J. W.; Kannan, K.; Berger, U.; de Voogt, P.; Field, J.; Franklin, J.; Giesy, J. P.; Harner, T.; Muir, D. C. G.; Scott, B.; Kaiser, M.; Jarnberg, U.; Jones, K. C.; Mabury, S. A.; Schroeder, H.; Simcik, M.; Sottani, C.; Van Bavel, B.; Karrman, A.; Lindstrom, G.; van Leeuwen, S. Analytical challenges hamper perfluoroalky research *Environmental Science & Technology*. 2004, *38,* 248A-255A.

(84) van Leeuwen, S. P. J.; Karrman, A.; Van Bavel, B.; de Boer, J.; Lindstrom, G. Struggle for quality in determination of perfluorinated contaminants in environmental and human samples *Environmental Science & Technology*. 2006, *40,* 7854-7860.

(85) van Leeuwen, S. P. J.; Swart, C. P.; van der Veen, I.; de Boer, J. Significant improvements in the analysis of perfluorinated compounds in water and fish: Results from an interlaboratory method evaluation study *Journal of Chromatography A*. 2009, *1216,* 401-409.

(86) Llobet, J. M.; Falco, G.; Bocio, A.; Domingo, J. L. Human exposure to polychlorinated naphthalenes through the consumption of edible marine species *Chemosphere*. 2007, *66,* 1107-1113.

(87) Llobet, J. M.; Bocio, A.; Domingo, J. L.; Teixido, A.; Casas, C.; Muller, L. Levels of polychlorinated biphenyls in foods from Catalonia, Spain: Estimated dietary intake *Journal of Food Protection*. 2003, *66,* 479-484.

(88) Marti-Cid, R.; Llobet, J. M.; Castell, V.; Domingo, J. L. Human dietary exposure to hexachlorobenzene in Catalonia, Spain *Journal of Food Protection*. 2008, *71,* 2148-2152.

(89) Marti-Cid, R.; Bocio, A.; Llobet, J. M.; Domingo, J. L. Intake of chemical contaminants through fish and seafood consumption by children of Catalonia, Spain: Health risks *Food and Chemical Toxicology*. 2007, *45,* 1968-1974.