Organohalogen contaminants in Greenland shark *(Somniosus microcephalus)*

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Abstract

The remote sub-Arctic/Arctic environment has due to human activities become a sink for organohalogen contaminants (OHCs). These OHC include traditional contaminants such as polychlorinated biphenyls (PCBs), DDTs and technical mixtures of polybrominated diphenyl ethers (PBDEs), all included in the Stockholm Convention list of persistent organic pollutants (POPs). Other OHCs, currently under evaluation to be included among the POPs i.e. short chain chlorinated paraffins (SCCPs) and hexabromocyclododecane (HBCDD) are also found in these environments as well as a whole range of other OHCs.
The main objective of this thesis is to increase the knowledge about the presence of OHCs in a high trophic Arctic shark species, the Greenland shark (*Somniosus microcephalus*). The Greenland shark is an opportunistic feeder, occasionally feeding at the top of the Arctic marine food chain. Furthermore may this species have a life span in excess of 100 years and is probably among the oldest of any fish species. These traits make the shark prone to accumulate elevated concentrations of OHCs.

This has shown to be true for the Greenland sharks studied and most of the targeted OHCs were determined in the species. The highest concentrations were observed for the DDTs, ranging up to 26 μg/g fat. Other OHCs reported that are of special interest are SCCPs and brominated flame retardants used as replacement products to PBDEs; pentabromoethylbenzene (PBEB) and 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE). Also a range of OHCs whose origin is assumed to be natural, were shown to be present in Greenland sharks.

This thesis is stressing the fact that even though the use of certain OHCs has been banned for decades they are still present at high concentrations in the deep waters of the Arctic. Therefore it is of major importance to continue to monitor the fate of traditional and emerging OHCs in the environment, and for this purpose the Greenland shark is an excellent species.

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List of papers

This thesis is based on the following four papers, which are referred to in the text by their Roman numerals. The two published articles are reproduced here with the kind permission of the publisher. Some unpublished results are also included in the thesis.

I. Dioxins and PCBs in Greenland shark *(Somniosus microcephalus)* from the North-east Atlantic.
Anna Strid, Hrönn Jörundsdottír, Olaf Päpke, Jörundur Svavarsson and Åke Bergman. (2007). Marine Pollution Bulletin, 54, 1514-1522.

II. Neutral and phenolic brominated organic compounds of natural and anthropogenic origin in North-east Atlantic Greenland shark *(Somniosus microcephalus.)*.
Anna Strid, Ioannis Athanassiadis, Maria Athanasiadou, Jörundur Svavarsson and Åke Bergman. (2010). Environmental Toxicology and Chemistry, 29, 2653-2659.

III. Brominated and chlorinated flame retardants in liver of Greenland shark *(Somniosus microcephalus)*.
Anna Strid, Christoffer Bruhn, Ed Sverko, Jörundur Svavarsson, Gregg Tomy and Åke Bergman. *Manuscript*.

IV. Selected organochlorine pesticides, PCBs and their methyl sulfonyl metabolites in Greenland shark (*Somniosus microcephalus*).
Anna Strid, Jörundur Svavarsson and Åke Bergman. *Manuscript*.

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Abbreviations

AMAP	Arctic Monitoring and Assessment Programme
ASE	Accelerated solvent extraction
BFR	Brominated flame retardant
BTBPE	1,2-Bis(2,4,6-tribromophenoxy)ethane
CPs	Chlorinated paraffins
DBP	Dimethyl bipyrrol
4,4'-DDD	1,1-dichloro-2,2-bis(4-chlorophenyl) ethane
4,4'-DDE	1,1-dichloro-2,2-bis(4-chlorophenyl) ethene
4,4'-DDT	1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane
diMeO-BB	Dimethoxylated brominated biphenyl
diMeO-BDE	Dimethoxylated brominated diphenyl ether
DL	Dioxin-like
DMSO	Dimethyl sulfoxide
ECD	Electron capture detector
ECNI	Electron capture negative ionization
EI	Electron ionization
GC	Gas chromatography
GPC	Gel permeation chromatography
HBCDD	Hexabromocyclododecane
HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane
HPLC	High performance liquid chromatography
HRMS	High resolution mass spectrometry
IUCN	International Union for the Conservation of Nature
KOH	Potassium hydroxide
LCCP	Long chain chlorinated paraffin
LOD	Limit of detection
LOQ	Limit of quantification
LRMS	Low resolution mass spectrometry
MAE	Microwave assisted extraction
MBP	Methyl bipyrrol
MCCP	Medium chain chlorinated paraffin
MeO-PBDEs	Methoxylated polybrominated diphenylethers
$MeSO_2$-PCBs	Methylsulfonyl-PCBs
MRM	Multiple reaction monitoring
OHCs	Organohalogen contaminants
OH-PBDEs	Hydroxylated polybrominated diphenyl ethers
OH-PCBs	Hydroxylated polychlorinated biphenyls
PBDEs	Polybrominated diphenyl ethers

PBEB	Pentabromoethylbenzene
PCBs	Polychlorinated biphenyls
PCDDs	Polychlorinated dibenzo-*p*-dioxins
PCDFs	Polychlorinated dibenzo furans
PFCs	Polyfluorinated compounds
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonate
PFOSA	Perfluorooctanesulfonamide
PLE	Pressurized liquid extraction
POP	Persistent organic pollutant
SCCP	Short chain chlorinated paraffin
SIM	Selected ion monitoring
TBP	Tribromophenol
TEF	Toxic equivalent factor
TEQ	Toxic equivalent
WHO	World Health Organization
w.w.	Wet weight
QA	Quality assurance
QC	Quality control

1 Introduction

Organohalogen contaminants (OHCs) existing for instance as industrial chemicals, pesticides or technical by-products, enter the environment in different ways. Industrial chemicals and pesticides may be released during their production, usage of products they are incorporated into and also at their disposal. Technical by-products may be formed and released during industrial activities and through incomplete incineration processes [1]. These human activities all lead to a constant input of OHCs in marine environments world wide where they, due to high lipophilicity and chemical stability, bioaccumulate in the food webs. In addition, natural compounds, with structural resemblance to anthropogenic OHCs, are also present in the marine environments [2]. These naturally occurring OHCs have also been shown to accumulate in the marine food chains. Elevated levels of OHCs are not only present in marine environments linked to industrialized areas, but also in remote areas such as sub-Arctic and Arctic regions where the input from local sources is minimal. OHCs are instead, depending on their chemical properties, transported to these remote areas mainly via long-range transport.

Wildlife (mussels, fish and bird eggs) from the Baltic Sea, one of the most contaminated marine environments globally, has been collected annually within the national Swedish contaminant programme for monitoring of OHCs since the late 1960s. This program is aimed to assess levels of contaminants, monitor long term time trends, spatial differences and also to monitor responses of measures already taken to reduce discharges of OHCs [3]. Concentrations of polychlorinated biphenyls (PCBs) have shown to decrease with 5-10% annually in for instance herring (*Clupea harengus)*, cod (*Gadus morhua*) and guillemot (*Uria aalge*) eggs since the seventies. Decreasing trends have also been observed for hexachlorobenzene (HCB) and hexachlorocyclohexanes (HCHs) [3]. Concentrations of polychlorinated dibenzo-*p*-dioxins (PCDDs) and furans (PCDFs) have shown decreasing trends in guillemot eggs until the mid eighties but have since then levelled of. However, no changes of the PCDD/F concentrations were observed in herring between 1990 and 2007 [3]. Time trends for polybrominated diphenyl ethers (PBDEs) and hexabromo-cyclododecane (HBCDD), examples of brominated flame retardants (BFRs), are also available showing decreasing trends for PBDEs since the mid nineties and increasing trends for HBCDD in guillemot eggs (3 % per year) between 1968 and 2007 [3]. Monitoring of OHCs in the Baltic is also important from a human exposure point of view since several fish species are sold commercially.

Data on OHCs as gathered by the Arctic Monitoring and Assessment Programme (AMAP) are presented to inform the governments of the eight

Arctic countries on current or potential threats caused by pollution and also to advice on possible actions. The first assessment was published in 1998 and the second in 2004 [4,5]. Several reviews have been published thereafter with up-dates, on for instance BFRs, exposure and effect assessments and temporal trends [6-9]. Elevated concentrations of certain OHCs are frequently reported in high trophic Arctic species and related to biological effects, such as marine mammals and birds, as recently reviewed by Letcher et al. 2010 [7]. It was concluded that data on levels and possible OHC mediated effects in Arctic fish were scarce.

Sharks are cartilaginous fish that together with rays form the subclass elasmobranches, which first appeared 400 million years ago. The number of now existing shark species is about 400, inhabiting all niches in the marine environment [10,11]. Sharks as a group is considered to be tertiary consumers, i.e. feeding at the top of the marine food chains, where they most likely play an important role affecting the structure and species composition [10,12]. In general are sharks very long lived, piked dogfish (*Squalus acanthias*) are known to live at least 30 years and possibly up to 100 years and the Greenland shark (*Somniosus microcephalus*) may have a life span in excess of 100 years [13,14]. Long lived species also reach maturity late, one example being the dusky shark (*Carcharhinus obscurus*) that reaches maturity at around 20 years [15]. Many species are so called “K-selected” meaning that being top predators they have few natural enemies and hence need to produce few young to maintain the population levels [10]. This means that population declines caused by overfishing or other factors may have a severe impact on such populations. Characteristics like long life spans and being top predators are also the basis for the ability to accumulate high levels of OHCs, possibly making them useful as monitoring species of marine pollutants. High concentrations of OHCs might also put extra stress on these already exposed species, i.e. causing effects on health and reproduction. Nothing is however hitherto known about any possible OHC mediated effects in sharks.

The Greenland shark, the main species of this thesis, is described together with three other shark species in the following sections (Chapter 1.1 and 1.2).

1.1 The Greenland shark (*Somniosus microcephalus*)

The Greenland shark *(Somniosus microcephalus)* (Figure 1.1) is the largest species within the Somniosidae family (sleeper sharks) and is also one of the largest shark species in the world [16]. Maximum total length may be up to 7.3 meters but most adults are between 2.4 and 4.3 meters. Females usually grow larger than the males [13,17]. This is foremost an Arctic species, present in areas seasonally covered by sea-ice, but is also known to move into more temperate waters in the North Atlantic [17,18].

Figure 1.1. A photo of two Greenland sharks (*Somniosus microcephalus*) collected from Icelandic waters in the North Atlantic. Photo: Jörundur Svavarsson.

The Greenland shark prefer cold and deep waters, and are present at depths down to 2200 meters in the southern part of its habitat [19]. They tend to stay at deeper depths during the summer and move into more shallow waters during the winter time (where the water is the coldest) [17]. Short term tagging experiments have also suggested that they remain at deeper depths during the mornings and move into more shallow waters during the afternoon and evenings. This behaviour have been seen in sharks studied under land fast sea ice in the Canadian Arctic as well as in sharks in the St. Lawrence estuary, Canada [18,20]. Horizontal movements have also been observed, as by Hansen 1963 that reported both site fidelity and large scale movements. The longest distance travelled was found to be in excess of 1100 km [14]. The information about the Greenland sharks migration behaviour is however very limited, but one can assume that at least some migrations across the North Atlantic and into the Arctic take place.

The previously mentioned tagging experiment also showed that this species may be very long lived. One tagged individual had grown from 262 to 270 cm in 16 years (0.5 cm/year) [14], indicating a very slow growth rate. The life span of the Greenland shark might therefore be over 100 years and accordingly among the longest living fish species in the world.

As most shark species, the Greenland shark is ovoviviparous, meaning that they give birth to living pups after the eggs have hatched inside the female. Each litter contains at least 10 pups measuring around 40 cm. Mating and birth of the Greenland shark have however never been observed [16,21].

The Greenland shark is an opportunistic feeder. Investigations of stomach contents have revealed everything from human waste, invertebrates and fish to marine mammals [21,22]. Recently was the stomach content of 22 Greenland sharks collected between 2001 and 2005 in Icelandic waters examined revealing mostly fish but also tissues from polar bears (*Ursus maritimus*) and some unidentified pinniped and cetacean species [23]. Some of the items found in the stomach of Greenland sharks caught around Iceland are shown in Figure 1.2.

Figure 1.2. Items found in the stomach of Greenland sharks collected from Icelandic waters. Photos: Jörundur Svavarsson and Anna Strid.

Fisk et al. 2002 suggested that the Greenland shark feed at a trophic level similar to turbot and ringed seal (about the fourth level), determined using stable isotopes of nitrogen. Based on stomach contents and OHC concentrations however, a higher trophic level was suggested [22]. Cortés 1999 has also placed the Greenland shark at about the fourth level, similar to marine mammals [12]. Greenland shark from Icelandic waters was recently assigned a trophic level of 4.3 based on stable isotopes of nitrogen [23].

The Greenland shark is nowadays of very limited importance for humans. In the past it was however hunted in Greenland, Iceland and Norway for its fatty liver and the oil was mainly used as lamp fuel. Inuits have also used the skin for making boots and the sharp lower dental bands as knives for cutting hair [13]. The largest catches of Greenland sharks were in the 1940s in west Greenland, where an estimated 50 000 sharks were landed. The Icelandic fishery reached a large scale in the 18th century, and peaked in 1867 when 13 100 barrels of oil were exported. Today they are mainly caught as by-catch in commercial fisheries, about 40 tonnes annually in Iceland, and is part of the traditional diet in Iceland [24]. A recent proposal from Greenland suggests that Greenland shark by-catch may be used as a source of energy; shark meat mixed with wastewater and macro-algae could be used to produce biogas to power isolated villages [16]. There are no population assessments available for this species, but they are listed as near threatened on the IUCN (International Union for the Conservation of Nature) red list [25].

1.2 Other shark species included in this thesis

1.2.1 *Piked dogfish (Squalus acanthias)*

Populations of piked dogfish (or spiny dogfish/spurdog) are found in many parts of the world. The piked dogfish prefer water temperatures between 7 and 15ºC and are found both inshore and offshore at depths down to at least 900 meters [13]. They feed primarily on teleost fish, but also on squids, crabs, shrimps and sea cucumbers among other things. In the eastern North Pacific subpopulation they may reach sizes of 1.6 meters while other populations are of smaller sizes. Males reach maturity at 60 to 70 cm and females between 80 and 100 cm [13]. Their trophic level have been calculated to 3.9 [12]. The piked dogfish is listed as vulnerable on the IUCN red list [26].

1.2.2 *Dusky shark (Carcharhinus obscurus)*

The dusky shark (or dusky whaler) is present in tropical and warm temperate waters worldwide. It is both coastal and pelagic in its distribution and is found both inshore and offshore close to the surface and down to depths of 400 meters, it is a highly migratory species that often migrate with season. Females

mature between 2.6 to 3 meters and reach at least 3.6 meters and males mature at around 2.8 meters and reach at least 3.4 meters. Similar to the Greenland shark, the diet of the dusky shark is highly variable including fish, other elasmobranches and squids, as well as whales [15]. Their trophic level has been calculated to 4.2, similar to the Greenland shark [12]. The dusky shark is among the slowest growing and late maturing sharks and is listed as vulnerable on the IUCN red list [27].

1.2.3 *Great hammerhead (Sphyrna mokarran)*

Great hammerheads are found in tropical waters world wide where they prefer coral reefs and continental shelves. They occur close inshore as well as offshore and are present at depths down to 80 meters. This species is considered to be nomadic and often migrates towards the poles in the summer. This is the largest member of the family Sphyrniade (hammerhead sharks). They may reach sizes of 5.5 to 6.1 meters, but most adults do not reach over 3.7 meters (females and males). Males mature at 2.3 to 2.7 meters and females at 2.5 to 3 meters [15]. Their diet is varied but stingrays, groupers and catfishes are among the favourites, and they have been assigned a trophic level of 4.3 [12,15]. The great hammerhead is listed as globally endangered by the IUCN red list based on evidence of an over 50% decline [28].

1.3 Thesis objectives

The aim of the thesis is to investigate the occurrence of industrial chemicals, pesticides, technical by-products and naturally occurring compounds in Greenland shark. This species is feeding at the highest trophical level and living far away from industrial sources of environmental contaminants, in the remote eastern North Atlantic. The high trophic level makes the Greenland shark prone to accumulate high concentrations of OHCs from their diet. Furthermore, the Greenland shark is possibly among the longest living fish species in the world, hence they may have accumulated these contaminants for a long period of time. There is a possibility that sharks larger than three meters might all have been alive since the large scale production of OHCs began and their releases into the environment started.

Efforts have also been made to investigate the metabolic capacity of the Greenland shark towards OHCs by investigating metabolites of some of the compounds studied. The correlation between sizes (a measure of age) of the individual sharks studied and concentrations of OHCs have been investigated. The thesis aims to improve the knowledge of OHCs in sharks in general and data on three other shark species (piked dogfish, dusky shark and great hammerhead) are included for comparative reasons, in this thesis.

2 Organohalogen contaminants studied and their occurrence in sharks

This chapter aims to briefly describe the OHCs included in the thesis. The contaminants studied are divided into four groups; industrial chemicals, technical by-products, pesticides and naturally occurring halogenated compounds (Figure 2.1). Metabolites of some of the OHCs have also been studied, see **Paper IV**. For more detailed descriptions, recent and comprehensive reviews are refereed to when possible. Several of the OHCs studied are defined as persistent organic pollutants (POPs) according to the Stockholm Convention [29]. Initially this list included twelve POPs that contracting parties agreed to eliminate or reduce usage of. Nine more were added to this list recently and three OHCs are currently undergoing evaluation for possible inclusion [29]. This chapter also aims to give an introduction to what is known about the studied OHCs in different shark species.

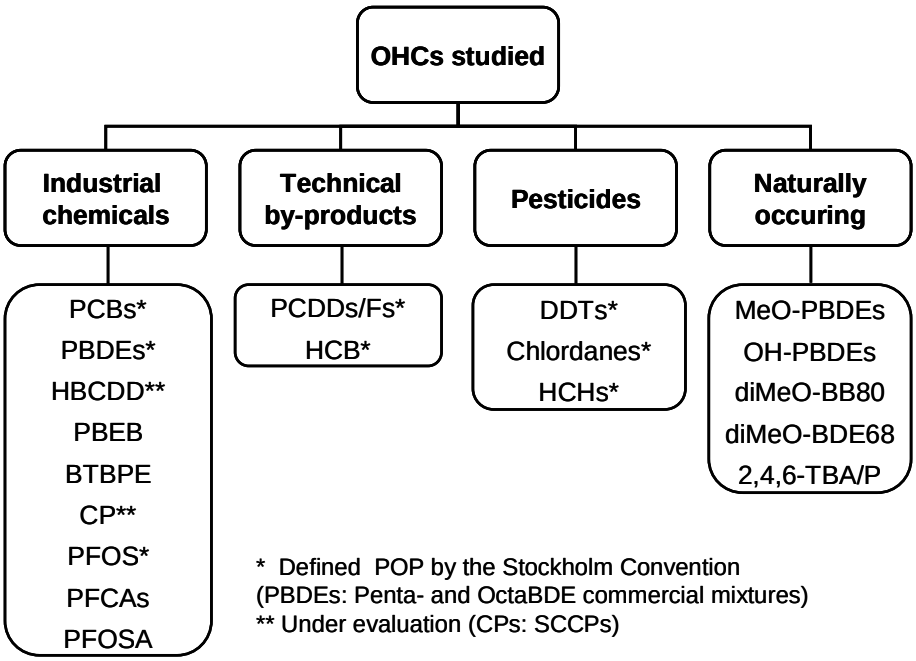

Figure 2.1. The different industrial chemicals, technical by-products, pesticides and naturally occurring organohalogen contaminants (OHCs) studied in this thesis. Some OHCs may be listed in several of the groups but placed where it is considered to be most relevant for the work presented herein.

2.1 Industrial chemicals

PCBs and PBDEs are both well known POPs and their general structures are shown in Figure 2.2. PCBs have been extensively used for their dielectric and fire resistant properties in transformers and capacitors ever since the technical production started in 1929, but also as heat transfer medium and as plasticizers in paint and sealants [30]. The use of PCBs is however restricted since the 1970s in most countries, and the environmental occurrence and toxicity are today comprehensively documented [5,31]. PCBs form hydroxylated and methyl sulfone metabolites *in vivo* (OH-PCBs and $MeSO_2$-PCBs, respectively) as described by Letcher et al. 2000 [32].

PBDEs have been used as additive BFRs in textiles, plastics and electronic equipment. Three different technical mixtures of PBDEs differing on the degree of bromination have been produced, i.e. Penta-, Octa- and DecaBDE. Today the use of all three mixtures is banned or restricted in both Europe and North America [33-35]. Like the previously mentioned PCBs, PBDEs have also been extensively studied. Levels and trends of PBDEs in both abiotic and biological samples have for instance been reviewed by de Wit et al. 2006 and Law et al. 2008 [6,36]. Toxic effects of PBDEs in humans and wildlife have for instance been reviewed by Darnerud 2003 and Vonderheide et al. 2008 [37,38]. PBDEs may undergo cytochrome P450 mediated formation to OH-PBDEs as shown in laboratory studies in fish, rat and in human hepatocytes, and reviewed by Hakk and Letcher 2003 [39-42]. In the environment, OH-PBDEs have been identified as metabolites in both fish and humans [43,44]. However, OH-PBDEs may also originate from natural sources as described in Chapter 2.4. Biotransformation of PBDEs may also include debromination of higher brominated congeners as shown in several fish species [45-47].

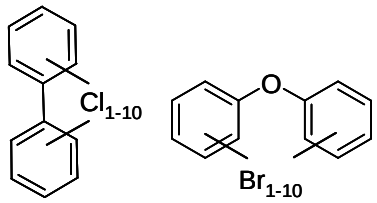

Figure 2.2. General structures of polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs).

The scientific interest in the environmental occurrence of potential replacement products to the technical PBDE mixtures have increased during recent years. Other BFRs studied in this thesis include HBCDD, pentabromoethylbenzene (PBEB) and 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE), their structures are all shown in Figure 2.3. HBCDD is an additive in polystyrene foam used as thermal insulation in buildings, and is also used as a flame retardant in textiles and electronic equipment. Commercial HBCDD formulations mainly consist of three stereoisomers (α-, β- and γ-HBCDD), with the α-HBCDD being the major stereoisomer in environmental samples as summarised in a review by Covaci et al. 2006 [48]. The environmental occurrence of HBCDD in Europe and Asia has also recently been reviewed by Law et al. 2008 [36].

PBEB have been reported to be used as an additive BFR during the 1970s and 1980s in the US [49]. In 2002, PBEB was listed as a low production volume chemical in Europe (10-1000 tonnes) used in the polymer industry [50]. BTBPE is an additive BFR that have been used for at least 25 years marketed as FF-680 by the Great Lakes Chemical Corporation and is now used as a replacement product for OctaBDE [51]. Existing data on environmental levels of both PBEB and BTBPE is scarce, but include air, sediment, fish and bird egg samples from industrialized areas [49,52-54]. Neither BTBPE nor PBEB have, to the best of my knowledge, been found in human samples. Low levels of BTBPE have been reported in Arctic bird eggs and beluga whale (*Delphinapterus leucas*) blubber, and PBEB only in bird eggs [55-57]. In addition, the two BFRs have also been found in an ice core from Svalbard [58]. Not much is known about the toxicity of these emerging BFRs. BTBPE have however shown to not be a potent thyroid disrupting BFR [59]. On the other hand may 2,4,6-tribromophenol, a BTBPE metabolite, be formed via ether cleavage of the compound [60], a compound that exerts endocrine and reproductive effects [61,62].

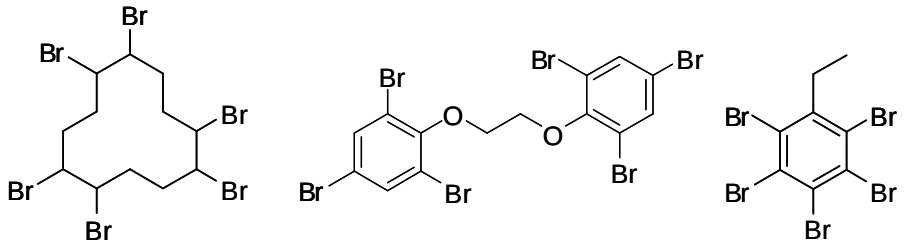

Figure 2.3. The general structure of hexabromocyclododecane (HBCDD) together with structures of 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE) and pentabromoethyl-benzene (PBEB).

Chlorinated paraffins (CPs) are *n*-alkanes with 10 to 30 carbons and substituted with chlorine atoms to about 40-70%, by mass. They have been used since the 1930s as for instance anti-wear lubricants in metal machinery, plasticizers and flame retardants in plastics but also as additives in paints and sealant [63]. CPs are divided into three categories depending on chain length: short chain CPs (SCCPs, C_{10-13}), medium chain CPs (MCCPs, C_{14-17}) and long chain CPs (LCCPs, C_{18-30}) [63]. SCCPs are listed as very toxic to the aquatic environment and the use of CPs is restricted through Directive 2002/45/EC within the European Union [64]. The serious and complex analytical challenges associated with the analysis of CPs are most likely the background for the very limited data available on CPs in humans and wildlife, an issue that is further discussed in Chapter 3. The environmental occurrence and toxicity data available have been reviewed by Bayen et al. 2006 and Feo et al. 2009 [65,66].

Perfluoroalkyl sulfonates (PFASs) and perfluoroalkyl carboxylic acids (PFCAs), grouped together as polyfluorinated alkyl compounds (PFCs), are industrial chemicals which have been used for over 50 years. Applications include use of PFCs as surfactants, in polymers and in fire fighting foams [67]. The PFCs are extremely stable compounds due to the strength of the carbon-fluorine bond. Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are the two best known PFCs, and their ubiquitous occurrence in the environment have been shown in several studies [68-70]. Perfluorinated-sulfonamides are used as surfactants but can also be found in pesticide formulations, *N*-ethyl perfluorooctanesulfonamide (*N*-EtPFOSA) is for instance an insecticide used against termites, cockroaches and ants [67]. *N*-EtPFOSA is metabolized via deethylation to perfluorooctanesulfonamide (PFOSA) and both compounds have been shown to be metabolic precursors of PFOS [71]. The structures of all PFCs mentioned are shown in Figure 2.4.

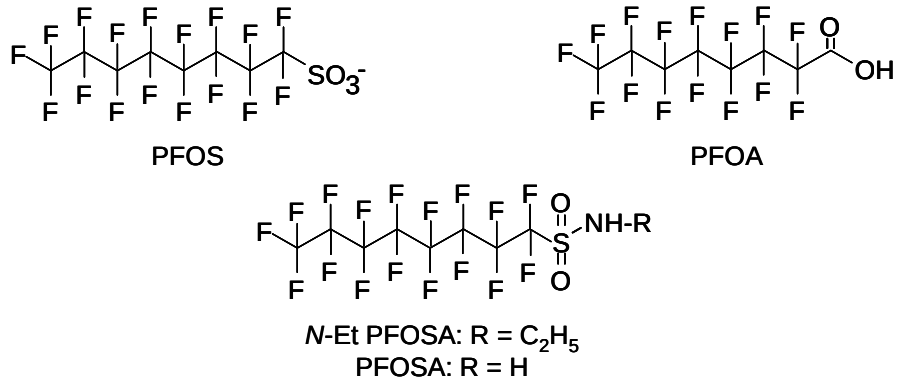

Figure 2.4. Structures and names of the polyfluorinated alkyl compounds (PFCs) discussed in this thesis; perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), *N*-ethyl perfluorooctanesulfonamide (*N*-EtPFOSA) and perfluoroctane-sulfonamide (PFOSA).

2.1.1 *Industrial chemicals in sharks*

PCBs followed by PBDEs are the most commonly studied industrial chemicals in different shark species. Some of the existing data are shown in Table 2.1 covering assessments from as many parts of the world as possible. Data on industrial chemicals in sharks from Australia are however, to my knowledge, limited to what is presented on dusky sharks and great hammerhead within this thesis, Chapter 4.

The highest PCB concentrations reported so far, 2.9-330 µg/g fat, have been observed in bull sharks (*Carcharhinus leucas*) from the east coast of Florida [72]. A high total concentration of PCBs, 35 µg/g fat, in one female bull shark from the southern part of Japan has also been reported [73]. Total PBDE concentrations are also the highest found in the literature in these two studies, with 12 to 4200 and 850 ng/g fat for the sharks from Florida and Japan, respectively [72,73]. The bull shark is a large (up to 3.4 meters) high trophic species, that commonly enters estuaries, river mouths, harbours and also migrates into rivers [15].

High concentrations of both PCBs and PBDEs have also been reported in Atlantic sharpnose shark (*Rhizoprionodon terraenovae*), sampled at the east coast of Florida. This shark species is feeding at a lower trophic level than the bull shark but both species prefer to inhabit estuaries and near shore waters [72]. PCBs have also been reported in lower trophic level species such as piked dogfish, bonnethead shark (*Sphyrna tiburto*) and leopard shark (*Triakis semifasciata*) from the US [72,74-76]. The highest PCB concentration in bonnethead sharks was found in samples from St. Simons sound, Georgia (3.5 µg/g fat), an area known to be heavily polluted with PCBs due to previous industrial activities [75].

Fewer studies are available from the eastern part of the North Atlantic and the Arctic. High concentration of PCBs has been reported in Greenland sharks from the Canadian Arctic and Iceland [22] (**Paper I**), whereas PBDE concentrations are low compared to reports from more industrialized regions (**Paper II**). Eastern North Atlantic data also include concentrations of PCBs and PBDEs in black dogfish (*Centroscyllium fabricii*), a deep water species, from west Greenland and Scotland [77,78].

PCB concentrations have been reported in seven different shark species from the Mediterranean, some included in Table 2.1 [79-83]. Serrano et al. 2000 reported PCB concentrations in eight different shark species from the northwest African Atlantic Ocean and the highest concentration was found in male Portuguese dogfish (*Centroscymnus coelolepis*) at 5.4 µg/g fat [84]. PCBs have been reported in muscle tissue from four different species caught in Brazilian

waters [85,86], with concentrations that in general were lower than those reported from sharks caught in the vicinity of industrialized cost lines.

Haraguchi et al. 2009 reported PCB and PBDE concentrations in four different shark species from southern coast of Japan, including the previous mentioned bull shark [73]. Low levels of PCBs were observed in bamboo sharks (*Chiloscyllium plagiosum*) from Hong Kong [87].

The only paper published on concentrations of HBCDD in sharks is by Johnson-Restrepo et al. 2007 reporting mean concentrations of 72 and 55 ng/g fat for bull shark and Atlantic sharpnose shark, respectively [88]. These concentrations are similar to the HBCDD data presented in Chapter 4 for the piked dogfish samples from Japan. Johnson-Restrepo et al. also reports concentrations of tetrabromobisphenol A, a reactive BFR, with the highest concentrations seen in bull shark (13 ng/g fat) [88].

PFCs have hitherto only been reported in two studies. Low levels of PFAS and PFCAs were reported in two species (bonnethead shark and Atlantic sharpnose shark) from the coast of Georgia [89]. Both muscle and liver tissue were analysed, with slightly higher concentration found in liver. PFOS was the major PFC in both species, with liver concentrations up to 2.8 and 6.3 ng/g w.w. for Atlantic sharpnose shark (n=5) and bonnethead shark (n=3), respectively [89]. A higher concentration of PFOS (18 ng/g w.w.) was reported in one liver sample from a hammerhead shark collected in the Ariake Sea, a shallow bay in the south western of Japan in 2000 [90]. PFCs are usually not reported on lipid weight basis due to the lack of lipophilicity. Instead they are accumulating due to protein binding and accordingly rather reported on wet weight (w.w.) basis making comparisons to other OHCs difficult. Information about PFCs in Greenland shark is presented in Chapter 4.

To summarize, high concentrations of PCBs are generally found in high trophic shark species regardless of sampling location. Available data on PBDEs indicate that shark samples from the US have higher concentrations compared to sharks from other locations. It is difficult to draw any conclusions regarding any of the other industrial chemicals discussed above, due to lack of data. Further, the data presented in **Paper III**, are so far the only published data on BTBPE, PBEB and SCCPs in any shark species.

2.2 Technical by-products

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs), commonly refereed to as "dioxins", are unintentional by-products formed in production of for instance PCBs, chlorinated phenols and pesticides. However, "dioxins" can also be formed during incomplete combustion processes [91]. Their general structures are shown in Figure 2.5.

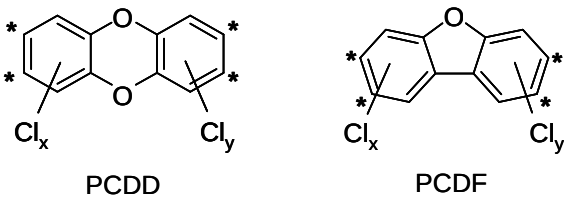

Figure 2.5. General structures of polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), congeners that are environmental relevant have their 2,3,7,8-positions occupied with chlorine (marked with an asterisk $^{(*)}$ in the structures above).

The dioxins are well known POPs that have been thoroughly studied, and the environmental occurrence in humans and wildlife have been reported from all parts of the world [5,92,93]. The PCDDs/Fs of particular concern, due to high toxicity and bioaccumulation, are those with 4-8 chlorine substituents and with chlorines in the 2,3,7,8-positions. These compounds make up a total of 17 PCDD/F congeners. The toxicity spectrum of PCDDs/Fs is described by others [91,94]. PCB congeners with a coplanar structure similar to the PCDDs/Fs share the same toxicological properties and are often referred to as dioxin-like (DL) and consists of four non-*ortho*- and eight mono-*ortho* PCB congeners [91].

PCDDs, PCDFs and DL-PCBs have, due to their pronounced toxicity and mode of action via one mechanism, been assigned toxic equivalent factors (TEF) used to calculate the total toxic equivalency (TEQ). The most recent review on TEF TEF values to apply, are given by van den Berg et al. 2006 [95]. The structure of CB-126, the most potent of the DL-PCBs is shown in Figure 2.6.

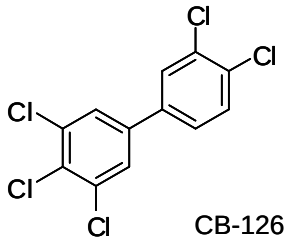

Figure 2.6. Structure of the most potent dioxin-like PCB congener; 3,3',4,4',5-pentachlorobiphenyl (CB-126).

Hexachlorobenzene (HCB) is formed as a by-product in various industrial processes, during waste incineration and energy production from fossil fuel. Moreover, HCB has also been used historically as a fungicide [96]. Production, emission, distribution and environmental levels of HCB have been extensively reviewed by Barber et al. 2005 [97].

2.2.1 *Technical by-products in sharks*

Existing data on PCDDs/Fs in sharks is very limited. Shark liver extracts of unspecified species, bought on the Taiwan market, have been analysed and PCDDs/Fs concentrations of 0.79 to 48 pg WHO-TEQ/g fat were reported [98]. Excluding that, to the best of my knowledge, is the only other study reporting concentrations of PCDDs/Fs in a shark species **Paper I** in this thesis. There are however some reports available regarding DL-PCBs. DL-PCBs have been reported in at least five different shark species from the Mediterranean [79,80,83], with the highest concentrations found in male smooth hammerhead (*Sphyrna zygaena*) livers [80]. DL-non-*ortho* and mono-*ortho* PCBs have also been reported in different shark species from the Atlantic Ocean northwest of Africa [84]. Eastern North Atlantic data includes concentrations of DL non-*ortho*- and mono-*ortho* PCBs in Greenland shark muscle and liver (**Paper I**). Webster et al. 2008 have also reported TEQ concentrations for five mono-*ortho* PCBs in liver of black dogfish from Scotland [78].

HCB has been reported from several parts of the world and are summarized in Table 2.2. Low levels have been reported in smooth hammerheads from Brazilian waters and in South African great whites (*Carcharodon carcharias*) [85,99]. Other data include two species from the Mediterranean [79], hammerhead sharks from Japan [100] and two deep water species from the eastern North Atlantic [77,101]. Fisk et al. 2002 have reported total concentrations of four chlorinated benzenes in Greenland sharks from the

Canadian Arctic [22]. The levels of HCB presented in **Paper IV** in Greenland shark are among the highest reported. Low levels of HCB were reported to be present in bonnethead sharks from four Florida estuaries [102]. The only data available from Australia is given in Chapter 4 on dusky shark and great hammerhead. Contrary to what has been observed in bull sharks regarding PCBs and PBDEs, there is no report in which a species was found to contain exceptionally high concentrations of HCB.

Table 2.1. Industrial chemicals reported in different shark species. Mean/median values are shown (ng/g fat) in general but in some cases are only concentration ranges shown. Information about the number of congeners included in ΣPCB and ΣPBDE is in most cases given in the references.

Species	Year	Location	Tissue	CB-153	ΣPCB	BDE-47	ΣPBDE	ΣHBCDD	Ref
Greenland shark female, *n*=10	2001-2003	Iceland	Muscle Liver	1200/900 1300/1000	4100/3200 4400/4000	32/24 32/24	53/35 60/41	na 6.0-13[b]	**PaperI** **PaperII**
Greenland shark mixed, *n*=15	1999	Cumberland Sound	Liver	500[a]/-	3400/-	na	na	na	[22]
Black dogfish unknown, *n*=na	1992	West Greenland	Liver	87/-	550/-	na	na	na	[77]
Black dogfish unknown, *n*=5	2006	West coast Scotland	Liver	na	270/270	na	12/13	na	[78]
Bull shark mixed, *n*=7	2002-2004	East coast Florida	Muscle	na	71000/-	480/-	1600/-	72/-	[72,88]
Atlantic sharpnose shark mixed, *n*=5	2004	East coast Florida	Muscle	na	5500/-	29/-	590/-	55/-	[72,88]
Bonnethead shark mixed, *n*=5	na	St. Simons sound Georgia	Liver	na	3500/-	na	na	na	[75]
Bonnethead shark mixed, *n*=5	na	Southern Florida	Liver	na	210/-	na	na	na	[75]
Leopard shark mixed, *n*=8 pools	1997	San Francisco Bay	Muscle	na	-/450[c]	na	na	na	[74]
Leopard shark unknown, *n*=1	na	San Francisco Bay	Whole	980	na	310	490		[76]
Smooth hammer-head mixed, *n*=3	2001	Brazil	Muscle	0.4-1.9[d]	4.5-19[d]	na	na	na	[85]
Bull shark female, *n*=1	2007	Southern Japan	Liver	11000	35000	440	850	na	[73]

Table 2.1 continued

Tiger shark Mixed, *n*=42	2007	Southern Japan	Liver	-/120	-/480	-/13	-/26	na	[73]
Silvertip shark mixed, *n*=8	2007	Southern Japan	Liver	-/72	-/280	-/6.2	-/12	na	[73]
Piked dogfish female, *n*=5	2005	Hokkaido Japan	Liver	28/27	120/110	10/11	17/17	150/110	**Chapter 4**
Bamboo shark mixed, *n*=20	2003-2004	Hong Kong China	Muscle	na	2.9/1.8[d]	na	na	na	[87]
Dusky shark male/female, *n*=2	2010	Australia	Liver	680/1700	1700/4300	73/130	140/250	nd	**Chapter 4**
Smallspotted dogfish mixed, *n*=11 pools	2000 2002	Adriatic sea Mediterranean	Liver	490/	1300/-	na	na	na	[82]
Blue shark mixed, *n*=44	1999-2001	Adriatic sea Mediterranean	Liver	910/-	2500/-	na	na	na	[83]
Kitefin shark mixed, *n*=64	1999 2001	Ionian sea Mediterranean	Liver	600/-	1800/-	na	na	na	[83]
Gulper shark female, *n*=25	1999	Adriatic sea Mediterranean	Muscle Liver	na na	1600/- 2200/-	na na	na na	na na	[79]
Longnose spurdog female, *n*=20	1999	Adriatic sea Mediterranean	Muscle Liver	na na	230/- 1700/-	na na	na na	na na	[79]
Kitefin shark female, *n*=1	1994-1995	African Atlantic Ocean	Liver	600[c]	2300	na	na	na	[84]
Portuguese dogfish male, *n*=3	1994 1995	African Atlantic Ocean	Liver	1400[c]/-	5400/-	na	na	na	[84]

na = not available, nd = not detected, [a] estimated value from graph, [b] *n*=3, [c] calculated from w.w., [d] ng/g w.w.

2.3 Pesticides

Pesticides investigated in this thesis include 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (4,4'-DDT) and related compounds, chlordanes and hexachlorocyclohexanes (HCHs).

DDT has been used as an insecticide since the 1930s, and the technical product mainly contains 4,4'-DDT but also 2,4'-DDT (15-20%) and 4,4'-DDD (up to 4%), all shown in Figure 2.7 [103,104]. 4,4'-DDD have also been manufactured as an insecticide itself [103]. The use of 4,4'-DDT is today restricted to malaria infected countries and is banned in most other countries [104,105]. The major transformation product of 4,4'-DDT is 1,1,1-trichloro-2,2-bis(4-chlorophenyl)-ethene (4,4'-DDE) (Figure 2.7), a highly persistent and bioaccumulative compound [104]. 4,4'-DDE have been linked to severe effects in predatory bird species, causing reproduction failure as seen in the Baltic Sea region [106]. Environmental levels, toxicity and effects of 4,4'-DDT and related compounds have been reviewed by others [7,103,104,107]. In a similar way as PCBs, 4,4'-DDE is metabolised to 3-$MeSO_2$-DDE, which has been reported in both human and wildlife tissue [108-110].

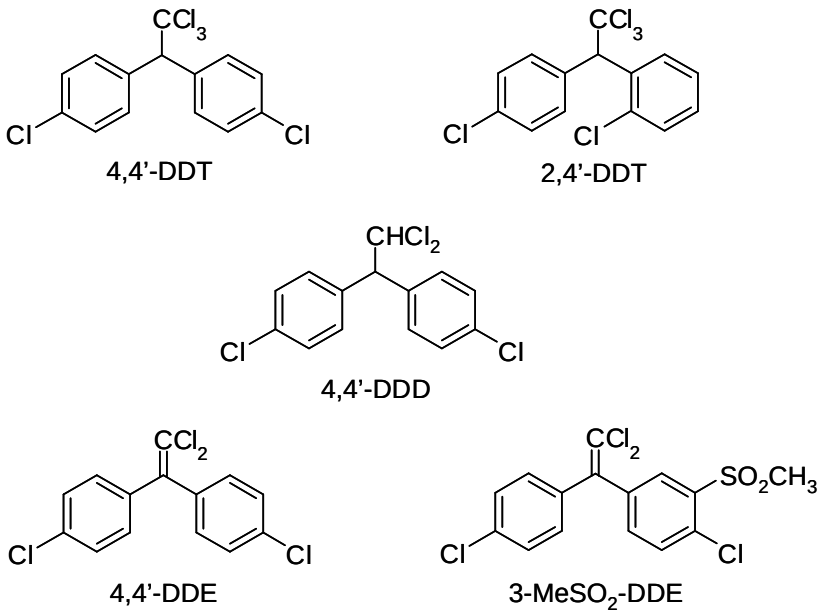

Figure 2.7. Structures of the main constituents in technical DDT mixtures (4,4'-DDT, 2,4'-DDT and 4,4'-DDD) together with the major degradation product 4,4'-DDE and its metabolite 3-$MeSO_2$-DDE.

Technical chlordane, has been used as an insecticide and includes at least 140 compounds, among which the major components are *cis*- and *trans*-chlordane and two minor are heptachlor and *trans*-nonachlor [111]. Oxychlordane and heptachlorepoxide are chlordane metabolites and are, together with *trans*-nonachlor, usually the major chlordane related compounds found in biota [112]. Structures of some chlordane related compounds are shown in Figure 2.8. Chlordane have been reviewed by for instance the World Health Organisation (WHO) 1984 and the Agency for Toxic Substances and Disease Registry (ATSDR) 1994 [113,114].

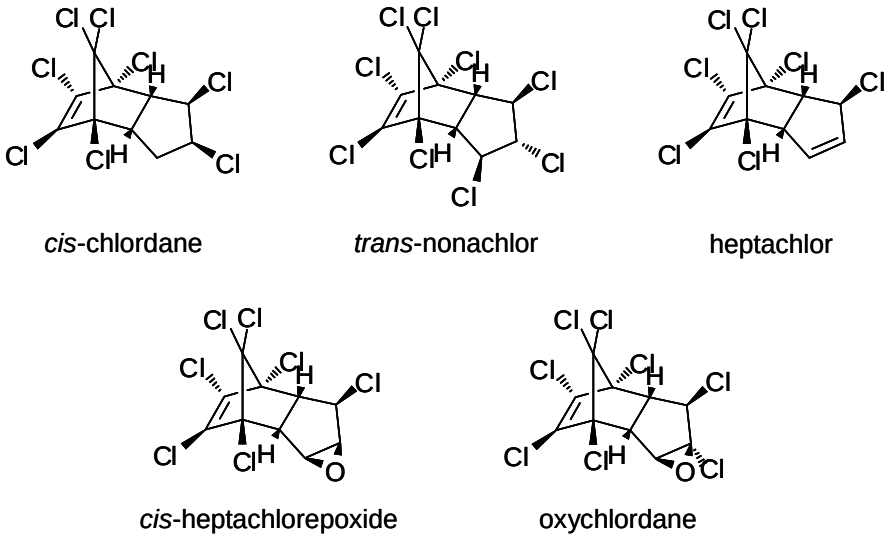

Figure 2.8. Structures of *cis*-chlordane, *trans*-nonachlor and heptachlor (components of technical chlordane mixtures) together with oxychlordane and *cis*-heptachlorepoxide (chlordane metabolites).

Technical HCH is an isomeric mixture with α-HCH (65-70%), β-HCH (7-10%) and γ-HCH (14-15%) as the major components (Figure 2.9) [115]. The γ-isomer, known as Lindane, is a broad-spectrum insecticide itself and is still in use in a few countries [116]. During the manufacture of Lindane, are both α-HCH and β-HCH formed as unwanted by-products [117], so even if technical HCH is no longer in use, these two isomers may still be released into the environment.

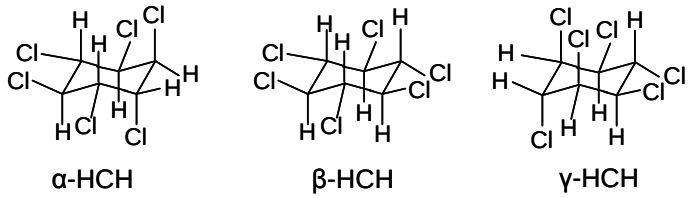

Figure 2.9. Structures of three isomers of hexachlorocyclohexanes (HCHs).

2.3.1 *Pesticides in sharks*

Concentrations of pesticides in different shark species are summarized in Table 2.2 covering data from as many parts of the world as possible and with the DDTs as the most frequently reported compounds. The highest mean and/or median concentrations have been reported in Greenland sharks from Iceland (**Paper IV**) and the Canadian Arctic together with hammerhead sharks from Japan [22,100]. Ongoing or recent exposure to DDT has been suggested to occur in bamboo sharks from Hong Kong [87] and great white sharks from South Africa [99]. This conclusions was drawn because of an observed 4,4'-DDE/ΣDDT ratio below 0.6 as suggested by Aguilar 1984 [118]. Ratios seen in kitefin sharks from the Ionian Sea in the Mediterranean also implied recent DDT exposure while sharks from other areas of the Mediterranean did not [79,82,83].

Deep water species from the Arctic and eastern North Atlantic, i.e. the Greenland shark and black dogfish, have shown 4,4'-DDE/4,4'-DDT ratios close to or below one [22,77], **Paper IV**. This can also been seen as an indication of a recent exposure to DDT but this seems unlikely for these species and the issue is further discussed in Chapter 5 and **Paper IV**.

HCHs have only been reported in few studies of sharks and then only present at fairly low concentrations, whereas chlordanes are more extensively studied (see Table 2.2). The number of compounds included in Σchlordane varies from study to study, making any comparisons difficult. The highest concentrations have however been observed in hammerhead sharks and Greenland sharks [22,100], **Paper IV.**

Table 2.2. Pesticides reported in different shark species. Mean/median values are shown (ng/g fat) in general but in some cases are only concentration ranges shown. Information about the number of congeners included in ΣDDTs, ΣHCHs and Σchlordanes (ΣCHLs) are given in the references.

Species	Year	Location	Tissue	ΣDDTs	ΣHCHs	ΣCHLs	HCB	Ref
Greenland shark female, *n*=10	2001-2003	Iceland	Muscle Liver	12000/9000 11000/8300	NA NA	1300/1200 1500/1400	120/120 72/96	**PaperIV**
Greenland shark mixed, *n*=15	1999	Cumberland Sound	Liver	7200/-	53/-	1800/-	52/-[c]	[22]
Black dogfish unknown, *n*=na	1992	West Greenland	Liver	950/-	12/-	66/-	37/-	[77]
Velvet belly unknown, *n*=10	1993	Nordfjord Norway	Liver	6000/-	nd	200/-	64/-	[101]
Bonnethead shark mixed, *n*=5	NA	St. Simons sound Georgia	Liver	150/-	nd	67/-	nd	[102]
Bonnethead shark mixed, *n*=5	NA	Eastern Florida	Liver	250/-	nd	370/-	nd	[102]
Leopard shark mixed, *n*=8 pools	1997	San Francisco Bay	Muscle	-/220[a]	na	-/46[a]	na	[74]
Smooth hammer-head mixed, *n*=3	2001	Brazil	Muscle	1.8-2.7[b]	0.10-0.11[b]	na	nd-0.06[b]	[86]
Great white mixed, *n*=3	2003	South Africa	Muscle Liver	76-200 970-3600	3.7-16 3.6-4.1	11-13 14-55	1.3-4.0 3.0-8.4	[99]
Hammerhead shark unknown, *n*=2	1999-2001	Ariake Sea Japan	Liver	8700	7.4	2400	140	[100]
Piked dogfish female, *n*=5	2005	Hokkaido Japan	Liver	300/260	25/25	37/32	21/21	**Chapter 4**
Bamboo shark mixed, *n*=20	2003-2004	Hong kong, China	Muscle	2.5/1.1[b]	0.01/0.02[b]	0.46/0.11	na	[87]

Table 2.2 continued

Dusky shark male/female, *n*=2	2010	Australia	Liver	1300/4000	nd	53/190	2.4/4.3	**Chapter 4**
Smallspotted dogfish mixed, *n*=11 pools	2000 2002	Adriatic sea Mediterranean	Liver	1200/-	na	na	na	[82]
Blue shark mixed, *n*=44	1999-2001	Adriatic sea Mediterranean	Liver	2400/-	na	na	na	[83]
Kitefin shark mixed, *n*=64	1999 2001	Ionian sea Mediterranean	Liver	4500/-	na	na	na	[83]
Gulper shark female, *n*=25	1999	Adriatic sea Mediterranean	Muscle Liver	2700/-[a] 5800/-a	na na	na na	194/-[a] 27/-[a]	[79]
Longnose spurdog female, *n*=20	1999	Adriatic sea Mediterranean	Muscle Liver	350/-[a] 2900/-[a]	na na	na na	52/-[a] 22/-[a]	[79]

na = not available, nd = not detected, [a] calculated from w.w, [b]ng/g w.w., [c]sum of four chlorobenzenes,

2.4 Naturally occurring OHCs

The worlds oceans have been shown to be a major source of naturally occurring OHCs, where many of these compounds are believed to be formed by algae, sponges or bacteria amongst other possible pathways [2]. Most of these naturally occurring OHCs contain bromine and/or chlorine [2], and during recent years there have been an increasing number of reports showing their presence at different trophic levels in the marine food webs.

The methoxylated and hydroxylated PBDEs (MeO-PBDEs and OH-PBDEs) are the most well known compounds within this group, identified as natural products in sponges, algae and cyanobacteria [119,120]. The first record of MeO-PBDEs in marine wild life is from Baltic Sea seal and fish [121]. Today there are several additional reports showing their presence in marine food webs globally [73,122-124]. The natural origin of 6-MeO-BDE47 and 2'-MeO-BDE68 (Figure 2.10) has been proven by ^{14}C measurements [125], and both have been quantified in a whale oil sample dated in 1929, collected prior to any large-scale production of industrial chemicals [126]. OH-PBDEs may however also be formed as metabolites of PBDEs (see Chapter 2.1). The presence of higher concentrations of OH-PBDE congeners compared to compounds of antrophogenic origin (PBDE and PCB metabolites) observed in red alga, mussels and fish is supporting the theory of natural production of OH-PBDEs in the marine environments [120,127,128]. Recently it has also been suggested that the OH-PBDEs may be metabolites (via demethylation) of naturally occurring MeO-PBDEs [129].

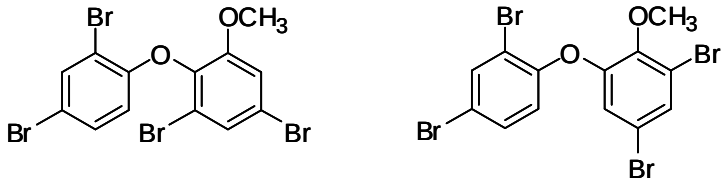

Figure 2.10. Structures of two methoxylated PBDE congeners of natural origin; 6-MeO-BDE47 and 2'-MeO-BDE68.

Other examples of naturally occurring OHCs are 2',6-diMeO-BDE68 and 2,2'-diMeO-BB80 along with a group of halogenated- methyl bipyrroles (MBPs) and dimethyl bipyrroles (DBPs) containing chlorine and/or bromine, shown in Figure 2.11 [130-134]. 2,4,6-Tribromophenol (TBP) together with other polybrominated phenols and anisoles are known natural products [135]. However, 2,4,6-TBP is also used as a BFR, intermediate in BFR production and

in wood preservation [136]. It is also a known biotransformation product of BFRs such as BTBPE and 2,3-dibromopropyl-2,4,6-tribromophenyl ether [60,137]. However, when present in the marine environment, 2,4,6-tribromophenol is most likely to be of biogenic origin [138].

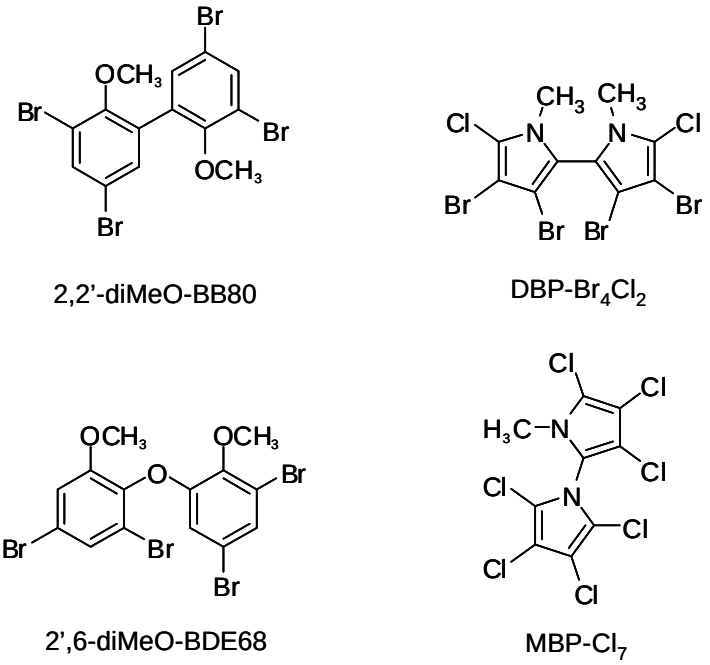

Figure 2.11. Structures of some OHCs assumed to be of natural origin; 2',6-dimethoxy-2,3',4,5'-tetrabromodiphenyl ether (2',6-diMeO-BDE68), 2,2'-dimethoxy-3,3',5,5'-tetrabromobiphenyl (2,2'-diMeO-BB80), 1,1'-dimethyl-3,3',4,4'-tetrabromo-5,5'-dichloro-2,2'-bipyrrole (DBP-Br_4Cl_2) and 1'-methyl-2,3,3',4,4',5,5'-heptachloro-1,2'-bipyrrole (MBP-Cl_7).

2.4.1 *Naturally occurring OHCs in sharks*

Data on naturally occurring OHCs in sharks are limited to two publications excluding **Paper II** and the information given in Chapter 4. 6-MeO-BDE47, 2'-MeO-BDE68, 2',6-diMeO-BDE68, 2,2'-diMeO-BB80, MBP-Cl_7 (Q1) and DBP- Br_4Cl_2 have all been shown to be present in four different shark species from the southern coast of Japan in the Pacific Ocean [73]. Low concentrations of 6-OH-BDE47, 2'-OH-BDE68 and 2,2'-diOH-BB80 have been reported in tiger sharks (*Galeocerdo cuvier*) and bull shark samples [139]. Low concentrations of 6-MeO-BDE47 and 2'-MeO-BDE68 have also been reported in commercial shark liver oil from New Zealand [140].

3 Analytical methods

3.1 Samples

The Greenland sharks used within this thesis were all females and collected from Icelandic waters in the eastern North Atlantic between 2001 and 2003 (Figure 3.1). Total length of the sampled sharks ranged between 3.5 and 4.8 meters. All sharks had accidentally been caught in trawls or entangled in long lines used for commercial fishing, and were not actively fished for either commercial or scientific purpose. Ten Greenland sharks were used in **Papers I** and **II**, and fifteen sharks for **Papers III** and **IV**. Muscle and liver tissue were used for **Papers I**, **II** and **IV** while in **Paper III** only liver samples were used.

Further, data on three other shark species is presented herein as hitherto unpublished results. Samples from piked dogfish (*n*=5) were provided by the Environmental specimen bank (*es-BANK*), Centre for Marine Environmental Studies (CMES), Ehime University in Matsuyama, Japan. Dusky shark (*n*=2) and great hammerhead (*n*=1) were caught as part of the New South Wales (NSW) ocean trap and line fishery under the Fisheries Management Act 1994 and kindly supplied by Dr Anthony Roach, Department of Environment, Climate Change and Water, NSW Government, Australia.

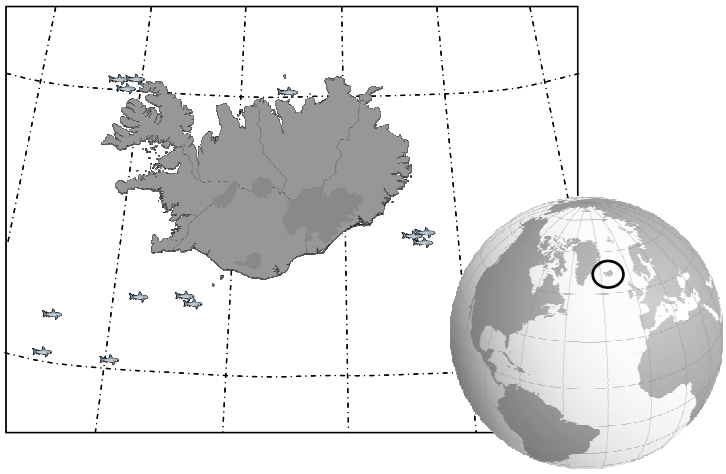

Figure 3.1. A map of Iceland showing the sampling location for thirteen of the fifteen individuals studied.

3.2 Extraction

Most OHCs analyzed within this thesis are associated with the lipids in the matrix and hence any technique that effectively extracts lipids could have been used for this purpose. Methods reported in the literature for extraction of fish and marine mammal tissues include Soxhlet extraction [72,141] and column extraction using different solvents [73,121]. Other techniques used are microwave assisted extraction (MAE) [142] and pressurized liquid extraction (PLE), also known as accelerated solvent extraction (ASE) [53,78,123]. The PFCs are however associated to proteins rather than to lipids, so other extraction techniques are used [143-145].

In **Paper I** and partly in **Paper II,** shark tissue was homogenized, mixed with sodium sulfate (three times the sample weight) for dehydration, and extracted with cyclohexane:dichloromethane in a glass column [146]. In **Papers II-IV** and for the other shark species included, a liquid-liquid extraction method was used as described by Jensen et al. 2003 [147]. Briefly, homogenized shark tissue was mixed with 2-propanol:diethyl ether to dehydrate the sample. Lipids and OHCs were thereafter extracted with 2-propanol and a hexane:diethyl ether mixture. The organic phase was washed with aqueous sodium chloride (0.9%) in 0.1 M phosphoric acid to protonate any potential phenolic compounds, certifying their partitioning into the organic phase.

PFCs were extracted with methanol, according to a method developed by Tomy et al. 2005 [144]. This quick and simple method was originally developed for biological samples such as liver from fish and marine mammals, aiming to eliminate a matrix dependent ion-suppression observed when using a method based on ion-pairing and extraction with methyl-*tert*-butyl ether [144,148]. The ion-pair method previously mentioned refers to a method developed by Hansen et al. 2001 [143].

3.3 Lipid removal and separation of substance groups

After extraction and lipid determination, which was done gravimetrically in **Papers I-IV**, it is necessary to remove all extracted lipids so the extracts are as clean as possible prior to instrumental analysis. Trying to separate OHCs into different fractions is crucial, especially when the analytes are present at very low concentrations. The different steps utilized for lipid removal and separation of substance classes used in **Papers I-IV** are shown in Figure 3.2 and 3.3.

Lipids were eliminated with concentrated sulfuric acid in the work done within **Papers II**, **III** and **IV**. This is a destructive lipid removal procedure and not all OHCs are stable in sulfuric acid (e.g. dieldrin and endrin), furthermore may

some compounds also partition into the acid (c.f. below). After the sulfuric acid treatment, a second lipid removal step was applied using activated silica gel impregnated with concentrated sulfuric acid.

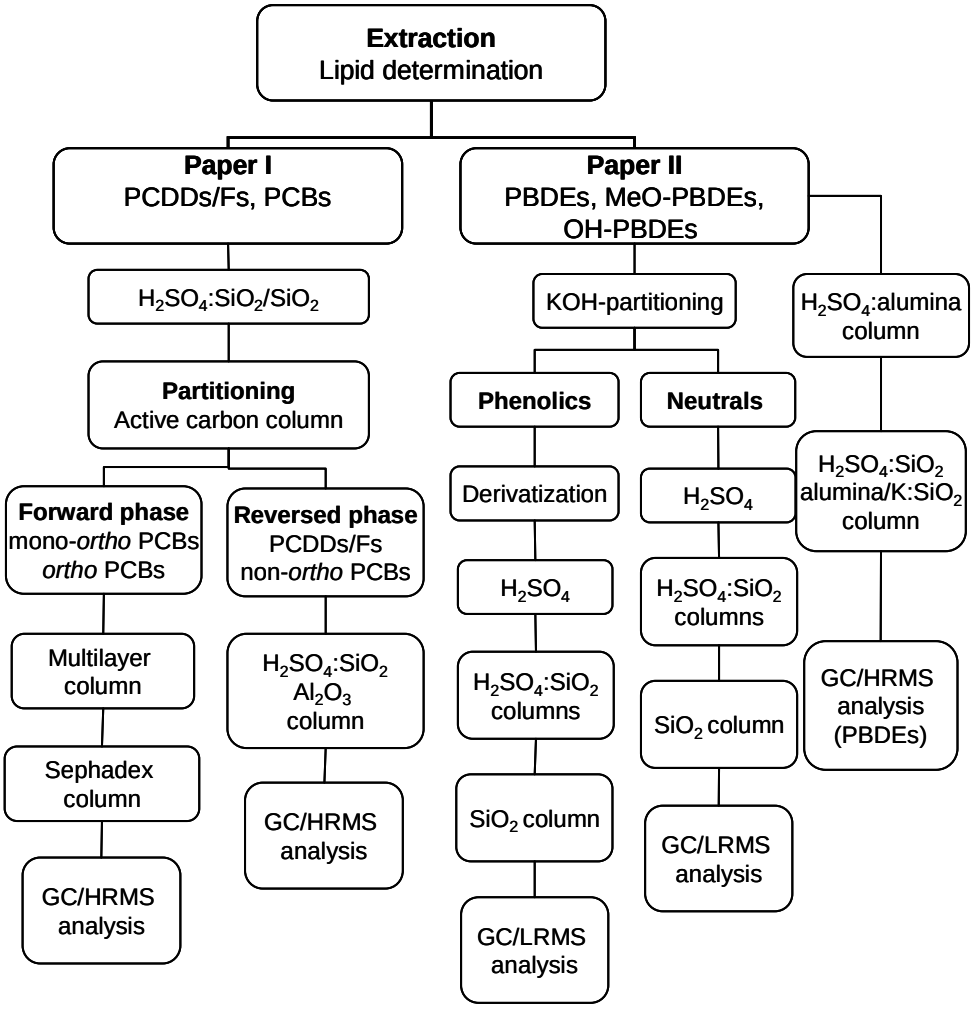

Figure 3.2. Scheme of the clean-up procedures used in **Papers I** and **II**.

In **Paper I** and partly in **Paper II,** lipids were removed by adding silica gel impregnated with sulfuric acid (2:1) to the samples and leaving them over night. The samples were then transferred to a glass column and cyclohexane was applied as mobile phase. Further clean-up included several steps as shown in Figure 3.2.

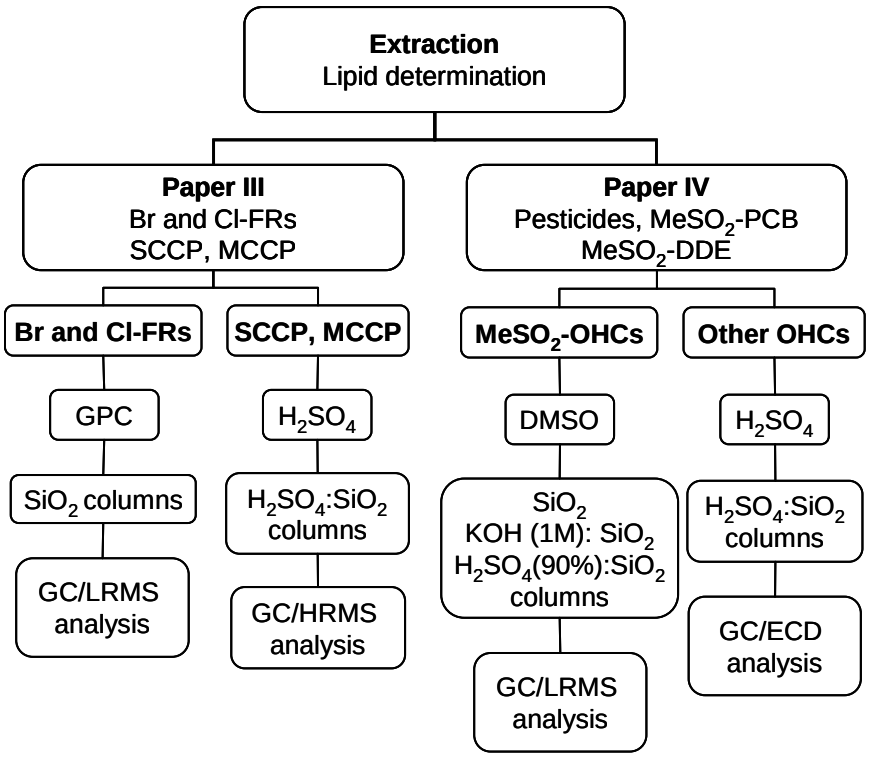

Figure 3.3. Scheme of the clean-up procedures used in **Papers III** and **IV**.

Gel permeation chromatography (GPC) was used for lipid removal for a fraction of the sample in cases when not all target compounds were assumed to be stable in sulfuric acid (**Paper III)**. GPC is a non-destructive method where separation is based on size and shape of the molecules. Molecules with large volumes are excluded from the pores in the cross-linked polystyrene GPC gel used (Bio Beads SX-3) while smaller compounds are retained in the pores. The choice of mobile phase together with planarity and polarity of molecules may also influence the separation of the analytes [149]. Further clean-up of the GPC fraction in **Paper III** was done using activated silica columns.

Another non-destructive method for removal of the bulk of lipids is to partition the lipids with acetonitrile. The principle behind this is that OHCs are dissolved fairly well in acetonitrile and lipids to a lesser degree [150].

The extraction method applied for PFCs does not extract lipids; hence no lipid removal step is required. The extraction was however followed by an ultracentrifuge step (13 500 rpm, 15 min), removing any possible co-extracted material [144].

3.3.1 *Separation of planar and non-planar compounds*

Planar compounds like PCDDs/Fs and non-*ortho* PCBs are usually present in environmental samples at much lower concentrations than *ortho* PCBs and pesticides. A prerequisite for high quality analysis is then separation of planar and non-planar compounds. Clean-up and separation of planar compounds may include alumina and/or Florisil columns in combination with active carbon columns as reviewed by Reiner 2010 and Hagberg 2010 [151,152]. PCDDs/Fs and non-*ortho* PCBs were separated from non-planar compounds using columns containing activated carbon as presented in **Paper I**. Non-planar compounds were eluted with cyclohexane:dichloromethane in the forward direction and planar compounds with toluene in the reversed direction [153].

3.3.2 *Separation of phenolic and neutral compounds*

Phenols were separated from neutral compounds using 0.5 M potassium hydroxide (KOH) in 50% ethanol [154]. To enhance the analysis of phenolic compounds by gas chromatography (GC) the OH-group was derivatized to decrease the polarity of the analytes. If not, the phenols will interact with the stationary phase in the GC column leading to non-optimized separations and an increase of the detection limits. Derivatization of phenolic compounds was achieved through methylation using diazomethane, giving stable derivates suitable for further clean-up and analysis, in this thesis. *Diazomethane must be handled with great care due to its toxicological risks and explosive properties. The use of diazomethane was approved by the Swedish work environment authority.*

3.3.3 *Separation of sulfone-containing compounds*

When treating a sample with concentrated sulfuric acid for lipid removal any sulfone-containing substances, such as $MeSO_2$-PCBs and 3-$MeSO_2$-DDE, are partitioned into the acidic phase. The sulfone group acts as a Lewis base and forms charged complexes with the acid. Adding water breaks these attractions and the sulfone-containing compounds can be re-extracted into an organic phase, for which hexane is commonly used [155].

Another way to isolate this group of compounds is by dry dimethyl sulfoxide (DMSO) partitioning [156]. Once partitioned into the DMSO, the sulfone containing compounds can be re-extracted by adding water, as applied in **Paper IV**. A draw-back using DMSO is the partitioning of 2,4'-DDD to both the hexane and DMSO phases [157]. During the work on **Paper IV**, it was also discovered that other compounds partitions into both phases. HCHs (α-, β-, γ-,

δ- and ε-isomers) partitioned to 30 - 80% into the DMSO and methoxychlor to about 40%. No PCBs and DDT related compounds other than 2,4'-DDD (40%) and 4,4'-DDD (60%) partitioned into the DMSO. So if analytes other than sulfone-containing compounds such as $MeSO_2$-PCBs and their parent compounds PCBs are to be analysed, separation using concentrated sulfuric acid is more appropriate. After separation may DMSO however be used as an additional clean-up step for the $MeSO_2$-fraction

3.3.4 *Isolation of chlorinated paraffins*

CPs are as previously mentioned extremely complex mixtures with thousands of isomers, enantiomers and diastereomers [158], and hence a great challenge to analyse. To date there is no technique available that can separate CPs into individual congeners, instead they are eluting over a wide time range leading to a broad hump in the chromatogram (Figure 3.4). This results in a number of possible co-eluting OHCs and it is important to isolate a fraction as free from interfering compounds as possible when CPs are to be assessed. Methods used for this purpose include fractionation on Florisil or silica gel columns, with the CPs eluting in the more polar fractions as reviewed by Santos et al. (2006) [159]. Photolytic clean-up has also been used, degrading most of the PCBs and partially also chlordane and toxaphene components [160]. High resolution mass spectrometry (HRMS) was used for analysis of CPs in **Paper III** allowing a less extensive clean-up as further discussed in Chapter 3.4.

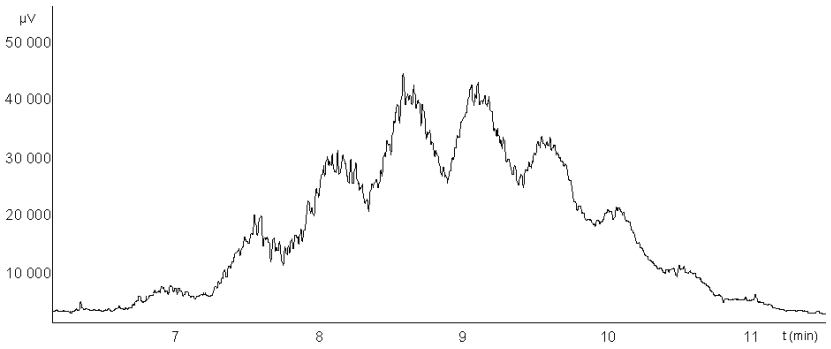

Figure 3.4. A GC (ECD) chromatogram of a commercial SCCP mixture (Hüls AG 60C, $C_{10\text{-}13}$, 60% Cl).

3.4 Instrumental analysis, identification and quantification

Analysis of the OHCs in **Papers I-IV** was achieved by GC in combination with electron capture detection (ECD), low resolution mass spectrometry (LRMS) or HRMS. Also high performance liquid chromatography (HPLC) was used for separation of analytes, together with MS/MS for detection and quantification of HBCDDs and PFCs (unpublished data presented in Chapter 4).

3.4.1 *GC (ECD)*

The ECD is very sensitive and selective towards halogenated compounds, and is commonly used for the analysis of well known OHCs present at high concentrations (e.g. PCBs and organochlorine pesticides) in environmental samples. The identification is solely based on comparisons to authentic reference standards, hence co-elutions cannot be ruled out. This method was used in **Paper IV** for the analysis of PCBs, HCB, DDTs and chlordanes, well known compounds present at fairly high concentrations. Quantifications were achieved using surrogate standards and single-point calibration. Calibration curves with dilution series of standards were run to keep track of the linear relationship of the analytes.

3.4.2 *GC/LRMS and HRMS*

The use of MS for detection allows gathering of more information on the analytes such as molecular weight and fragmentation patterns. MS is therefore useful for structural elucidation of "unknown peaks". Two different ionization techniques were applied in this thesis. Electron capture negative ionization (ECNI) which is a soft ionization method that gives little fragmentation of the analytes. A buffer gas, typically methane or ammonia, is bombarded with electrons creating low energy electrons. Negative ions are then formed when the analytes capture these electrons. GC/LRMS (ECNI) operated in selected ion monitoring mode (SIM) using ^{79}Br and ^{81}Br is commonly used for analysis PBDEs and other brominated compounds like MeO-PBDEs. The method was originally described by Buser [161] and it gives low detection limits but unfortunately no structural information. On the other hand, electron ionization (EI), a highly reproducible technique, gives important structural information due to characteristic fragmentation patterns of each compound analysed. When LRMS is used, the detection limits are higher with EI compared to ECNI. If HRMS is used, the selectivity increases and the detection limits are lowered.

GC/LRMS (ECNI) was applied for analysis of brominated compounds in **Papers II** and **III**, monitoring ^{79}Br and ^{81}Br. GC/LRMS (ECNI) was used for analysis of $MeSO_2$-PCBs and 3-$MeSO_2$-DDE, due to assumed low levels of

these metabolites (**Paper IV).** Authentic reference standards were applied for identification of analytes. Quantifications were conducted using surrogate standards and single-point calibration.

Environmental levels of PCDDs/Fs and non-*ortho* PCBs are usually very low (pg/g) requiring HRMS analysis. GC/HRMS (EI) was used in **Paper I** and also in **Paper II** for analysis of PBDEs. SIM was used for monitoring of the ions: $[M+2]^+$ and $[M]^+$ (PCDDs/Fs and PCBs) and $[M-2Br]^+$ and $[M]^+$ (PBDEs). Quantifications were done using ^{13}C labelled surrogate standards and a five point calibration curve.

GC/HRMS (ECNI) was applied for analysis of SCCPs and MCCPs in the Greenland shark samples (**Paper III)**. Using HRMS operating at a resolution of ~12000 is considered enough to separate CPs from interfering compounds with similar retention times [158]. It also eliminates the problem with so-called self-interferences, i.e. isomers with similar nominal mass that cannot be separated with LRMS [158]. When using ECNI the response is depending on the degree of chlorination, and lower chlorinated congeners ($Cl_{<5}$) are poorly detected [162]. This makes the choice of a suitable quantification standard important. Technical mixtures is mainly used for this purpose and large variations have been determined when mixtures with different degree of chlorination were used [162-164]. Preferably should the pattern observed in the standard be as close as possible to the pattern seen in the samples. If EI is used, the response is not depending on the degree of chlorination but no congener or homologue information can be obtained due to high fragmentation [162].

In **Paper III,** quantification was done by selecting the largest peak corresponding to the $[M-Cl]^-$ ion of the most abundant formula group present (i.e. congeners with the same number of carbon and chlorine atoms) and comparing the response to that of the commercial mixture used as external standard, in accordance with Tomy et al. 1997 [158].

3.4.3 *LC/MS/MS*

For separation of non-volatile, ionic or thermally unstable analytes HPLC is more appropriate than GC. HPLC in combination with MS/MS operated in negative ion electrospray mode was used in this thesis for the analysis of PFCs and HBCDD. Multiple reaction monitoring (MRM) was used, allowing monitoring of an additional transition of the molecular ion for confirmation. When HBCDD is analysed using LC it is possible to do isomer specific analysis, something which is not possible using GC/MS since thermal rearrangements only allow the determination to total HBCDD [165].

3.4.4 *Limit of detection and quantification*

It is of critical importance to define the limit of detection (LOD) and the limit of quantification (LOQ). If an analyte is present in the blank samples, that amount usually defines the LOQ, while if not present, the LOD and LOQ values of an analyte depend on other factors such as noise from the matrix and the condition of the instruments. For the GC/HRMS analysis (**Papers I** and **II),** the LOQs were set to two times the average value of the blank samples or equal to the LOD determined at a signal-to-noise ratio of 3 from the matrix and the instrumental background in each sample. In **Papers II** and **IV** the GC/LRMS LOD values were determined as the lowest amount injected that was required for detection of a signal-to-noise ratio of three and LOQ determined as 3 times the LOD (or equal to the concentrations in the blank samples). In **Paper III** was yet another definition used for determining LOQ: the average amount in the blank samples plus three times the standard deviation of the blank samples.

3.5 Quality assurance/quality control (*QA/QC*)

In this section are some of the control measurements done to assure the quality of the analyses described.

3.5.1 *Solvent blank samples*

Contamination from solvents, instrumentation, and laboratory equipment etcetera is controlled by analysing procedural blank samples covering the whole or part of the analytical procedure. During the work presented in this thesis contamination of blank samples has not been a problem except in **Paper III** where a contamination of both SCCPs and MCCPs lead to high LOQ values.

3.5.2 *Surrogate standards and recoveries*

A surrogate standard should preferably be a compound that has similar physical and chemical characteristics as the analytes without being present in the samples as an environmental contaminant. The surrogate standard should be added as early as possible in the analytical chain to get an accurate recovery as a measure of the quality of the method used. The use of ^{13}C-labelled surrogate standard is considered to be an ideal choice for this purpose, preferably one standard for each congener. This method is however mainly restricted to the use of EI in combination with HRMS. ^{13}C-labelled BDE-209 can however be used as surrogate standard for BDE-209 when using GC/LRMS (ECNI) as described by Björklund et al. (2003) [166]. ^{13}C-labelled standards were used for the analysis of PCBs, PCDDs/Fs and PBDEs as described in **Papers I** and **II**. The analysis of PFCs and HBCDD also included labelled standards.

In **Paper II**, 4-OH-BDE121 was used as surrogate standard for brominated phenolic compounds. Low recoveries were seen in some samples, especially in liver. A recovery test was then performed to investigate the reason for the low recoveries observed. Extracted Greenland shark liver fat was spiked with a mixture of OH-PBDEs including 4-OH-BDE121 (10 ng each) and treated in different ways. The low levels of OH-PBDEs already present in liver samples were considered to be negligible. Sample A was spiked and treated in the same way as in **Paper II**. Sample B was spiked and treated with acetonitrile prior to the KOH-partitioning to reduce the amount of lipids. Sample C was partitioned with KOH and then spiked and derivatized. The highest recoveries were obtained in sample C and it was concluded that the dervatization step was working properly. The conclusions were that matrix effects during the KOH-partitioning caused the low recoveries obtained in some samples and that treating the sample with acetonitrile gave somewhat higher recoveries. The results are shown in Table 3.1.

Table 3.1. Recoveries (%) of OH-PBDEs in three liver fat samples treated in different ways, as described in the text above.

	Sample A	**Sample B**	**Sample C**
6-OH-BDE47	20	63	76
2'-OH-BDE68	28	66	95
6-OH-BDE85	24	59	75
6-OH-BDE90	61	58	95
6-OH-BDE99	66	55	96
2-OH-BDE123	51	62	93
6-OH-BDE137	57	68	90
4-OH-BDE121	18	63	73

3.5.3 *Reference material*

Reference material should be used when possible to confirm the accuracy of the extraction methods, clean-up procedures and instrumental analysis. This reference material should resemble the actual sample as much as possible. In **Papers I, II** and **IV** samples from a fish oil pool were used as laboratory reference material (LRM), and for comparison of the accuracy between the results obtained from different laboratories. The analysis of PFCs also included samples from a fish muscle homogenate spiked with known amounts of PFCs [167].

3.5.4 *Comparisons using different analytical methods*

Results obtained for the same sample using different analytical methods is also a measure of the quality of the analysis. A comparison of PBDE concentrations obtained with GC/LR- and HRMS is included in **Paper II**, showing good agreement. During the work on **Paper IV** some PCB congeners were quantified and compared to concentrations obtained with GC/HRMS in the same samples (**Paper I**), also indicating similar results of the two methods. A comparison is shown in Figure 3.5.

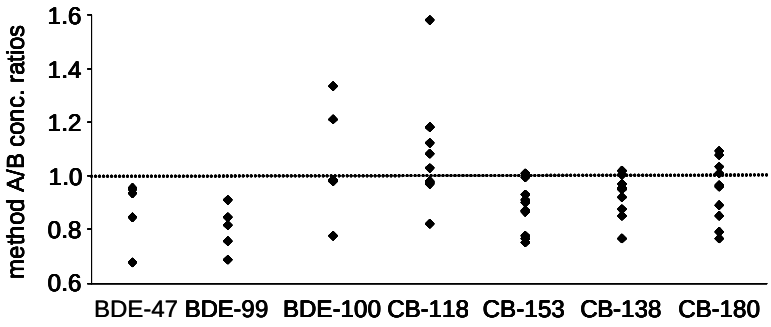

Figure 3.5. A comparison of results obtained with two different methods. For PBDEs; GC/LRMS / GC/HRMS and PCBs GC (ECD) / GC/HRMS.

4 Additional results

Some unpublished data related to this thesis are presented in this chapter. The aim is to contribute to the understanding of the presence of OHCs in the Greenland shark and in some other shark species studied for comparison.

4.1 Additional results: Greenland shark

PFOS, PFCAs (C_8-C_{12}) and PFOSA were analysed in muscle and liver tissue from ten and fifteen Greenland sharks, respectively. Low levels of PFOS were detected in liver but not above the MDL (2.3 ng/g w.w.). The only PFC quantified was PFOSA, present at low concentrations (up to 2.6 ng/g w.w.) in seven of the 15 liver samples. Concentrations of α-, β- and γ-HBCDD were determined in three liver samples. The total concentration of HBCDDs (sum of the three isomers) ranged between 6.0 and 13 ng/g fat with α-HBCDD as the far most abundant isomer (Figure 4.1).

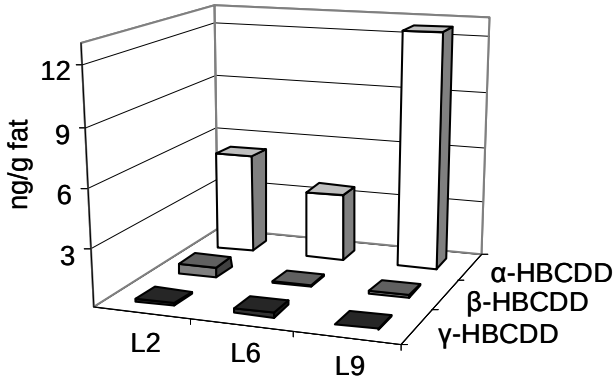

Figure 4.1. Concentrations of HBCDD isomers in three liver samples.

Efforts were made to identify MBPs and DBPs in Greenland shark liver. By comparing GC/LRMS (ECNI) fullscan spectra of authentic reference standards with spectras obtained from the sharks it was possible to identify one MBP (MBP-Cl_7, Q1) and one DMP (DMP-Br_6). In the GC (ECD) analysis (**Paper IV),** Q1 was shown to partly co-elute with *trans*-nonachlor (Figure 4.2). In a few samples it was possible to quantify Q1 and the concentrations ranged between 86 and 330 ng/g fat.

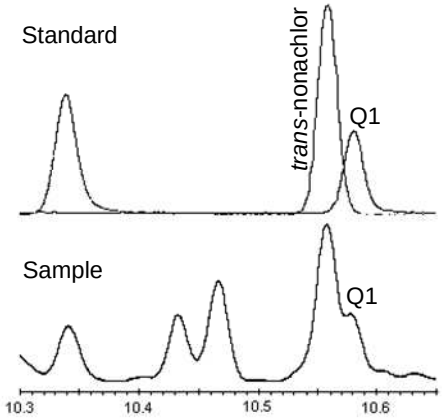

Figure 4.2. GC (ECD) chromatogram showing the retention time of *trans*-nonachlor and Q1 in a standard (upper) and the presence of 1'-methyl-2,3,3',4,4',5,5'-heptachloro-1,2'-bipyrrole (MBP-Cl_7, Q1) in Greenland shark (lower chromatogram).

4.2 Additional results: piked dogfish, dusky shark and great hammerhead shark

Liver tissue from the piked dogfish, dusky shark and great hammerhead were analysed for PCBs, DDTs, HCHs, *trans*-nonachlor, PBDEs, MeO-PBDEs and also for brominated phenolic compounds. Extraction and clean-up procedures followed the methods used in **Paper II.** The results for neutral chlorinated and brominated OHCs are summarized in Table 4.1. Brominated phenolic compounds detected in piked dogfish and dusky shark are shown in the GC/MS (ECNI) chromatograms (Figure 4.3). Concentrations of the identified compounds are however not reported. The results presented herein are further discussed in Chapter 5.

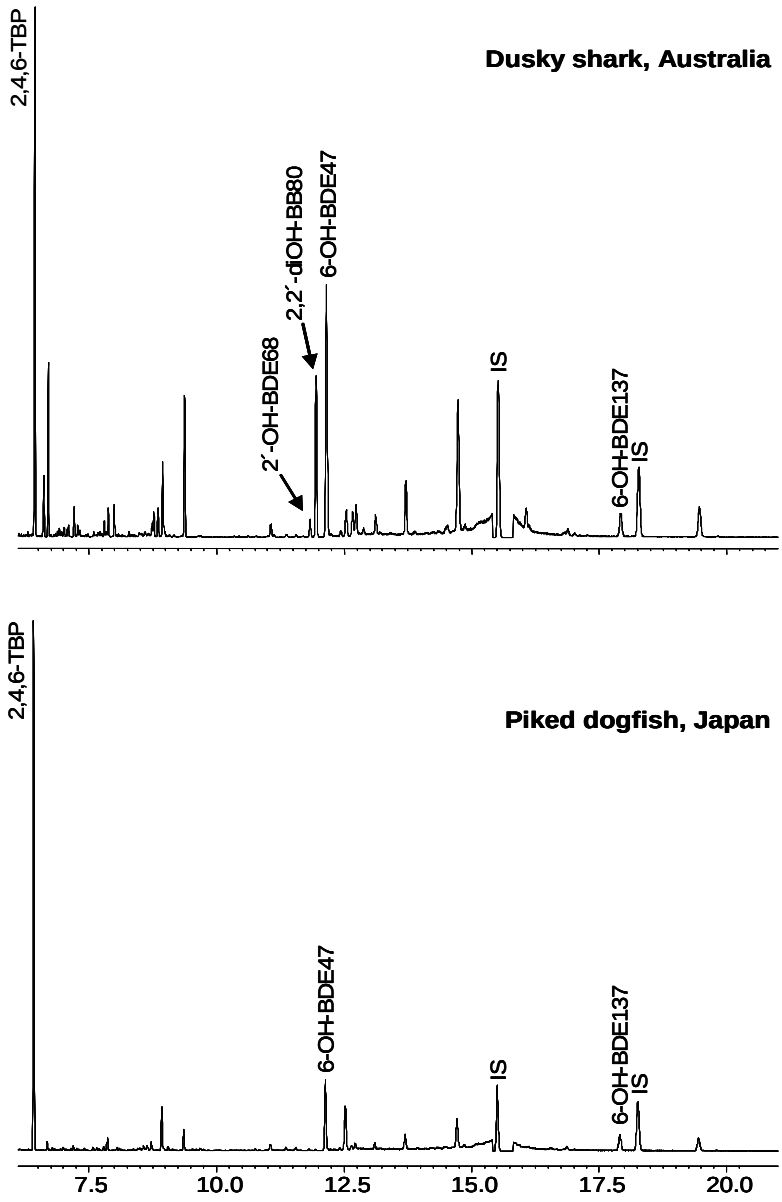

Figure 4.3. GC/MS (ECNI) chromatograms (bromide ions, m/z 79, 81) showing the phenolic fraction of dusky shark and piked dogfish (liver tissue).

Table 4.1. Concentrations (ng/g fat) of chlorinated and brominated OHCs in liver of PD (piked dogfish), DS (dusky shark) and GH (great hammerhead). For PD is mean values and ranges (min-max) presented.

	PD female (*n*=5)		**DS** male (*n*=1)	**DS** female (*n*=1)	**GH** female (*n*=1)
Size (m)	1.0	(0.83-1.3)	3.2	3.4	2.7
Lipid (%)	51	(42-65)	74	77	53
Chlorinated OHCs					
CB-28	3.6	(3.2-4.6)	1.7	1.4	nd
CB-52	6.4	(4.9-8.9)	0.99	1.1	0.17
CB-101	18	(13-24)	7.4	4.0	5.6
CB-118	29	(21-38)	130	320	6.9
CB-138	25	(20-31)	470	1200	9.1
CB-153	28	(23-38)	680	1700	24
CB-180	7.9	(6.3-9.9)	390	1100	8.9
ΣPCB_7	120	(98-150)	1700	4300	55
4,4'-DDE	170	(140-240)	1200	3900	16
4,4'-DDD	50	(38-65)	32	29	nd
4,4'-DDT	82	(46-120)	39	56	0.92
ΣDDT	300	(250-430)	1300	4000	17
α-HCH	7.1	(6.1-8.1)	nd	nd	nd
β-HCH	16	(14-18)	nd	nd	nd
γ-HCH	2.8	(2.5-3.0)	nd	nd	nd
ΣHCH	25	(23-29)	-	-	-
trans-nonachlor	37	(27-54)	53	190	0.87
HCB	21	(17-26)	2.4	4.3	3.9
Brominated OHCs					
BDE-17	0.10	(0.07-0.15)	nd	nd	nd
BDE-28	0.79	(0.62-1.0)	1.0	1.5	0.16
BDE-47	10	(7.9-12)	73	130	3.4
BDE-66	0.47	(nd-0.78)	2.0	3.0	0.35
BDE-99	1.3	(0.62-2.0)	18	38	0.10
BDE-100	2.4	(1.7-2.7)	37	55	1.5
BDE-153	0.52	(0.18-0.93)	4.5	8.6	0.11
BDE-154	1.2	(0.57-2.2)	8.1	13	0.29
ΣPBDE	17	(12-20)	140	250	5.6
ΣHBCDD	150	(63-250)	nd	nd	nd
2,4,6-TBA	3.9	(1.3-12)	7.3	2.0	8.7
6-MeO-BDE47	11	(9.0-15)	15	13	21
2'-MeO-BDE68	1.8	(1.1-2.7)	21	49	31
6-MeO-BDE90	nd		3.2	0.63	0.12
6-MeO-BDE99	0.51	(0.34-0.73)	1.7	0.83	0.08
2',6-diMeO-BDE68	2.2	(0.93-3.2)	8.0	9.2	12
2,2'-diMeO-BB80	2.0	(0.96-3.0)	1000	150	42

5 Discussion

This chapter is aimed to draw the attention to some of the main results from **Papers I-IV** and the additional results presented in Chapter 4, and to discuss them in some detail.

5.1 Greenland shark as a monitoring species of OHCs

The results presented in **Papers I-IV** confirm the initial hypothesis this thesis is based on, which was that the Greenland shark would be useful for assessments of environmental contaminants in our environment in general and in the remote sub-Arctic and Arctic environment in particular. All target OHCs, with very few exceptions, have been found as contaminants in the Greenland sharks assessed herein with the directed chemicals analyses performed. Several of the OHCs reported are of special interest since this is the first time ever reported in a shark species and hence further discussed below.

Among those OHCs reported for the first time in any shark species, are PCDDs/Fs (**Paper I**) and in **Paper III,** PBEB, BTBPE and SCCPs. There is in general very little information available about the environmental fate of PBEB and BTBPE, and the levels presented in **Paper III** are among the highest reported in Arctic wildlife so far. SCCPs are, as previously mentioned, suggested to be included in the Stockholm Convention list of POPs. The present results may thus be used in support of such an action since it is evident that SCCP are indeed transported to the remote Arctic/eastern North Atlantic environment, and bioaccumulate in the Greenland shark.

The occurrence of "dioxins" presented in **Paper I** are from a human exposure point of view, of special interest since the Greenland shark muscle tissue is part of the traditional human diet in Iceland, although only eaten occasionally linked to a certain season and consumed in low volumes. Figure 5.1 shows the concentrations of DL-PCBs and PCDDs/Fs expressed as pg WHO-TEQ/ g w.w. based on calculations using WHO TEF values from 1998 [168]. All but two individual Greenland shark muscle samples exceeds the EU maximum limit for human consumption, 8 pg WHO-TEQ/g w.w. [169]. The median value (14 pg WHO-TEQ/g w.w.) is comparable to concentrations reported in fish (salmon and herring) from the Baltic Sea [170]. Liver TEQ concentrations are also included in Figure 5.1 for comparison, showing a similar specific liver retention of the PCDDs/Fs and DL non-*ortho* PCBs as reported in other species [171-173].

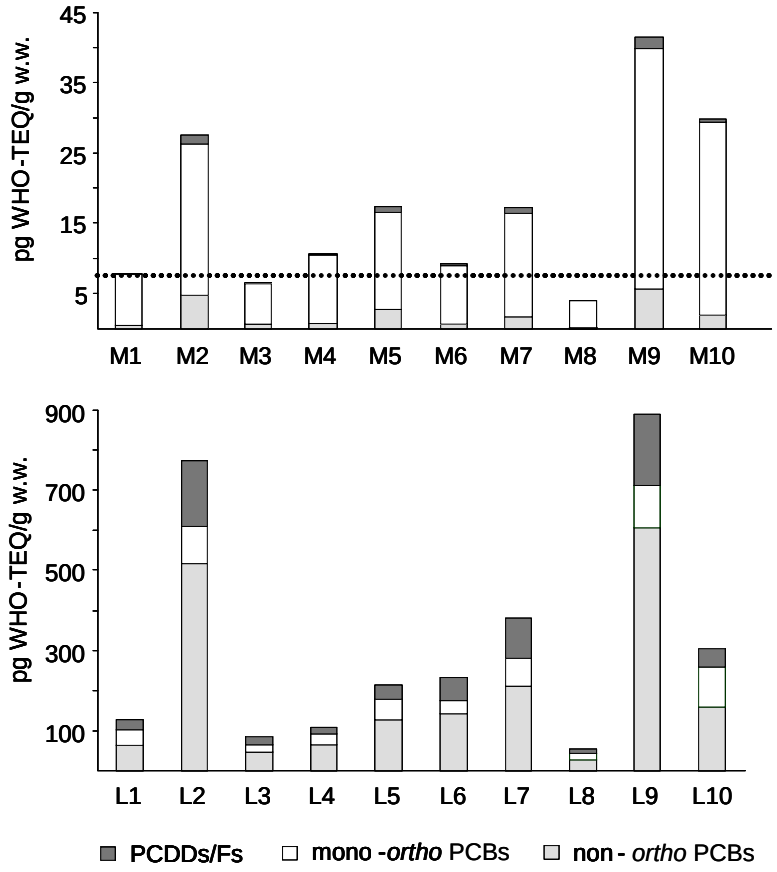

Figure 5.1. Concentrations of dioxins expressed as pg WHO-TEQ/g w.w. in Greenland shark muscle (M) and liver (L). The dotted line represents the EU maximum limit for fish muscle intended for human consumption.

It needs to be mentioned that both the biology and the behaviour of the Greenland shark are very poorly known. Still some, but uncertain data, are available as e.g. the slow growth rate, 0.5 to 1 cm/year, suggested for the Greenland shark [14]. Accordingly there may be a substantial age difference among the sharks studied in this thesis with lengths between 3.5 and 4.8 meters. One of the aims of this thesis was to determine age (size) related accumulation of OHCs in Greenland shark. As shown in Figure 5.2 there are large variations in concentration for several of the OHCs studied, differences that could not be related to the size of the sharks.

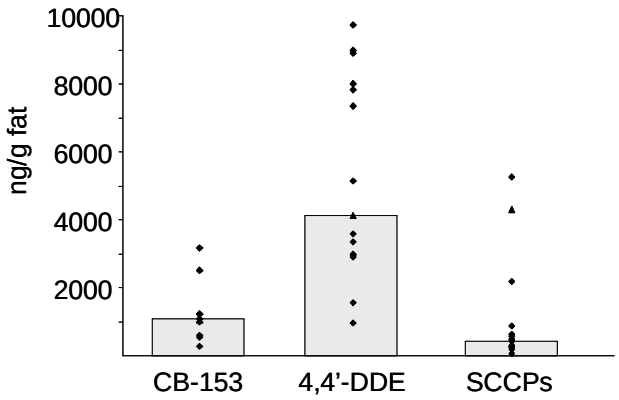

Figure 5.2. Individual variations in concentrations for some organohalogen contaminants (OHCs) in Greenland shark liver (the bars show the median concentrations).

The stomach content of 22 Greenland sharks from Icelandic waters were investigated by McMeans et al. 2010 [23]. Teleost fishes were found to contribute the most and redfish (*Sebastes marinus/mentella*) were found in 55% of the stomachs. Fragments of unidentified mammals, pinnipeds and dolphins/porpoises were found in 4.5%, 14% and 14% of the stomachs, respectively. Most interestingly, polar bear tissues (leg and skin) was present in 4.5% of the stomachs [23]. Hence, fish seems to be the major part of the Greenland shark diet while high trophic species, like seals, are consumed less frequent but definitely a significant part of the diet. The large concentration differences observed for the OHCs investigated could be explained by the reported opportunistic feeding behaviour of the Greenland shark. Accordingly, large adult Greenland sharks are suitable to use as a monitoring species when the aim solely is to study what OHCs are present in the deep Arctic waters. This data will contribute to the understanding of long range transport, persistency and bioaccumulativity of chemicals.

5.2 Metabolic capacity of the Greenland shark

The biology of sharks is poorly known in general, therefore is the knowledge of their metabolism of anthropogenic compounds very limited as well. The metabolism in these species has been suggested to be "primitive" and their metabolic rates seem to be lower than in the more advanced teleost fish, as reviewed by Ballantyne 1997 [174]. This seems to be true for deep water species while more active sharks in temperate waters may have metabolic rates similar to the teleost fish [174]. One explanation for this could to be that sharks have lower blood concentrations of hemoglobin and lower hematocrite values compared to teleost fish and hence a lower capacity for oxygen delivery [174,175]. Enzymes used to metabolise exogenous compounds are present in elasmobranches, Cytochrome P450 and glutathione S-transferase have for instance been found in liver as well as in other tissues [176,177]. It has also been suggested that the large sizes of their livers should allow a great capacity to eliminate xenobiotics [178]. However, a dosage experiment where ^{14}C 4,4'-DDT were administered to piked dogfish showed that 4,4'-DDT was stored in liver and probably not metabolised or excreted at any appreciable rate [179]. The metabolism of OHCs in livers of elasmobranches may therefore be very slow.

The pattern of DDTs reported in **Paper IV** might be an indication of a similar limited metabolic capacity of the Greenland shark. The ratios between 4,4'-DDE and 4,4'-DDT in Greenland shark muscle and liver is shown in Figure 5.3 together with ratios reported in other species from the eastern North Atlantic. The highest 4,4'-DDE/4,4'-DDT ratios observed is in black guillemot (*Cepphus grylle*) followed by harbour seal (*Phoca vitulina*) and gray seal (*Halichoerus grypus*) [180,181]. This is in accordance with an enhanced metabolic capacity for those species compared to fish. It is worth to stress that the 4,4'-DDE/4,4'-DDT ratios are not really different among any of the fish species, sharks included (Figure 5.3). The ratio determined in red fish, one of the major prey species for Greenland sharks from the eastern North Atlantic, was also low [23,77]. The lowest 4,4'-DDE/4,4'-DDT ratio was seen in black dogfish, with an average ratio below one [77].

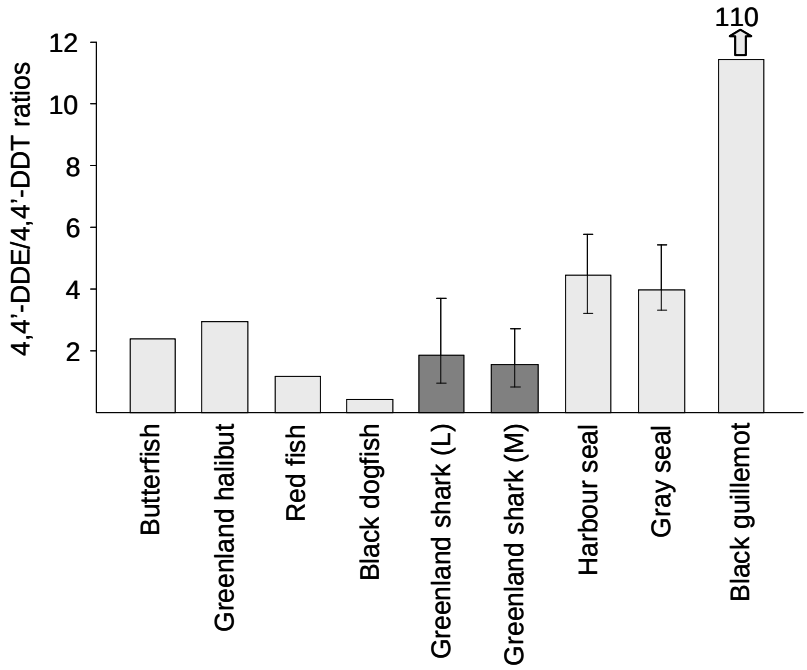

Figure 5.3. 4,4'-DDE/4,4'-DDT ratios in different species from the eastern North Atlantic [77,180,181]. For Greenland shark and the two seal species are medians and ranges shown, for the other species included are ratios calculated from average concentrations.

The median values for 4,4'-DDT reported in Greenland shark muscle (2700 ng/g fat) and liver (2200 ng/g fat) is considerable higher than in any of the other species included in Figure 5.3 [77,180,181]. As presented in **Paper IV,** all components of technical DDT and their metabolites are present in the Greenland shark. This is probably not the result of a lower capacity to metabolise DDTs compared to other deep water fish and shark species from the eastern North Atlantic but rather a reflection of exposure over a long period of time due to the Greenland sharks assumed long life span. The piked dogfish samples from Japan included in Chapter 4 had 4,4'-DDE/4,4'-DDT ratios ranging between 3.1 and 7.5 and even higher ratios were seen in dusky shark and great hammer head (up to 140 in dusky shark). This supports the hypothesis that sharks living in temperate waters possess a better metabolic capacity relative to slow deep water species.

The occurrence and concentrations of $MeSO_2$-PCBs and 3-$MeSO_2$-DDE in Greenland shark are given in **Paper IV**. It is questionable if the presence of these metabolites is a result of metabolic formation in the shark or due to bioaccumulation from ingested mammals. Bioaccumulation of $MeSO_2$-PCBs have for instance shown to occur in the polar bear food chain [109]. The knowledge about $MeSO_2$-PCBs in fish is quite limited but metabolic formation

have shown to occur in fish [182,183]. The capacity to metabolise PCBs to sulfones is considered to be low in fish compared to marine mammals and birds [184]. Accordingly, the low ΣMeSO$_2$-PCB/ΣPCB and 3-MeSO$_2$-DDE/4,4'-DDE ratios in Greenland shark (**Paper IV**) compared to Arctic marine mammals was expected [109,110]. The source of MeSO$_2$-compounds in the Greenland shark remain to be confirmed but possibly their presence is a result of both accumulation from their diet and slow metabolic formation.

No difference was seen between muscle and liver concentrations of MeSO$_2$-PCBs in Greenland shark. This is different from marine mammals where higher concentrations have been reported in liver than in other tissues, believed to be the result of protein binding [185-187]. It has however also been suggested to depend on the lipid composition of the liver, i.e. that the slightly less hydrophobic MeSO$_2$-PCBs, compared to PCBs, might be partitioned more efficiently into polar lipids in liver tissue than the parent compounds [186]. Polar lipids such as phospholipids and cholesterol are however only minor constituents in shark livers as reviewed by Ballantyne 1997 [174]. This might explain the lack of retention of MeSO$_2$-compounds in Greenland shark liver.

5.3 Comparison of some OHCs in the shark species studied

The highest concentrations for PCBs, DDTs and PBDEs were determined in the two dusky sharks from Australia comparing data from the other shark species investigated (Table 4.1). The highest ∑PBDE concentration was 250 ng/g fat in one of the dusky sharks, similar to the highest concentration in Greenland shark liver (200 ng/g fat). The dusky shark is, like the Greenland shark considered to be an opportunistic feeder, a behaviour that may induce relatively large variation in OHC concentrations. The OHC concentrations were rather similar between the five piked dogfish individuals analysed and reported in Chapter 4. Possibly this indicates a different feeding behaviour. The piked dogfish might therefore possibly be a suitable species in food web studies and for estimations of size/age related accumulation of OHCs in sharks.

It is notable that HBCDD was detected at rather high concentrations in the piked dogfish from Japan. Concentrations were ranging between 63 and 250 ng/g fat (mean 150 ng/g fat) and exceeded the concentrations of ΣPBDE by a factor of 3.9 to 12. This is in accordance with a general trend observed in marine mammals from Japan during recent years, reflecting an increased use of HBCDD over PBDEs in Japan [188]. The HBCDD concentrations in the piked dogfish were much higher than in Greenland shark, and were in fact similar to concentrations reported in shark species from the US [88], see Table 2.2.

Geographical differences were also observed for the HCHs, which only was present in piked dogfish from Japan. Concentrations ranged between 24 and 29 ng/g fat, slightly higher compared to what has been reported in hammerhead sharks, also from Japan [100] (Table 2.2). Levels of HCHs have shown not to be decreasing in marine mammals from Japan between 1978 and 2003 implying input to the Japanese environment from other Asian countries where HCHs have been used in large amounts [189]. China has, for instance, consumed the highest amount of technical HCH globally as reviewed by Li et al. 2005 [116], something that may serve as a potential explanation for the situation.

Neutral brominated compounds, assumed to be of natural origin, were present in all four species studied (Chapter 4 and **Paper II**). This is however not further discussed in this thesis. Brominated phenolic compounds, also of assumed natural origin, identified in both liver of dusky shark and piked dogfish are 2,4,6-tribromophenol, 6-OH-BDE47 and 6-OH-BDE137 (see Figure 4.3). 2'-OH-BDE68 and 2,2'-diOH-BB80 were also identified in both species although only at trace levels in piked dogfish. As shown in Figure 4.3 there are still several unidentified peaks in the fraction containing phenolic compounds, in both species. Only 2,4,6-TBP, 2'-OH-BDE68 and 6-OH-BDE47 were found in low levels in the Greenland sharks (**Paper II**). The only other publication available for brominated phenolic compounds in sharks, is a study on tiger shark and bull shark from Japan reporting the presence of 6-OH-BDE47, 2'-OH-BDE68 and 2,2'-diOH-BB80 [139]. The brominated phenolic compounds discussed are all assumed to be of natural origin in the shark species studied and the differences observed may be an indication of geographical differences for production of these compounds. It must be pointed out that metabolic formation of 6-OH-BDE47 from BDE-47 cannot be ruled out to contribute, at least to some extent, to the levels of this particular phenolic compound.

6 Concluding remarks

The work presented in this thesis expand our knowledge on environmental contaminants and sharks, where data otherwise are quite limited. The contribution is in particular related to the Greenland shark. The thesis contributes to the knowledge of organohalogen contaminants in this deep water species in the remote sub-Arctic/Arctic environment. The screening of traditional OHCs and in some cases their metabolites together with some new and emerging OHCs in Greenland shark presented here is by far the most extensive dataset available for any shark species in the scientific literature so far.

Most of the targeted contaminants were present in the Greenland sharks studied, and in some individuals at quite elevated concentrations. The sizes of the sharks used for the analyses ranged between 3.5 and 4.8 meters. Assuming a growth rate of 0.5 to 1 cm/year there could be a substantial age difference among the individuals studied. Even so could no size/age related accumulation be indicated. All sharks sampled for this thesis were most likely born prior to the large scale production and use of OHCs. Further, the Greenland sharks studied have all consumed marine mammals like seals more or less frequently, and this opportunistic feeding behaviour is probably reflected in the concentration differences observed in the different studies (**Papers I-IV**). Fish are in general assumed to have a lower capacity of biotransformation of OHCs compared to marine mammals and birds, and based on the results presented herein, this appears to be true also for a deep water shark species like the Greenland shark. More active shark species, present in temperate waters, may however have an enhanced capacity to metabolize OHCs.

The main conclusion of this thesis is that the Greenland shark and similar species seem to have a great potential to be used for screening of novel and emerging environmental contaminants, to alert us regarding environmental contamination by chemicals in the marine environments. A full scan GC/MS (ECNI) chromatogram is shown in Figure 6.1 indicating the occurrence of a large number of hitherto unknown chemicals present in the Greenland shark. Only a fraction of all contaminants present in the shark has been elucidated and included in this thesis, leaving much more to be done in the future.

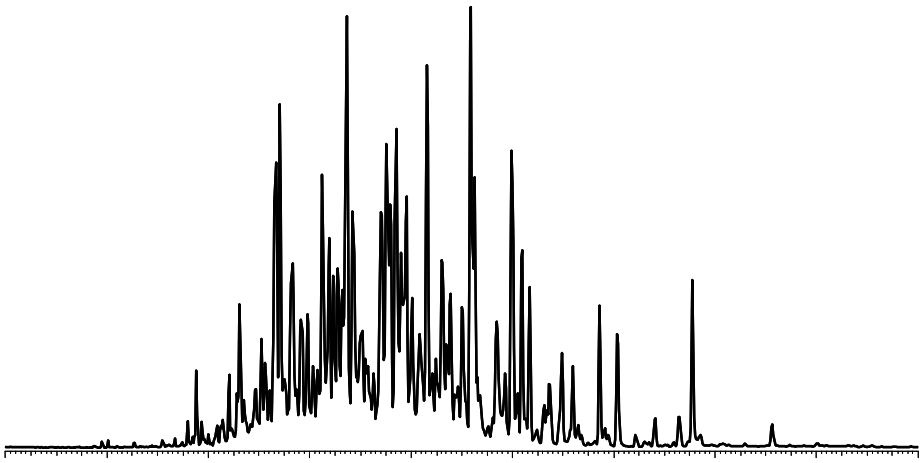

Figure 6.1. A GC/MS (ECNI) full scan chromatogram from a Greenland shark liver sample.

Other conclusions; It is clear from the present work that it is not possible to use the Greenland shark for neither temporal trend studies nor food web studies regarding uptake and fate of POPs. The reason is that the Greenland shark is a long lived shark species with highly opportunistic feeding behaviour. Further, a low metabolic capacity may also influence the species potential for temporal trend studies. It is probably also difficult to use species like this for food web studies since the diet will differ between individuals.

Future studies on Greenland shark should however include smaller/younger sharks since that would probably be a more accurate reflection of the present contamination status. It should also be noted that the possible impact the OHCs present may have on the health and reproduction of the Greenland shark is beyond the scope of this thesis. This is however something that also should be considered in future investigations.

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8 References

1. Breivik K., Alcock R., Li Y.F., Bailey R.E., Fiedler H., Pacyna J.M. (2004). Primary sources of selected POPs: regional and global scale emission inventories. *Environmental Pollution*, 128, 3-16.

2. Gribble G.W. (2003). The diversity of naturally produced organohalogens. *Chemosphere*, 52, 289-297.

3. Bignert, A, Danielsson S., Nyberg E., Asplund L., Eriksson U., Berger U., Haglund P. (2009). Comments concerning the national Swedish contaminant monitoring program in marine biota 2009. Swedish Museum of Natural History. http://www.nrm.se/download/18.6321786f122df65955f80002587/Marina+programmet+2009.pdf.

4. AMAP (1998): AMAP Assessment Report: Arctic Pollution Issues. Arctic Monitoring and Assessment Programme (AMAP), Oslo, Norway, pp xii-859.

5. AMAP (2004): AMAP Assessment 2002: Persistent Organic Pollutants in the Arctic. Arctic Monitoring and Assessment Programme (AMAP), Oslo, Norway, pp xvi-310.

6. de Wit C.A., Alaee M., Muir D.C.G. (2006). Levels and trends of brominated flame retardants in the Arctic. *Chemosphere*, 64, 209-233.

7. Letcher R.J., Bustnes J.O., Dietz R., Jenssen B.M., Jorgensen E.H., Sonne C., Verreault J., Vijayan M.M., Gabrielsen G.W. (2010). Exposure and effects assessment of persistent organohalogen contaminants in arctic wildlife and fish. *The Science of the Total Environment*, 408, 2995-3043.

8. Riget F., Bignert A., Braune B., Stow J., Wilson S. (2010). Temporal trends of legacy POPs in Arctic biota, an update. *The Science of the Total Environment*, 408, 2874-2884.

9. de Wit C.A., Herzke D., Vorkamp K. (2010). Brominated flame retardants in the Arctic environment - trends and new candidates. *The Science of the Total Environment*, 408, 2885-2918.

10. Chami M., Fowler S.L., Musick J.A., Bräutigam, A, Fordham S. (1998). Sharks and their relatives - ecology and conservation. IUCN/SCC Shark Specialist group. IUCN, Gland, Switzerland and Cambridge, UK. iv-39

11. Fowler S.L., Cavanagh R.D., Chami M., Burgess G.H., Cailliet G.M., Fordham S., Simpfendorfer C.A., Musick J.A. (2005). Sharks, rays and chimaeras: The

status of the chondrichthyan fishes. IUCN/SCC Shark Specialist group. IUCN, Gland, Switzerland and Cambridge, UK. x-461

12. Cortés E. (1999). Standardized diet composition and trophic levels of sharks. *ICES Journal of Marine Science*, 707-717.

13. Compagno L.J.V. (1984). FAO species catalogue. Vol 4. Sharks of the world. An annotated and illustrated catalogue of shark species known to date. Part 1 Hexanchiformes to Lamniformes. FAO Fisheries Synopsis 125.

14. Hansen P.M. (1963). Tagging experiments with the Greenland shark (*Somniosus microcephalus* (Bloch and Schneider)) in Subarea 1. *International Commission Northwest Atlantic Fisheries Special Publication*, 4, 172-175.

15. Compagno L.J.V. (1984). FAO species catalogue. Vol 4. Sharks of the world. An annotated and illustrated catalogue of shark species known to date. Part 2 Carcharhiniformes. FAO Fisheries Synopsis 125.

16. Greenland shark (*Somniosus microcephalus*) (2010). The Greenland shark and elasmobranch education and research group. http://www.geerg.ca/gshark1.htm

17. Bigelow HB, Schroeder WC (1948): Sharks. Tee-Van J (ed) In: Fishes of the Western North Atlantic, Part 1. Yale University, New Haven, Connecticut, pp 59-546.

18. Skomal G.B., Benz G.W. (2004). Ultrasonic tracking of Greenland sharks (*Somniosus microcephalus*) under Arctic ice. *Marine Biology*, 145, 489-498.

19. Herdendorf C.E., Berra T.B. (1995). A Greenland shark from the wreck of the SS Central America at 2200 meters. *Transactions of the American Fisheries Society*, 950-953.

20. Stokesbury Michael J.W., Harvey-Clark Chris, Gallant Jeffrey, Block Barbara A., Myers Ransom A. (2005). Movement and environmental preferences of Greenland sharks (*Somniosus microcephalus*) electronically tagged in the St. Lawrence Estuary, Canada. *Marine Biology*, 159-165.

21. Yano K., Stevens J.D., Compagno L.J.V. (2007). Distribution, reproduction and feeding of the Greenland shark *Somniosus (Somniosus) microcephalus*, with notes on two other sleeper sharks, *Somniosus (Somniosus) pacificus* and *Somniosus (Somniosus) antarcticus*. *Journal of Fish Biology*, 70, 374-390.

22. Fisk A.T., Tittlemier S., Pranschke J., Norstrom R.J. (2002). Using anthropogenic contaminants and stable isotopes to assess the feeding ecology of Greenland shark. *Ecology*, 83, 2162-2172.

23. McMeans B.C., Svavarsson J., Dennard S., Fisk A.T. (2010). Diet and resource use among Greenland sharks (*Somniosus microcephalus*) and teleosts sampled

in Icelandic waters, using $\delta^{13}C$, $\delta^{15}N$, and mercury. *Canadian Journal of fisheries and aquatic sciences*, 67, 1428-1438.

24. Greenland shark (2010). Icelandic fisheries, Icelandic Ministry of Fisheries and Agriculture. http://www.fisheries.is/main-species/cartilaginous-fishes/greenland-shark/

25. Kyne P.M., Sherill-Mix S.A., and Burgess G.H. (2006). *Somniosus microcephalus* (2010). IUCN 2010. IUCN Red list of threatened species. Version 2010.2. www.iucnredlist.org

26. Fordham S., Fowler S.L., Coelho R., Goldman K.J., and Francis M. (2006). *Squalus achantias* (2010). IUCN 2010. IUCN Red list of threatened species. Version 2010.2. www.iucnredlist.org

27. Musick J.A., Grubbs R.D., Baum J., and Cortés E. (2007). *Carcharihinus obscurus* (2010). IUCN 2010. IUCN Red list of threatened species. Version 2010.2. www.iucnredlist.org

28. Denham J., Stevens J., Simpfendorfer C.A., Heupel M.R., Cliff G., Morgan A., Graham R., Ducroq M, Dulvy N.D., Seisay M., Asber M., Valenti S.V., Litvinov F., Martins P P., Lemine Ould Sidi M., Tous P., and Bucal D. (2007). *Sphyrna mokarran* (2010). IUCN 2010. IUCN Red list of threatened species. Version 2010.2. www.iucnredlist.org

29. UNEP. Stockholm Convention on persistent organic pollutants (POPs). (2010) Stockholm Convention. http://chm.pops.int/

30. Waid S. (1986): PCBs and the Environment I-III. CRC Press.

31. WHO (2002): Global assessment of the state-of-the-science of endocrine disruptors. Damstra, T., Barlow, S., Bergman, Å., Kavlock, R., and van der Kraak, G. International programme on chemical safety, WHO.

32. Letcher RJ, Klasson Wehler E, Bergman Å (2000): Methyl sulfone and hydroxylated metabolites of polychlorinated biphenyls. Paasivirta J (ed) In: New Types of Persistent Halogenated Compounds. 1 edn. Springer-Verlag, Berlin, pp 315-359.

33. Cox P., Efthymiou P. (2003). Directive 2003/11/EC of the European parliament and of the council of February 6 2003 amending for the 24th time Council Directive 76/669/EEC relating to restrictions on the marketing and use of certain dangerous substances and preparations (pentabromodiphenyl ether, octabromodiphenyl ether). *Official Journal of the European Union*, 42, 45-46.

34. Cox P., Drys G. (2003). Directive 2002/95/EC of the European Parliament and of the Council of 27 January 2003 on the restriction of the use of certain

hazardous substances in electrical and electronic equipment. *Official Journal of the European Union*, 37, 19-23.

35. U.S.Environmental Protection Agency (US EPA). Polybrominated diphenyl ethers (PBDEs) Action Plan (2009). US EPA. http://www.epa.gov/oppt/existingchemicals/pubs/pbdes_ap_2009_1230_final.pdf

36. Law R.J., Herzke D., Harrad S., Morris S., Bersuder P., Allchin C.R. (2008). Levels and trends of HBCD and BDEs in the European and Asian environments, with some information for other BFRs. *Chemosphere*, 73, 223-241.

37. Darnerud P.O. (2003). Toxic effects of brominated flame retardants in man and in wildlife. *Environment International*, 29, 841-853.

38. Vonderheide A.P., Mueller K.E., Meija J., Welsh G.L. (2008). Polybrominated diphenyl ethers: Causes for concern and knowledge gaps regarding environmental distribution, fate and toxicity. *The Science of the Total Environment*, 400, 425-436.

39. Kierkegaard A., Burreau S., Marsh G., Klasson Wehler E., de Wit C., and Asplund L. (2001). Metabolism and distribution of 2,2',4,4'-tetrabromo[^{14}C]diphenyl ether in pike (*Esox lucius*) after dietary exposure. *Organohalogen Compounds*, 52, 58-61.

40. Marsh G., Athanasiadou M., Athanassiadis I., Sandholm A. (2006). Identification of hydroxylated metabolites in 2,2',4,4'-tetrabromodiphenyl ether exposed rats. *Chemosphere*, 63, 690-697.

41. Stapleton H.M., Kelly S.M., Pei R., Letcher R.J., Gunsch C. (2009). Metabolism of polybrominated diphenyl ethers (PBDEs) by human hepatocytes in vitro. *Environmental Health Perspectives*, 117, 197-202.

42. Hakk H., Letcher R.J. (2003). Metabolism in the toxicokinetics and fate of brominated flame retardants - a review. *Environment International*, 29, 801-828.

43. Athanasiadou M., Cuadra S.N., Marsh G., Bergman Å., Jakobsson K. (2008). Polybrominated diphenyl ethers (PBDEs) and bioaccumulative hydroxylated PBDE metabolites in young humans from Managua. *Environmental Health Perspectives*, 116, 400-408.

44. Valters K., Li H., Alaee M., D'Sa I., Marsh G., Bergman Å., Letcher R.J. (2005). Polybrominated diphenyl ethers and hydroxylated and methoxylated brominated and chlorinated analogues in the plasma of fish from the Detroit River. *Environmental Science and Technology*, 39, 5612-5619.

45. Browne E.P., Stapleton H.M., Kelly S.M., Tilton S.C., Gallagher E.P. (2009). In vitro hepatic metabolism of 2,2',4,4',5-pentabromodiphenyl ether (BDE 99) in Chinook salmon *(Onchorhynchus tshawytscha)*. *Aquatic Toxicology*, 92, 281-287.

46. Stapleton H.M., Letcher R., Baker J.E. (2004). Debromination of polybrominated diphenyl ether congeners BDE-99 and BDE-183 in the intestinal tract of the common carp (*Cyprinus carpio*). *Environmental Science and Technology*, 38, 1054-1061.

47. Tomy G.T., Palace V.P., Halldorson T., Braekevelt E., Danell R., Wautier K., Evans B., Brinkworth L., Fisk A.T. (2004). Bioaccumulation, Biotransformation, and Biochemical Effects of Brominated Diphenyl Ethers in Juvenile Lake Trout (*Salvelinus namaycush*). *Environmental Science and Technology*, 38, 1496-1504.

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

49. Hoh E., Zhu L., Hites R.A. (2005). Novel Flame Retardants, 1,2-Bis(2,4,6-tribromophenoxy)ethane and 2,3,4,5,6-Pentabromoethylbenzene, in United States' Environmental Samples. *Environmental Science and Technology*, 39, 2472-2477.

50. OSPAR commision. The OSPAR list of chemicals of possible concern 2002. (2010) www.ospar.org.

51. Great Lakes Chemical Corporation: Report on Firemaster 550, 2004. (2010) Great Lakes Chemical Corporation. http://www.pu2pu.com/htdocs/customers/greatlakes/firemaster.htm

52. Gauthier L.T., Hebert C.E., Weseloh D.V.C., Letcher R.J. (2007). Current-use flame retardants in the eggs of herring gulls (*Larus argentatus*) from the Laurentian Great Lakes. *Environmental Science and Technology*, 41, 4561-4567.

53. Ismail N., Gewurtz S.B., Pleskach K., Whittle D.M., Helm P.A., Marvin C.H., Tomy G.T. (2009). Brominated and chlorinated flame retardants in Lake Ontario, Canada, lake trout (*Salvelinus namaycush*) between 1979 and 2004 and possible influences of food-web changes. *Environmental Toxicology and Chemistry*, 28, 910-920.

54. Shi T., Chen S.J., Luo X.J., Zhang X.L., Tang C.M., Luo Y., Ma Y.J., Wu J.P., Peng X.Z., Mai B.X. (2009). Occurrence of brominated flame retardants other than polybrominated diphenyl ethers in environmental and biota samples from southern China. *Chemosphere*, 74, 910-916.

55. Karlsson M., Eriksson I., van Bavel B., Jensen J.-K., Dam M. (2006). Levels of brominated flame retardants in Northern Fulmar (*Fulmarus glacialis*) eggs from the Faroe Islands. *Science of the Total Environment*, 367, 840-846.

56. Tomy G.T., Ismail N., Pleskach K., Danell R., and Stern G. (2007). Temporal and spatial trends of brominated and chlorinated flame retardants in beluga (*Delphinapterus leucas*) from the Canadian Arctic. *4th International workshop on brominated flame retardants (BFR 2007), 24-27 April, 2007*

57. Verreault J., Gebbink W.A., Gauthier L.T., Gabrielsen G.W., Letcher R.J. (2007). Brominated Flame Retardants in Glaucous Gulls from the Norwegian Arctic: More Than Just an Issue of Polybrominated Diphenyl Ethers. *Environmental Science and Technology*, 41, 4925-4931.

58. Hermanson M.H., Isaksson E., Forsstrom S., Teixeira C., Muir D.C.G., Pohjola V.A., van de Wal R.S.V. (2010). Deposition History of Brominated Flame Retardant Compounds in an Ice Core from Holtedahlfonna, Svalbard, Norway. *Environmental Science and Technology*, 44, 7405-7410.

59. Tomy G.T., Palace V.P., Pleskach K., Ismail N., Oswald T., Danell R., Wautier K., Evans B. (2007). Dietary Exposure of Juvenile Rainbow Trout (Oncorhynchus mykiss) to 1,2-bis(2,4,6-tribromo- phenoxy)ethane: Bioaccumulation Parameters, Biochemical Effects, and Metabolism. *Environmental Science and Technology*, 41, 4913-4918.

60. Hakk H., Larsen G., Bowers J. (2004). Metabolism, tissue disposition, and excretion of 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE) in male Sprague-Dawley rats. *Chemosphere*, 54, 1367-1374.

61. Hamers T., Kamstra J.H., Sonneveld E., Murk A.J., Kester M.H.A., Andersson P.L., Legler J., Brouwer A. (2006). In Vitro Profiling of the Endocrine-Disrupting Potency of Brominated Flame Retardants. *Toxicological Sciences*, 92, 157-173.

62. Norman Halden A., Nyholm J.R., Andersson P.L., Holbech H., Norrgren L. (2010). Oral exposure of adult zebrafish (*Danio rerio*) to 2,4,6-tribromophenol affects reproduction. *Aquatic Toxicology*, 100, 30-37.

63. WHO (1996). Environmental Health Criteria 181. Chlorinated paraffins. International Program on Chemical Safety, World Health Organization. Geneva, Switzerland

64. Cox P., Matas I Palou J. (2002). Directive 2002/45/EC of the European parliament and of the council of June 2002 amending for the twentieth time Council Directive 76/769/EEC relating to restrictions on the marketing and use of certain dangerous substances and preparations. *Official Journal of the European Communities*, L177, 21-22.

65. Bayen S., Obbard J.P., Thomas G.O. (2006). Chlorinated paraffins: A review of analysis and environmental occurrence. *Environment International*, 32, 915-929.

66. Feo M.L., Eljarrat E., Barcelo D., Barcelo D. (2009). Occurrence, fate and analysis of polychlorinated n-alkanes in the environment. *Trends in Analytical Chemistry*, 28, 778-791.

67. Key B.D., Howell R.D., Criddle C.S. (1997). Florinated organics in the biosphere. *Environmental Science and Technology*, 31, 2445-2454.

68. Butt C.M., Berger U., Bossi R., Tomy G.T. (2010). Levels and trends of poly- and perfluorinated compounds in the arctic environment. *The Science of the Total Environment*, 408, 2936-2965.

69. Giesy J.P., Kannan K. (2001). Global distribution of perfluorooctane sulfonate in wildlife. *Environmental Science and Technology*, 35, 1339-1342.

70. Houde M., Martin J.W., Letcher R.J., Solomon K.R., Muir D.C.G. (2006). Biological Monitoring of Polyfluoroalkyl Substances: A Review. *Environmental Science and Technology*, 40, 3463-3473.

71. Tomy G.T., Tittlemier S.A., Palace V.P., Budakowski W.R., Braekevelt E., Brinkworth L., Friesen K. (2004). Biotransformation of N-Ethyl Perfluorooctanesulfonamide by Rainbow Trout (*Onchorhynchus mykiss*) Liver Microsomes. *Environmental Science and Technology*, 38, 758-762.

72. Johnson-Restrepo B., Kannan K., Addink R., Adams D.H. (2005). Polybrominated diphenyl ethers and polychlorinated biphenyls in a marine foodweb of coastal Florida. *Environmental Science and Technology*, 39, 8243-8250.

73. Haraguchi K., Hisamichi Y., Kotaki Y., Kato Y., Endo T. (2009). Halogenated bipyrroles and methoxylated tetrabromodiphenyl ethers in tiger shark *(Galeocerdo cuvier)* from the southern coast of Japan. *Environmental Science and Technology*, 43, 2288-2294.

74. Davis J.A., May M.D., Greenfield B.K., Fairey R., Roberts C., Ichikawa G., Stoelting M.S., Becker J.S., Tjeerdema R.S. (2002). Contaminant concentrations in sport fish from San Francisco Bay, 1997. *Marine Pollution Bulletin*, 44, 1117-1129.

75. Gelsleichter J., Szabo N.J., Belcher C.N., Ulrich G.F. (2008). Organochlorine contaminants in bonnethead sharks (*Sphyrna tiburo*) from Atlantic and Gulf estuaries on the US east coast. *Marine Pollution Bulletin*, 56, 359-363.

76. Holden A., She J., Tanner M., Lunder S., Sharp R., Hooper K. (2003). PBDEs in the San Francisco Bay area: measurements in fish. *Organohalogen Compounds*, 61, 255-258.

77. Berg V., Ugland K.I., Hareide N.R., Aspholm P.E., Polder A., Skaare J.U. (1997). Organochlorine contamination in deep-sea fish from the Davis Strait. *Marine Environmental Research*, 44, 135-148.

78. Webster L., Walsham P., Russell M., Neat F., Phillips L., Dalgarno E., Packer G., Scurfield J.A., Moffat C.F. (2009). Halogenated persistent organic pollutants in Scottish deep water fish. *Journal of Environmental Monitoring*, 11, 406-417.

79. Storelli M.M., Marcotrigiano G.O. (2001). Persistent organochlorine residues and toxic evaluation of polychlorinated biphenyls in sharks from the Mediterranean Sea (Italy). *Marine Pollution Bulletin*, 42, 1323-1329.

80. Storelli M.M., Ceci E., Storelli A., Marcotrigiano G.O. (2003). Polychlorinated biphenyl, heavy metal and methylmercury residues in hammerhead sharks: contaminant status and assessment. *Marine Pollution Bulletin*, 46, 1035-1039.

81. Storelli M.M., Storelli A., Marcotrigiano G.O. (2003). Coplanar polychlorinated biphenyl congeners in the liver of Galeus melastomus from different areas of the Mediterranean Sea. *Bulletin of Environmental Contamination and Toxicology*, 71, 276-282.

82. Storelli M.M., Barone G., Santamaria N., Marcotrigiano G.O. (2006). Residue levels of DDTs and toxic evaluation of polychlorinated biphenyls (PCBs) in *Scyliorhinus canicula* liver from the Mediterranean Sea (Italy). *Marine Pollution Bulletin*, 52, 696-700.

83. Storelli M.M., Storelli A., Marcotrigiano G.O. (2005). Concentrations and hazard assessment of polychlorinated biphenyls and organochlorine pesticides in shark liver from the Mediterranean Sea. *Marine Pollution Bulletin*, 50, 850-855.

84. Serrano R., Fernandez M., Rabanal R., Hernadez M., Gonzalez M.J. (2000). Congener-specific determination of polychlorinated biphenyls in shark and grouper livers from the Northwest African Atlantic Ocean. *Archives of Environmental Contamination and Toxicology*, 217-224.

85. de Azevedo e Silva C.E., Azeredo A., de Cassia Lima Dias A., Costa P., Lailson-Brito J., Malm O., Guimaraes J.R.D., Torres J.P.M. (2009). Organochlorine compounds in sharks from the Brazilian coast. *Marine Pollution Bulletin*, 58, 294-298.

86. Eduardo de Azevedo e Silva C., Azeredo A., Lailson-Brito J., Torres J.P.M., Malm O. (2007). Polychlorinated biphenyls and DDT in swordfish (*Xiphias*

gladius) and blue shark (*Prionace glauca*) from Brazilian coast. *Chemosphere*, 67, 48-53.

87. Cornish A.S., Ng W.C., Ho V.C.M., Wong H.L., Lam J.C.W., Lam P.K.S., Leung K.M.Y. (2007). Trace metals and organochlorines in the bamboo shark *Chiloscyllium plagiosum* from the southern waters of Hong Kong, China. *The Science of the Total Environment*, 376, 335-345.

88. Johnson-Restrepo B., Adams D.H., Kannan K. (2008). Tetrabromobisphenol A (TBBPA) and hexabromocyclododecanes (HBCDs) in tissues of humans, dolphins, and sharks from the United States. *Chemosphere*, 70, 1935-1944.

89. Senthil Kumar K., Zushi Y., Masunaga S., Gilligan M., Pride C., Sajwan K.S. (2009). Perfluorinated organic contaminants in sediment and aquatic wildlife, including sharks, from Georgia, USA. *Marine Pollution Bulletin*, 58, 621-629.

90. Nakata H., Kannan K., Nasu T., Cho H.S., Sinclair E., Takemura A. (2006). Perfluorinated contaminants in sediments and aquatic organisms collected from shallow water and tidal flat areas of the Ariake Sea, Japan: environmental fate of perfluorooctane sulfonate in aquatic ecosystems. *Environmental Science and Technology*, 40, 4916-4921.

91. de Wit CA, Strandell M (2000): Levels, sources and trends of dioxins and dioxin-like substances in the Swedish environment - The Swedish dioxin survey. Swedish Environmental Protection Agency, Stockholm, Sweden.

92. Domingo J.L., Bocio A. (2007). Levels of PCDD/PCDFs and PCBs in edible marine species and human intake: a literature review. *Environ Int*, 33, 397-405.

93. LaKind J.S. (2007). Recent global trends and physiologic origins of dioxins and furans in human milk. *Journal of Exposure Science and Environmental Epidemiology*, 17, 510-524.

94. Mandal P.K. (2005). Dioxin: a review of its environmental effects and its aryl hydrocarbon receptor biology. *Journal of Comparative Physiology, B: Biochemical, Systemic, and Environmental Physiology*, 175, 221-230.

95. van den Berg M., Birnbaum L.S., Denison M., De Vito M., Farland W., Feeley M., Fiedler H., Hakansson H., Hanberg A., Haws L., Rose M., Safe S., Schrenk D., Tohyama C., Tritscher A., Tuomisto J., Tysklind M., Walker N., Peterson R.E. (2006). The 2005 World Health Organization Reevaluation of Human and Mammalian Toxic Equivalency Factors for Dioxins and Dioxin-Like Compounds. *Toxicological Sciences*, 93, 223-241.

96. WHO (1997): Environmental Health Criteria 195. Hexachlorobenzene. International Program on Chemical Safety, World Health Organization, Geneva, Switzerland.

97. Barber J.L., Sweetman A.J., van Wijk D., Jones K.C. (2005). Hexachlorobenzene in the global environment: Emissions, levels, distribution, trends and processes. *The Science of the Total Environment*, 349, 1-44.

98. Hsu M.S., Liao C.Y., Cai Y.J., Ling Y.C. (2006). PCDD/Fs and WHO-PCBs in nutritional supplements sold on Taiwan market. *Organohalogen Compounds*, 68, 1916-1919.

99. Schlenk D., Sapozhnikova Y., Cliff G. (2005). Incidence of organochlorine pesticides in muscle and liver tissues of South African great white sharks *Carcharodon carcharias*. *Marine Pollution Bulletin*, 50, 208-211.

100. Nakata H., Hirayama Y., Sakai Y., Takemura A. (2003). Variations in organochlorine concentrations and patterns with growth and trophic status of tidal flat and coastal species from the Ariake Sea, Japan. *Organohalogen Compounds*, 62, 186-189.

101. Berg V., Polder A., Skaare J.U. (1998). Organochlorines in deep-sea fish from the Nordfjord. *Chemosphere*, 38, 275-282.

102. Gelsleichter J., Manire C.A., Szabo N.J., Cortes E., Carlson J., Lombardi-Carlson L. (2005). Organochlorine Concentrations in Bonnethead Sharks (*Sphyrna tiburo*) from Four Florida Estuaries. *Archives of Environmental Contamination and Toxicology*, 48, 474-483.

103. ATSDR (2002). Toxicological profile for DDT, DDE and DDD. U.S Department of health and human services. Public health service. Agency for toxic substances and disease registry. www.atsdr.cdc.gov/toxprofiles.

104. WHO (1989): Environmental Health Criteria 83. DDT and its derivatives - Environmental aspects. International Program on Chemical Safety, World Health Organization, Geneva, Switzerland.

105. WHO (2006). Indoor residual spraying. Use of indoor spraying for scaling up malaria control and elimination. World Health Organisation. Geneva, Switzerland

106. Helander B., Olsson A., Bignert A., Asplund L., Litzén K. (2002). The role of DDE, PCB, Coplanar PCB and eggshell parameters for reproduction in the white-tailed Sea Eagle (*Haliaeetus albicilla*) in Sweden. *Ambio*, 31, 386-403.

107. Turusov V., Rakitsky V., Tomatis L. (2002). Dichlorodiphenyltrichloroethane (DDT): Ubiquity, persistence, and risks. *Environmental Health Perspectives*, 110, 125-128.

108. Chu S., Covaci A., Jacobs W., Haraguchi K., Schepens P. (2003). Distribution of methyl sulfone metabolites of polychlorinated biphenyls and p,p'-DDE in human tissues. *Environmental Health Perspectives*, 111, 1222-1227.

109. Letcher R.J., Norstrom R.J., Muir D.C.G. (1998). Biotransformation versus Bioaccumulation: Sources of Methyl Sulfone PCB and 4,4'-DDE Metabolites in the Polar Bear Food Chain. *Environmental Science and Technology*, 32, 1656-1661.

110. McKinney M.A., De Guise S., Martineau D., Beland P., Lebeuf M., Letcher R.J. (2006). Organohalogen contaminants and metabolites in beluga whale (*Delphinapterus leucas*) liver from two Canadian populations. *Environmental Toxicology and Chemistry*, 25, 1246-1257.

111. Dearth M.A., Hites R.A. (1991). Complete Analysis of technical chlordane using negative ionization mass spectrometry. *Environmental Science and Technology*, 25, 245-254.

112. Vetter W. (2001). Enantioselective fate of chiral chlorinated hydrocarbons and their metabolites in environmental samples. *Food Reviews International*, 17, 113-182.

113. ATSDR (1994). Toxicological profile for Chlordane. U.S Department of health and human services. Public health service. Agency for toxic substances and disease registry.

114. WHO (1984): Environmental Health Criteria 34. Chlordane. International Program on Chemical Safety, World Health Organization, Geneva, Switzerland.

115. WHO (1991): Environmental Health Criteria 124. Lindane. International Program on Chemical Safety, World Health Organization, Geneva, Switzerland.

116. Li Y.F., Macdonald R.W. (2005). Sources and pathways of selected organochlorine pesticides to the Arctic and the effect of pathway divergence on HCH trends in biota: a review. *The Science of the Total Environment*, 342, 87-106.

117. WHO (1992): Environmental Health Criteria 123. Alpha- and Beta-hexachlorocyclohexanes. International Program on Chemical Safety, World Health Organization, Geneva, Switzerland.

118. Aguilar A. (1984). Relationship of DDE/ΣDDT in marine mammals to the chronology of DDT input into the ecosystems. *Canadian Journal of fisheries and aquatic sciences*, 41, 840-844.

119. Handayani D., Edrada R.A., Proksch P., Wray V., Witte L., van Soest R.W.M., Kunzmann A., Soedarson O. (1997). Four new bioactive polybrominated diphenyl ethers of the sponge *Dysidea herbacea* from west Sumatra, Indonesia. *Journal of Natural Products*, 60, 1313-1316.

120. Malmvärn A., Zebühr Y., Kautsky L., Bergman A., Asplund L. (2008). Hydroxylated and methoxylated polybrominated diphenyl ethers and polybrominated dibenzo-p-dioxins in red alga and cyanobacteria living in the Baltic Sea. *Chemosphere*, 72, 910-916.

121. Haglund P.S., Zook D.R., Buser H.R., Hu J. (1997). Identification and quantification of polybrominated diphenyl ethers and methoxy-polybrominated diphenyl ethers in Baltic biota. *Environmental Science and Technology*, 31, 3281-3287.

122. Covaci A., Losada S., Roosens L., Vetter W., Santos F.J., Neels H., Storelli A., Storelli M.M. (2008). Anthropogenic and naturally occurring organobrominated compounds in two deep-sea fish species from the Mediterranean Sea. *Environmental Science and Technology*, 42, 8654-8660.

123. Kelly B.C., Ikonomou M.G., Blair J.D., Gobas F.A.P.C. (2008). Hydroxylated and methoxylated polybrominated diphenyl ethers in a Canadian Arctic marine food web. *Environmental Science and Technology*, 42, 7069-7077.

124. Losada S., Roach A., Roosens L., Santos F.J., Galceran M.T., Vetter W., Neels H., Covaci A. (2009). Biomagnification of anthropogenic and naturally-produced organobrominated compounds in a marine food web from Sydney Harbour, Australia. *Environment International*, 35, 1142-1149.

125. Teuten E.L., Xu L., Reddy C.M. (2005). Two abundant bioaccumulated halogenated compounds are natural products. *Science*, 307, 917-920.

126. Teuten E.L., Reddy C.M. (2007). Halogenated organic compounds in archived whale oil: A pre-industrial record. *Environmental Pollution*, 145, 668-671.

127. Löfstrand K., Malmvärn A., Haglund P., Bignert A., Bergman Å., Asplund L. (2010). Brominated phenols, anisoles, and dioxins present in blue mussels from the Swedish coastline. *Environmental Science and Pollution Research*, 10.1007/s11356-010-0331-1

128. Löfstrand K., Athanassiadis I., Bergman Å., and Asplund L. (2008). 6-Hydroxylated-2,2´,4,4´-tetrabromodiphenyl ether in herring (Clupea harengus) plasma from the Baltic Sea. *Organohalogen Compounds*, 70, 1625-1628.

129. Wan Y., Wiseman S., Chang H., Zhang X., Jones P.D., Hecker M., Kannan K., Tanabe S., Hu J., Lam M.H.W., Giesy J.P. (2009). Origin of hydroxylated brominated diphenyl ethers: Natural compunds or man-made flame retardants? *Environmental Science and Technology*, 43, 7536-7542.

130. Tittlemier S., Borrell A., Duffe J., Duignan P.J., Fair P., Hall A., Hoekstra P., Kovacs K.M., Krahn M.M., Lebeuf M., Lydersen C., Muir D., O'Hara T., Olsson M., Pranschke J., Ross P., Siebert U., Stern G., Tanabe S., Norstrom R. (2002). Global Distribution of Halogenated Dimethyl Bipyrroles in Marine

Mammal Blubber. *Archives of Environmental Contamination and Toxicology*, 43, 244-255.

131. Marsh G., Athanasiadou M., Athanassiadis I., Bergman Å., Endo T., Haraguchi K. (2005). Identification, quantification, and synthesis of a novel dimethoxylated polybrominated biphenyl in marine mammals caught off the coast of Japan. *Environmental Science and Technology*, 39, 8684-8690.

132. Vetter W., Jun W. (2003). Non-polar halogenated natural products bioaccumulated in marine samples. II. Brominated and mixed halogenated compounds. *Chemosphere*, 52, 423-431.

133. Teuten E.L., Pedler B.E., Hangsterfer A.N., Reddy C.M. (2006). Identification of highly brominated analogues of Q1 in marine mammals. *Environmental Pollution*, 144, 336-344.

134. Vetter W., Alder L., Kallenborn R., Schlabach M. (2000). Determination of Q1, an unknown organochlorine contaminant, in human milk, Antarctic air, and further environmental samples. *Environmental Pollution*, 110, 401-409.

135. Gribble G.W. (2000). The natural production of organobromine compounds. *Environmental Science and Pollution Research International*, 7, 37-49.

136. WHO IPCS (2005). Tribromophenols (2,4,6-) and other Simple Brominated Phenols. Concise International Chemical Assessment Document, No 66, Geneva, Switzerland

137. Von Recke R., Vetter W. (2007). Synthesis and Characterization of 2,3-Dibromopropyl-2,4,6-tribromophenyl Ether (DPTE) and Structurally Related Compounds Evidenced in Seal Blubber and Brain. *Environmental science & technology*, 41, 1590-1595.

138. Führer U., Ballschmiter K. (1998). Bromochloromethoxybenzenes in the marine troposphere of the Atlantic ocean: a group of organohalogens with mixed biogenic and anthropogenic orgin. *Environmental Science and Technology*, 32, 2208-2214.

139. Kato Y., Okada S., Atobe K., Endo T., Matsubara F., Oguma T., Haraguchi K. (2009). Simultaneous Determination by APCI-LC/MS/MS of Hydroxylated and Methoxylated Polybrominated Diphenyl Ethers Found in Marine Biota. *Analytical Chemistry*, 81, 5942-5948.

140. Melcher J., Olbrich D., Marsh G., Nikiforov V., Gaus C., Gaul S., Vetter W. (2005). Tetra- and tribromophenoxyanisoles in marine samples from Oceania. *Environmental Science and Technology*, 39, 7784-7789.

141. Coelhan M. (1999). Determination of Short-Chain Polychlorinated Paraffins in Fish Samples by Short-Column GC/ECNI-MS. *Analytical Chemistry*, 71, 4498-4505.

142. Vetter W., Stoll E., Garson M.J., Fahey S.J., Gaus C., Müller J.F. (2002). Sponge halogenated natural products found at parts-per-million levels in marine mammals. *Environmental Toxicology and Chemistry*, 21, 2014-2019.

143. Hansen K.J., Clemen L.A., Ellefson M.E., Johnson H.O. (2001). Compound-specific, quantitative characterization of organic fluorochemicals in biological matrices. *Environmental Science and Technology*, 35, 766-770.

144. Tomy G., Halldorson T., and Tittlemier S. (2005). Methanolic extraction of poly- and perfluorinated alkyl acids from biota. *Organohalogen compounds*, 67, 787-789.

145. Powley C.R., George S.W., Ryan T.W., Buck R.C. (2005). Matrix Effect-Free Analytical Methods for Determination of Perfluorinated Carboxylic Acids in Environmental Matrixes. *Analytical Chemistry*, 77, 6353-6358.

146. Smith L.M., Stalling D.L., Johnson J.L. (1984). Determination of part-per-trillion levels of polychlorinated dibenzofurans and dioxins in environmental samples. *Analytical Chemistry*, 56, 1830-1842.

147. Jensen S., Häggberg L., Jörundsdóttir H., Odham G. (2003). A quantitative lipid extraction method for residue analysis of fish involving nonhalogenated solvents. *Journal of Agricultural and Food Chemistry*, 51, 5607-5611.

148. Tomy G.T., Budakowski W., Halldorson T., Helm P.A., Stern G.A., Friesen K., Pepper K., Tittlemier S.A., Fisk A.T. (2004). Fluorinated Organic Compounds in an Eastern Arctic Marine Food Web. *Environmental Science and Technology*, 38, 6475-6481.

149. Bicking M.K.L., Wilson R.L. (1991). High performance size exclusion chromatography in environmental method development. 1. The effect of mobile phase and temperature on the retention of dioxins, furans and polychlorinated biphenyls. *Chemosphere*, 22, 421-435.

150. Jensen S., Athanasiadou M., and Bergman Å. (1992). A technique for separation of xenobiotics from large amounts of lipids. *Organohalogen Compounds*, 8, 79-80.

151. Hagberg J. (2009). Analysis of brominated dioxins and furans by high resolution gas chromatography/high resolution mass spectrometry. *Journal of Chromatography A*, 1216, 376-384.

152. Reiner E.J. (2010). The analysis of dioxins and related compounds. *Mass Spectrometry Reviews*, 29, 526-559.

153. Päpke O. (1997). Determination of PCDD/Fs in human blood. A fast and sensitive method. *Organohalogen Compounds*, 31, 212-214.

154. Hovander L., Athanasiadou M., Asplund L., Jensen S., Klasson Wehler E. (2000). Extraction and cleanup methods for analysis of phenolic and neutral organohalogens in plasma. *Journal of Analytical Toxicology*, 24, 696-703.

155. Jensen S., Jansson B. (1976). Methyl sulfone metabolites of PCB and DDE. *Ambio*, 5, 257-260.

156. Bergman Å., Athanasiadou M., Bergek S., Haraguchi K., Jensen S., Klasson Wehler E. (1992). PCB and PCB methyl sulfones in mink treated with PCB and various PCB fractions. *Ambio*, 21, 570-576.

157. Cantillana T. (2009). Thesis: Toxicologically important DDT metabolites. Department of Environmental Chemistry, Stockholm University.

158. Tomy G.T., Stern G.A., Muir D.C.G., Fisk A.T., Cymbalisty C.D., Westmore J.B. (1997). Quantifying C10-C13 Polychloroalkanes in Environmental Samples by High-Resolution Gas Chromatography/Electron Capture Negative Ion High-Resolution Mass Spectrometry. *Analytical Chemistry*, 69, 2762-2771.

159. Santos F.J., Parera J., Galceran M.T. (2006). Analysis of polychlorinated n-alkanes in environmental samples. *Anal.Bioanal.Chem.*, 386, 837-857.

160. Friden U., Jansson B., Parlar H. (2004). Photolytic clean-up of biological samples for gas chromatographic analysis of chlorinated paraffins. *Chemosphere*, 54, 1079-1083.

161. Buser H.R. (1986). Selective detection of brominated aromatic compounds using gas chromatography/negative chemical ionization mass spectrometry. *Analytical Chemistry*, 58, 2913-2919.

162. Zencak Z., Borgen A., Reth M., Oehme M. (2005). Evaluation of four mass spectrometric methods for the gas chromatographic analysis of polychlorinated n-alkanes. *Journal of Chromatography A*, 1067, 295-301.

163. Coelhan M., Saraci M., Parlar H. (2000). A comparative study of polychlorinated alkanes as standards for the determination of C10-C13 polychlorinated paraffins in fish samples. *Chemosphere*, 40, 685-689.

164. Tomy G.T., Westmore J.B., Stern G.A., Muir D.C.G., Fisk A.T. (1999). Interlaboratory study on quantitative methods of analysis of C10-C13 polychloro-n-alkanes. *Analytical Chemistry*, 71, 446-451.

165. Peled M., Scharia R., and Sondack D. (1995). Thermal rearrangement of hexabromo-cyclododecane (HBCD). *Industrial Chemistry Library*, 7, 92-99.

166. Björklund J., Tollbäck P., Östman C. (2003). Mass spectrometric characteristics of decabromodophenyl ether and the application of isotopic dilution in the electron capture negative ionization mode for the analysis of polybrominated diphenyl ethers. *Journal of Mass Spectrometry*, 38, 394-400.

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

168. van den Berg M., Birnbaum L., Bosveld B.T.C., Brunström B., Cook P., Feeley M., Giesy J., Hanberg A., Hasegawa R., Kennedy S.W., Kubiak T., Larsen J.C., van Leeuwen F.X.R., Liem A.K.D., Nolt C., Peterson R.E., Poellinger L., Safe S., Schrenk D., Tillitt D., Tysklind M., Younes M., Waern F., Zacharewski T. (1998). Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. *Environmental Health Perspectives*, 106, 775-792.

169. Kyprianou M. (2006). Commission Regulation (EC) No 199/2006 on 3 February 2006 amending Regulation (EC) No 466/2001 setting maximum levels for certain contaminants in foodstuffs as regards dioxins and dioxin-like PCBs. *Official Journal of the European Communities*, L 32:34-38.

170. Ankarberg E., Bjerselius R., Aune M., Darnerud P.O., Larsson L., Andersson A., Tysklind M., Bergek S., Lundstedt-Enkel K., Karlsson L., Törnkvist A., and Glynn A. (2004). Study of dioxin and dioxin-like PCB levels in fatty fish from Sweden 2000-2002. *Organohalogen Compounds*, 66, 2035-2039.

171. Iwata H., Watanabe M., Okajima Y., Tanabe S., Amano M., Miyazaki N., Petrov E.A. (2004). Toxicokinetics of PCDD, PCDF, and Coplanar PCB Congeners in Baikal Seals, *Pusa sibirica*: Age-Related Accumulation, Maternal Transfer, and Hepatic Sequestration. *Environmental Science and Technology*, 38, 3505-3513.

172. Korhonen M., Verta M., Lehtoranta J., Kiviranta H., Vartiainen T. (2001). Concentrations of polychlorinated dibenzo-*p*-dioxins and furans in fish downstream from a Ky-5 manufacturing. *Chemosphere*, 43, 587-593.

173. Wan Y., Hu J., An W., Zhang Z., An L., Hattori T., Itoh M., Masunaga S. (2006). Congener-Specific Tissue Distribution and Hepatic Sequestration of PCDD/Fs in Wild Herring Gulls from Bohai Bay, North China: Comparison to Coplanar PCBs. *Environmental Science and Technology*, 40, 1462-1468.

174. Ballantyne James S. (1997). Jaws: The inside story. The metabolism of elasmobranch fishes. *Comparative Biochemistry and Physiology*, 118b, 703-742.

175. Wells R.M.G., Weber R.E. (1983). Oxygenational properties and phosphorylated metabolic intermediates in blood and erythrocytes of the dogfish, *Squalus acanthias*. *Journal of Experimental Biology*, 103, 95-108.

176. Andersson T., Nilsson E. (1989). Characterization of cytochrome P-450-dependent activities in hagfish, dogfish, perch and spectacle caiman. *Comparative Biochemistry and Physiology B*, 94B, 99-105.

177. Sugiyama Y., Yamada T., Kaplowitz N. (1981). Glutathione S-transferases in elasmobranch liver. Molecular heterogeneity, catalytic and binding properties, and purification. *Biochemical journal*, 199, 749-756.

178. Pritchard J.B., Anderson J.B., Rall D.P., Guarino A.M. (1980). Comparative hepatic and renal handling of phenol red and indocyanine green by cyclostome elasmobranch, and teleost fish. *Comparative Biochemistry and Physiology C*, 65C, 99-104.

179. Dvorchik B.H., Maren T.H. (1972). Fate of p,p'-DDT [2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane] in the dogfish, *Squalus acanthias*. *Comparative Biochemistry and Physiology A*, 42, 205-211.

180. Skarphedinsdottir H., Gunnarsson K., Gudmundsson G.A., Nfon E. (2010). Bioaccumulation and Biomagnification of Organochlorines in a Marine Food Web at a Pristine Site in Iceland. *Archives of Environmental Contamination and Toxicology*, 58, 800-809.

181. Vetter W., Hummert K., Luckas B., Skirnisson K. (1995). Organochlorine residues in two seal species from Western Iceland. *The Science of the Total Environment*, 170, 159-164.

182. Bright D.A., Grundy S.L., Reimer K.J. (1995). Differential bioaccumulation of non-ortho-substituted and other PCB congeners in coastal arctic invertebrates and fish. *Environmental Science and Technology*, 29, 2504-2512.

183. Stapleton H.M., Letcher R.J., Baker J.E. (2001). Metabolism of PCBs by the deepwater sculpin (*myoxocephalus thompsoni*). *Environmental Science and Technology*, 35, 47470-4752.

184. Boon J.P., Eijgenraam F., Everaarts J.M., Duinker J.C. (1989). A structure-activity relationship (SAR) approach towards metabolism of PCBs in marine animals from different trophic levels. *Marine Environmental Research*, 27, 159-176.

185. Bergman Å., Norstrom R.J., Haraguchi K., Kuroki H., Béland P. (1994). PCB and DDE methyl sulfones in mammals from Canada and Sweden. *Environmental Toxicology and Chemistry*, 13, 121-128.

186. Karlson K., Ishaq R., Becker G., Berggren P., Broman D., Colmsjö A. (2000). PCBs, DDTs and methyl sulfone metabolites in various tissues of harbour porpoises (*Phocoena phocoena*) from Swedish waters. *Environmental Pollution*, 110, 29-46.

187. Larsson C., Norström K., Athanassiadis I., Bignert A., König W.A., Bergman Å. (2004). Enantiomeric specificity of methylsulfonyl-PCBs and distribution of bis(4-chlorophenyl) sulfone, PCB, and DDE methyl sulfones in grey seal tissues. *Environmental Science and Technology*, 38, 4950-4955.

188. Tanabe S., Ramu K., Isobe T., Takahashi S. (2008). Brominated flame retardants in the environment of Asia-Pacific: an overview of spatial and temporal trends. *Journal of Environmental Monitoring*, 10, 188-197.

189. Isobe T., Ochi Y., Ramu K., Yamamoto T., Tajima Y., Yamada T.K., Amano M., Miyazaki N., Takahashi S., Tanabe S. (2009). Organohalogen contaminants in striped dolphins (Stenella coeruleoalba) from Japan: Present contamination status, body distribution and temporal trends (1978-2003). *Marine Pollution Bulletin*, 58, 396-401.