General Introduction:

Occurrence, accumulation and toxicity

of perfluorinated alkylated substances

1.1. Perfluorinated Alkylated Substances (PFAS)

1.1.1. Background

Since the early '30s, production of chemicals has increased from 1 million tons to over 400 million tons nowadays. A large amount of these chemical substances finds their way to the estuarine and marine environment, and causes a severe impact on the aquatic ecosystem. Over 100 000 chemicals are released in such volumes that they pose a threat to both environmental and human health. Still, precise knowledge on the distribution, on the exposure and accumulation patterns and on the toxicological mode of action of many of them is scarce.

Due to their environmental persistence and tendency to bioaccumulate, halogenated organochemicals, heavy metals and pesticides have been a topic of several research studies (1,2). The group of perfluorinated alkylated substances (PFAS), however, has received less attention. Nevertheless, PFAS such as perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), as well as their related products, are being produced over 50 years and have a broad application spectrum in industry and households. These compounds are stable and chemically inert and therefore, always considered as being safe for humans and environment. The presence of fluorine in human blood has been reported for the first time in 1968 (3). However, it took several decades before other research studies reported on the occurrence of perfluorinated substances in both occupationally and non-occupationally exposed humans (4-6). Since 2001, with the first major study of Giesy and Kannan (7) on the global distribution of PFAS, perfluorinated compounds have been identified in environmental samples (8,9), in several tissues in wildlife (7, 10-14) and in the blood and seminal plasma of humans (15,16). From then onwards, public concerns about the persistence and the toxicological effects of PFAS have increased.

1.1.2. Structure and physicochemical properties

The chemicals of the PFAS group include both oligomers and polymers and comprise several hundreds of compounds (17). Monomers are characterized on one hand by a carbon chain with variable length, to which fluorine atoms are covalently bound, and on the other hand by a functional group like a sulfonyl (perfluorinated sulfonates: e.g. figure 1.1.) or

carboxyl group (perfluorinated carboxylic acids). In nature, organofluorines are composed of a carbon-hydrogen chain in which only one carbon-hydrogen bond is replaced by a carbon-fluorine. In contrast, in man-made perfluorinated alkylated substances, all carbon-hydrogen bonds can be replaced with carbon-fluorine bonds. The C-F bond is thermodynamically one of the strongest bonds known and given its binding energy, it is expected that many PFAS will be resistant to hydrolysis, photolysis, biodegradation or metabolism (18,19).

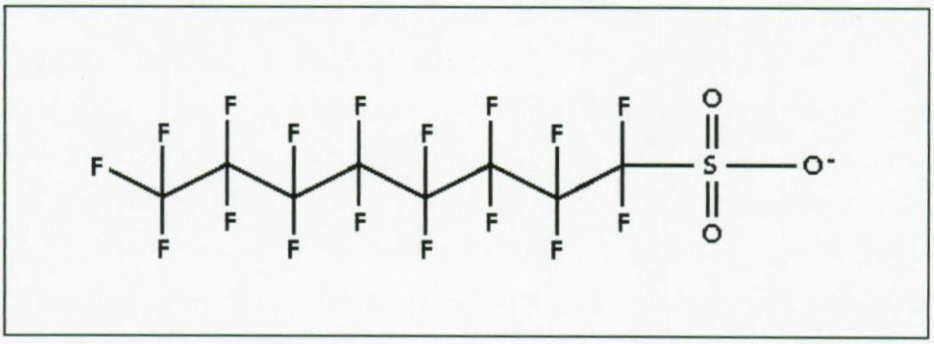

Figure 1.1. Chemical structure of perfluorooctane sulfonate (PFOS).

The majority of PFAS have low vapour pressures and special surface-active properties (20). Perfluorinated compounds have both hydrophobic properties in one end of the molecule (perfluorinated carbon tail) and polar/hydrophilic properties in the other functional end and therefore, they repel both water and oil and act as surfactants. Furthermore, PFAS will not accumulate in fatty tissues, but preferentially bind to blood proteins and accumulate in liver and kidney. These "special" solubility profiles of e.g. PFOS and PFOA make environmental fate predictions based on octanol-water partition coefficients irrelevant for these chemicals (21).

1.1.3. Production and applications of PFAS

Perfluorinated alkylated substances are made by three different production processes (22), namely (1) direct fluorination using electro-chemical fluorination (ECF) especially used by the 3M Company, (2) telomerisation which is mainly used by DuPont, Daikin, Clariant and Ashai Glass and (3) oligomerisation (the chemical process of creating oligomers from larger or smaller molecules). However, the first two mentioned processes are the ones which are normally used (23,24).

Due to the specific physicochemical properties, PFAS have been produced and manufactured over 50 years and are used in a wide variety of applications. Perfluorinated carboxylic acids like PFOA are primarily used as non-reactive processing aids in the production of other fluorinated or perfluorinated compounds (25). PFOA is also the major compound from which Teflon is made, a non-stick coating used for saucepans (26) and also Goretex contains PFOA (27). The use of the sulfonated perfluorochemicals can be divided into 3 main categories, (1) surface treatment applications, (2) paper protection applications and (3) performance chemical applications. The unique properties of repelling both water and oil are used in surface treatment of fabrics (carpets, textile), metal, glass and leather and in paper products such as food containers, folding cartons and bags. Performance chemicals have a broad application spectrum in industry and commerce as surfactants (fire-fighting foams), cleaning agents (shampoos, carpet cleaners), coating additives, photographic plates and antistatic agents, pesticides and insecticides (e.g. Sulfuramid, an insecticide used to control cockroaches, termites and ants) (28,29).

As no information on cumulative production volumes have been released so far by the fluorochemical industry, it is hard to make exact estimations on the production volumes or emissions of PFAS over the past 50 years. Based on a production of 300 000 ton in 2000 by 3M alone, the total annual world production is likely to have been more than 500 000 ton (30). The largest global manufacturer of both PFOS and PFOA has for long been the 3M Company. However, increasing concerns about the persistence of PFOS and its potential role in increasing risk of health effects, have lead to the announcement of the 3M Company to phase-out the production of PFOS-based chemicals from December 2000 and to stop external sale of PFOA. The thought behind this decision is that if manufacturing is

the main source of PFOS in the environment, then the phase-out should result in a substantial reduction of PFOS in the environment. However, the problem might not be solved just by phasing out some of the perfluorinated chemicals by only one manufacturer. On one hand, there has already been a shift of the buyers to other producers on the European and Chinese market that have carried on the production of these compounds (31). On the other hand, if PFOS is coming from existing products or from landfills there could still be a significant input of PFOS related chemicals into the environment in to the future (21).

After the voluntary manufacturing phase-out of PFOS by the main producer, perfluorobutane sulfonate (PFBS) was announced as official successor for the PFOS related products (32). PFBS is a chemical that is the potential degradation product of certain substances based on perfluorobutane sulfonyl fluoride chemistry. PFBS is a fully fluorinated four-carbon organic molecule produced synthetically by electrochemical fluorination, other processes, and from the degradation or metabolism of other four-perfluorocarbon products or derivatives. According to the industry, PFBS-based surfactants offer improved environmental properties. Studies performed by 3M laboratories, showed that PFBS is not metabolized but is excreted rapidly and has very low toxicity in acute and repeat-dose tests. Further, it does not affect reproductive function or prenatal development. Although it is persistent in the environment, PFBS has not been shown to accumulate in biota and until present, no PFBS traces were confirmed in biota samples yet.

1.2. PFAS levels in the aquatic environment

1.2.1. Pathways to the estuarine and marine environment

Estuarine and coastline ecosystems are of major economic importance, but due to large industrial and domestic waste water discharges, the structure and functions of these habitats are often disturbed. The North Sea is often considered as one of the most polluted seas in the world (33-35). A significant input of pollutants to the North Sea comes from the extension of the discharge plume of several large rivers, like the Western Scheldt, the

Rhine, the Meuse and the Thames (36). Since the oceans serve as a sink for a variety of environmental pollutants, monitoring PFAS in this ecosystem is crucial.
Given the wide range of applications and products in which PFAS are used, pathways to the estuarine and marine environment include direct and indirect pollution sources such as wastewater discharges (37), leachates from consumer products and from waste products in landfills (38), atmospheric losses during combustion and from certain domestic and commercial uses, wash-off from various application such as in fire-fighting foams, or emissions from wearing PFAS treated materials. Especially the use of fire-fighting foams on offshore oil platforms, may form a potential direct water pollution risk.

1.2.2. Levels in coastal seawater

Data on the occurrence of perfluoroalkylated chemicals in the marine abiotic environment are still scarce. Most of the data available are for the freshwater environment (ground-, drinking and surface water). There, the main pollution source of groundwater and surface waters by perfluorinated alkylated substances is the use of fire-fighting foams to fight hydrocarbon-fuelled fires or accidental spills of the products. In these cases, the wastewater enters surface water and groundwater without prior treatment and leads to groundwater and soil contamination, and eventually sometimes to drinking water pollution (39-41). One of the reasons why little information is available on concentrations of PFAS in the marine abiotic environment is that the compounds' unique physicochemical properties create several challenges to analytical chemists endeavouring to measure PFAS at trace levels. Background contamination in the analytical blanks is one of the major problems. These problems lead to high limits of detections of perfluorinated substances in water samples, in the range of several tens to hundreds of ng/L to a few µg/L (42a).

Recently, many research studies have been performed in coastal and open water in Asia. Yamashita et al. (43) reported on perfluorinated alkylated substances in surface water samples taken shortly after a major fire in an oil storage facility near a Pacific coast city, Tomakomai (Japan), which was fought with aqueous film forming foams (AFFF) containing both perfluorinated carboxylates (PFCAs) and sulfonates. Mean concentrations of PFOS (42.3 ng/L), PFHS (6.9 ng/L) and PFBS (0.22 ng/L) were higher than mean

concentration of the measured carboxylates, where mean concentrations ranged from <0.1 ng/L (PFDoA) to 7.9 ng/L (PFNA). Two months after the fire, water concentrations of all PFAS decreased dramatically, from 2- to 13-fold, indicating that the compounds are removed by dilution and exchange of coastal and offshore waters. However, more hydrologic information such as tidal and/or water currents is necessary to explain the discharge from PFAS from coastal waters to open ocean (44). Further, after two months, relatively high levels were measured in snow, suggesting that some PFAS were released into the air and deposited to land through wet deposition. Other studies (8,45) on coastal water concentrations from several Asian countries (China, Hong Kong and Korea) reported on concentrations ranging from 0.023 to 9.7 ng/L (PFOS), from <0.005 to 1.4 ng/L (PFHS) and from 0.24 to 15.3 ng/L (PFOA). Other PFAS were also detectable in about 90% of the samples that were analysed from coastal waters of Hong Kong and South China, but at concentrations less than those for PFOS and PFOA (45). Highest levels in coastal water in Asia, however, were measured in Tokyo Bay with concentrations ranging from 1.8 to 192 ng/L for PFOA and from 0.4 tot 58 ng/L for PFOS (44). In general, PFAS levels in coastal waters are probably associated with urban and industrial areas.

In 2003, several locations in the North Sea have been sampled and water samples were analysed for 7 different perfluorinated compounds (46). In all samples PFOS and PFOA were the predominant compounds. Coastal water concentrations in the German Bight were up to 7 ng PFOS /L and up to 14 ng PFOA /L, while the other detectable compounds (PFHxA, PFHxS, PFHpA, PFNA, PFDA and PFOSA) ranged from 1 to 3 ng/L. The highest concentrations were encountered at the mouth of the river Elbe with PFOS values up to 20 ng/L. At this location, the compounds followed also a different distribution pattern, namely higher PFOS than PFOA levels.

In a screening on water samples originating from Finland, Denmark, Iceland and Faroe Islands, PFOA was the predominant compound (up to 70% of the PFAS burden) with a median concentration of 5.2 ng/L (47,48). PFOA was followed by PFHxA in most of the samples. The median PFOS concentration was below 1 ng/L. However, in one sample from Finland PFOS concentrations up to 20 ng/L were detected. Overall, seawater samples from Finland and Denmark showed the highest PFAS levels, indicating the influence of the

population density in these countries, although the difference with countries with smaller populations is low (around factor 2).

In contrast to biota samples, where PFOS is the predominant compound, higher concentrations of PFOA are observed in almost all water samples. This is probably due to the lower water solubility of PFOS compared to the carboxylic acids (47), and it also suggests that the bioaccumulation potential of PFOA is relatively lower than that of PFOS (44).

1.2.3. Levels in open ocean waters

Studies on PFAS levels in open ocean water are scarce. In general, concentrations are lower than in coastal waters and are measured in pg/L (parts per quadrillion) quantities. Taniyasu et al. (44) reported on a method for ultra-trace level analysis of PFAS making a global survey of open ocean pollution possible. PFOA was the predominant fluorochemical, ranging in concentrations from 15 to 62 pg/L in surface water of the Central to Eastern Pacific Ocean, from 136 to 142 pg/L in the Western Pacific Ocean, from 160 to 338 pg/L in the North Atlantic Ocean and from 100 to 439 pg/L in the Mid Atlantic Ocean. Offshore of Japan concentrations of PFOA from 137 to 1060 pg/L were measured. Concentrations of perfluorinated sulfonates ranged between 1.1 and 78 pg PFOS /L and between 0.1 and 12 pg PFHxS/L. In general, the "background" surface water levels of perfluorinated compounds in the Pacific Ocean were 5 to 10 times lower than in the Atlantic Ocean. Concentrations in deep water (>1000m depth) were an order of magnitude lower than those in surface water, ranging from below the detection limit to 36 pg/L for PFOA and from 3.2 to 24 pg/L for PFOS.

Water samples were taken at 5m depth in the open sea of the North Sea (46). At most stations only PFOA could be detected at concentrations of 0.5 ng/L. All other target compounds were below the detection limits.

1.2.4. Levels in marine sediment

So far, investigators are still not sure if there are losses of PFOS and/or PFOA in water due to sorption to sediments (49,50). The relative importance of sediments as potential source of PFAS to the marine food chain has still to be evaluated.

Surficial sediment samples from the outlets of various rivers and creeks in the San Francisco Bay area were taken and analysed in 2004 by Higgings et al. (51). Data from the survey suggest widespread occurrence of PFAS in sediments at the low ng/g to sub-ng/g level, with concentrations of total perfluoroalkyl sulfonyl-based chemicals ranging between 0.06 and 5.53 ng/g. Concentrations of total perfluorocarboxylates ranged from zero to 6.51 ng/g. PFOS, PFDS, *N*-MeFOSAA, *N*-EtFOSAA, PFOA and PFDA were the most commonly detected PFAS. Perfluorocarboxylates measured in sediment showed a dominance of even chain-lengths. This observation might suggest that the source of perfluorocarboxylates in the sediments was the biodegradation of fluorotelomeralchohols (52).

In a recent Dutch screening project sediment samples from the North Sea were investigated for PFOS and PFOA (9). The levels of PFOS were highest with concentrations ranging from <0.4 to 4.57 ng/g dry weight. In 50% of the samples, PFOA could be measured at concentrations between <0.4 and 3.05 ng/g dry weight.

1.3. PFAS levels in aquatic organisms

During the past few years, research has revealed that PFOS is the predominant compound detectable in living organisms. Other PFAS, such as PFOSA, PFHxS, PFOA and long-chain perfluorocarboxylates, are also detectable but often at lower concentrations. In the following sections, the biodegradation, the bioaccumulation potential and the age/gender distribution of PFAS is described into detail. More information on specific levels of perfluorinated chemicals in biota (invertebrates, fish, birds and mammals) from the marine and estuarine environment is given in sections 1.3.4. to 1.3.7.

1.3.1. Biodegradation

PFOS is a stable end-product of various sulfonated perfluorochemicals, such as *n*-methyl perfluorooctanesulfonamidoethanol [*N*-MeFose; $C_8F_{17}SO_2N(CH_3)CH_2CH_2OH$] and is not biodegradable. The reason for its persistence is that the perfluorocarbon chain, with the very strong C-F bindings, is extremely resistant to heat, UV-radiation and to chemical attacks by acids and bases, and to reducing and oxidizing agents. On the other hand, the functional end-groups like sulfonamides and alcohol, will be more readily transformed in the environment and in biota, and the more complex compounds will finally be degraded to the most persistent sulfonates (e.g. PFOS) and carboxylates (e.g. PFOA) (18, 19, 53, 54).

PFOSA, PFOA and PFHxS are intermediates in the production of several PFAS. PFOSA and PFOA are also products used in various applications. In addition, *N*-ethyl-N-(2-hydroxyethyl)-perfluorooctanesulfonamide can undergo *N*-deethylation to form *N*-(2-hydroxyethyl)-perfluorooctanesulfonamide that can be metabolized by *N*-deethylation into perfluorooctanesulfonamide (PFOSA), which is used as an insecticide (Sulfuramid). PFOSA itself can undergo metabolisation to PFOS (55,56). Plausible precursors molecules that could break down to yield perfluorocarboxylates are fluorotelomer alcohols (FTOHs). Recently, it has been shown that FTOHs can be broken down in the atmosphere to form PFCAs (57). Dinglasan et al. (52) used a mixed microbial system in which 8:2 FTOH was broken down to telomere acids ($CF_3(CF_2)_7CH_2COOH$; $CF_3(CF_2)_6CFCHCOOH$) and PFOA. These metabolic reactions may serve as probable sources of PFOA and other carboxylic fluorinated acids that have been detected in wildlife.

1.3.2. Bioaccumulation potential

Analysis of existing data shows that organisms are bioaccumulating PFAS and especially PFOS. Bioaccumulation has been described as a process in which a substance accumulates in an organism. This can happen through two different pathways: (1) uptake from the water, namely bioconcentration, and (2) uptake through food, namely biomagnification. Bioaccumulating substances are of great concern because or their potential to attain toxicological significant tissue and organ residu concentrations in higher-trophic-level species, such as fish, birds, mammals and humans (58). Unlike most persistent organic

pollutants such as organochlorines, perfluorinated compounds do not accumulate in fatty tissues, but they concentrate preferentially in blood, liver, kidney and gallbladder (21). However, PFAS also have high affinity for plasma proteins, such as albumin (59).

Modelling technologies often use the octanol/water partition coefficient to predict the bioconcentration potential of chemicals. However, since perfluorinated substances are characterised by special solubility profiles, octanol/water partition coefficients are not suitable for these compounds (see section 1.1.2.). So far, for perfluorinated substances, we have to rely on both laboratory experiments and field studies of marine and freshwater organisms.

In 2001, Giesy and Kannan (7,60) reported biomagnification factors (BMF) in the range of 11 to 23, with a mean value of 18. These estimations were based on the concentrations of PFOS measured in livers of mink. Under laboratory conditions, mink were fed carp at levels of 10, 20 and 40% from a bay in Michigan, containing an average concentration of 120 ng PFOS/g wet wt (240-300 ng/g wet wt). With an increase in the proportion of carp in the diet (and thus with PFOS), an increase in hepatic PFOS concentrations in mink was seen; suggesting a dose-related increase.

In a preliminary laboratory study on the dietary accumulation of PFAS in juvenile rainbow trout (61), bioaccumulation factors (BAF) ranged from 0.038 to 1.0 and increased with length of the perfluorinated chain. These results indicate that PFAS dietary exposure will not result in biomagnification in juvenile rainbow trout, because their rate of depuration is relatively high and their natural feeding rate is too low. However, it cannot be assumed that the same will be the case in mature rainbow fish or other species.

In addition, another study of Martin et al. (62), calculated bioconcentration factors in juvenile rainbow trout, exposing the fish to a PFAS diluted solution in a flow-through exposure design for 12 days. Carboxylates and sulfonates with perfluoroalkyl chain lengths shorter than 7 and 6 carbons respectively, were considered to have insignificant bioconcentration factors (BCFs). However, for the other PFAS, BCFs increased with increasing chain lengths and ranged from 4.0 to 23000. Given equal chain lengths,

sulfonates were taken up at a greater rate than carboxylates. The same trend was seen in the dietary exposure (61).

Moody et al. (39) reported that perfluorinated compounds have a significant potential to bioaccumulate, based on PFOS concentrations, measured under field circumstances. To calculate a bioaccumulation factor (BAF), the authors used environmental data (water and fish PFOS concentrations) collected from Etobicoke Creek, a small creek close to the airport of Toronto, which suffered from an accidental spill of 22000L of fire fighting foam in June 2000. The BAF range for PFOS in fish liver tissue was calculated to be approximately 6300-125000. One reason for the extreme high BAF, in comparison with the earlier reported BMF, may be that the creek fish accumulated other PFOS-based compounds which could be able to break down to PFOS, but which were not measured in the water samples and therefore not accounted for.

Wild fish from different coastal regions of Japan were used to estimate bioconcentration factors (63). BCFs calculated from PFOS concentrations in livers of two marine fish and one freshwater fish, ranged from 274 to 41600 and were in the range of the other bioconcentration factors.

Food web biomagnification in Lake Ontario was described by Martin et al. (64). Diet weighted BAFs were calculated that accounted for the abundance of each prey item in the diet of trout. Weighted BAFs were 2.9 (PFOS), 1.4 (PFOSA), 2.3 (PFNA) and 3.5 (PFUA), suggesting that bioaccumulation is occurring at the top of the food chain. However, the authors are not convinced that biomagnification appears also at the lower trophic level in the pelagic food web. One invertebrate species, namely *Diporeia*, seemed to be the most heavily contaminated organism in the food web with respect to PFAS, but also occupied the lowest trophic level of all species. Therefore, it may be argued that the relative amount of PFAS observed in higher trophic animals, is simply a function of how much *Diporeia* is consumed, and not, or not solely, a consequence of biomagnification.

A recent study in fluorinated organic compounds in an arctic marine food web, described biomagnification factors for PFOS between 0.4 (cod:zooplankton) and 9 (glaucus gull:cod) (65). The results indicate that PFOS biomagnifies in the marine food web when liver concentrations of PFOS are used for seabirds and marine mammals. However, the authors also commented that biotransformation is one of the confounding factors that may complicate the study of the bioaccumulation potential of perfluorinated compounds. In particular, N-EtPFOSA and PFOSA may be transformed to PFOS, and so, influence the final PFOS levels in higher-trophic-level animals. Therefore, a better understanding of biotransformation, formation pathways and precursor molecules, would improve assessments of PFAS bioaccumulation. A similar remark is made by Kannan et al. (66), who reported a bioconcentration factor of approximately 1000 in benthic invertebrates (based on whole-organisms) and a BMF of 10 to 20 in mink of bald eagles, based on liver tissue and relative to their prey items, but noted that PFOS-based (partially) metabolized molecules may serve as a contributory source of PFOS for higher trophic-level organisms.

1.3.3. Age/gender distribution

A general observation in all biomonitoring surveys was the lack of age or sex-related differences in concentrations of perfluorinated alkylated substances. No statistically significant age differences were observed in birds, otters, ringed and grey seals, and bottlenose dolphins (54, 60, 67, 68).

Only few studies reported on significant differences in some species. In polar bears from east Greenland, a significant increase in concentration was seen up to the age of six for PFOS, PFNA and perfluorinated carboxylates with a chain length of 10 to 14 carbons (69). Polar bears from the South Hudson Bay in Northern America showed age trends for PFOS and PFUA (70). The same study also indicated a significant increase in PFOS levels with age in bears from Alaska, the Northwest Territories and Greenland. PFOS concentrations in adult female mink from Massachusetts were 2-fold greater than those measured in juvenile females, which could be explained by a difference in feeding rates (60).

No gender-specific differences could be discerned in birds from Japan and Korea (71). Gender was also not correlated to concentrations of any fluorinated compound measured

in polar bears by Smitwick et al. (69,70) or in mink analysed by Kannan et al. (60). However, PFOS concentrations in blood of male grey seals from the Baltic Sea were significantly higher than those found in the blood of females (54,68).

The present results show the difference between the accumulation pattern of perfluorinated compounds and that of other persistent organic pollutants like PCBs and DDTs. Generally, lipophilic contaminants tend to accumulate in fatty tissues over a period of time, which results in higher concentrations in older organisms. However, with these organic compounds, adult females generally contain lower contaminant levels than males. In adult females there is a transfer of lipophilic compounds to their offspring during lactation, due to their affinity for milk. This forms a major excretory route for females. As PFOS does not accumulate in lipid-rich tissues like milk and blubber, but binds to specific proteins in liver, kidney and blood plasma, a similar transfer from mother to calf is not obvious. It seems that PFOS, like butyltin compounds, binds to specific proteins in liver and blood plasma and they are expected to enter the enterohepatic circulation due to their ionic properties (30). This process is not gender-specific. Further, it has been suggested that accumulation of PFAS is controlled by a dynamic equilibrium between uptake and elimination or that there is a protein turnover (54).

1.3.4. Marine invertebrates

In general, the information on the exposure levels on marine invertebrates is scarce. The few data available show that PFOS has been detected in oysters collected in the Gulf of Mexico and Chesapeake Bay (USA) (28), in mussels from north-central Portuguese estuaries (72) and in shrimp, clams and zooplankton samples from the Eastern Canadian Arctic (65).

Bivalves (oysters and mussels) have been widely used in biomonitoring studies to investigate the pollution burden in marine coastal and estuarine ecosystems (O'Connor, 1996). Kannan et al. (28) reported on PFOS concentrations in American oysters (*Crassostrea virginica*) collected between 1996 and 1998. PFOS was found in 64% of the samples analysed. Concentrations ranged from <10 to 99.54 ng/g wet wt (values were

converted from dry weight to wet weight, taking into account the percentage of water of the samples, supplied by the authors). Except for one oyster sample (out of 20 oysters), none of the oysters collected from Chesapeake Bay contained detectable concentrations of PFOS, suggesting that this is a non-contaminated estuary.

The results of the study performed on mussels (*Mytilus galloprovincialis*) from Portuguese estuaries indicate that the animals are significantly polluted by PFOS (72). Whole body burden values ranged from 36.8 to 125.9 ng/g wet wt. No significant differences could be detected between the estuaries, moreover, PFOS concentrations could not be related to the proximity of potential sources of this compound. Beside whole body values, target tissues such as digestive gland, gills and haemolymph, were also investigated. PFOS concentrations in analysed tissues were considerably higher than in whole body samples. Gills presented significantly lower levels (364.7 ± 21.8 ng/g wet wt) than haemolymph (479.5 ± 26.4 ng/g wet wt) and digestive gland (476.3 ± 29.0 ng/g wet wt). Furthermore, significant differences were found between mature and immature mussels. Non-mature animals showed lower PFOS concentrations in whole body tissues and digestive gland, however, looking at haemolymph, PFOS levels were higher in non-mature animals. No difference in PFOS concentrations were seen in gill tissue. The authors make the suggestion that the PFOS burden load seems to be related, at least to a certain degree and in some tissues, to maturation state. Therefore, gills seem to be the most appropriate tissue to use in biomonitoring studies, since their PFOS level is independent of maturation state.

Mixed zooplankton samples from Frobisher Bay in the Eastern Canadian Arctic contained 1.1 to 2.6 ng PFOS/g wet wt and 1.7 to 3.4 ng PFOA/g wet wt (65). PFOS was detected in 4 of the 5 shrimp samples (*Pandalus borealis* and *Hymenodora glacialis*) analysed (<0.06 to 0.9 ng/g wet wt) and PFOA was detected in only 3 samples (0.2 to 0.5 ng/g wet wt). PFOS concentrations in clams (*Mya truncata* and *Serripes groenlandica*) ranged from 0.08 to 0.6 ng/g wet wt. PFOA was not detectable in any of the clams. Concentrations of *N*-EtPFOSA were highest in clams (1.9 to 85.9 ng/g wet wt), followed by shrimp (<0.59 to 44.8 ng/g wet wt) and zooplankton (<0.59 to 0.65 ng /g wet wt). PFOSA was not detected in any of the marine invertebrates investigated in this study. It could not be readily explained why levels of PFOS and PFOA are higher in mixed zooplankton samples (up to

5-fold) than in shrimp and clams. The authors suggest that, probably, shrimp and clams have a greater benthic association and exposure to PFOS and PFOA may be greater in the water column resulting in higher concentrations in zooplankton.

1.3.5. Marine fish

The first paper published on levels of PFOS in wildlife, also included a few marine fish species, namely the yellow-fin tuna from the Northern North Pacific Ocean and the blue-fin tuna (*Thunnus thynnus*) originating from the Mediterranean Sea (7). Liver tissue of the yellow-fin tuna contained concentrations below the limit of quantification (<7 ng/g wet wt). Concentrations in liver of tuna from the Mediterranean Sea ranged between 21 and 87 ng/g wet wt.

In addition, levels of different PFAS in blood and liver of blue-fin tuna (*Thunnus thynnus*) and swordfish (*Xiphias gladius*) from the Mediterranean Sea, and in liver of Atlantic salmon collected from the Northern Baltic Sea, were reported in a later paper by Kannan et al. (54). Blood concentrations in tuna and swordfish ranged from 27 to 52 and from 4 to 21 ng PFOS/mL, respectively. 92% of the fish samples contained quantifiable concentrations of PFOS. Liver concentrations of PFOS in tuna (21-87 ng/g wet wt; 7) were higher than liver concentrations in swordfish (<1-13 ng/g wet wt). Although FOSA was found in all blood samples from both tuna (13-19 ng/mL) as swordfish (1.1-28 ng/mL), none of the liver samples contained detectable concentrations of FOSA (<38 ng/g wet wt). Whereas, the mean concentration of FOSA in blood of tuna (15 ng/mL) was 2.6-fold less than that of PFOS (40 ng/mL), mean FOSA concentration of swordfish blood (15 ng/mL) was 2 times greater than that of PFOS (7.2 ng/mL). However, no explanation for this observation was given. PFHxS was measured in one swordfish liver sample at a concentration of 10 ng/g wet wt. PFOA was not detected in any of the samples. None of the salmon livers contained quantifiable concentrations of any of the target fluorochemicals (quantification limits between 8 and 19 ng/g wet wt). All salmons were adult female fish collected during the spawning period. As they fast for 4 months prior to spawning, the lack of quantifiable concentrations may be linked to this long fasting period. However, this would also mean that these fish do not accumulate PFAS in their tissues during their life.

Eight marine fish species (herring, sculpin, arctic char, cod, flounder, eelpout, long rough dab and dab) from Sweden, Denmark, Iceland and Faroe Islands were analysed for eight fluorochemicals (47). As in other marine fish, PFOS usually represented the predominant PFAS contaminant. Concentrations were up to 60 ng/g wet wt in cod from Sweden and eelpout from Denmark and levels of PFOS in Danish flounders and herrings were around 20 ng/g wet wt. However, PFOSA was found in higher levels than PFOS in sculpins of the Faroe Islands. In all the Icelandic samples, PFDS (median 10 ng/g wet wt) and PFHxA (>1 ng/g wet wt) were present. The carboxylic acids PFHxA, PFHpA and PFOA were only quantifiable in fish from Denmark. Overall, marine fish from the Faroe Islands showed the lowest concentrations. The high variability in the PFAS distribution was explained by differences in trophic levels, feeding habits, sampling locations as well as uptake and possible transformation mechanisms. Compared to freshwater fish from the same countries (with PFOS concentrations up to 551 ng/g wet wt in Finnish spike), the lower levels in marine fish could be explained by dilution effects with distance to primary sources.

Between 2000 and 2001, a preliminary screening was conducted on shorthorn sculpin (*Myoxocephalus scorpius*) from Greenland (74). The concentration of PFOS in pooled fish liver tissue was higher in samples from east Greenland (13-18ng/g wet wt) compared to west Greenland (not detectable). A similar geographical trend was reported earlier in sculpins for DDTs and PCBs (75,76). However, PFOSA could only be quantified in the pooled sample of west Greenland (9 ng/g wet wt). PFOA and PFHxS were not detected in any of the samples.

Although only one liver sample of a sculpin (*Myoxocephalus scorpioides*) was analysed in an Arctic food chain study by Martin et al. (14), FOSA was the predominant fluorochemical present at a concentration of 18 ng/g wet wt, followed by PFOS (12 ng/g wet wt) and PFNA (2.2 ng/g wet wt). The general odd/even pattern of carboxylates that was found in marine mammals and birds, was also found in the sculpin sample, namely that the odd-chain-length PFCA concentration exceeded the concentration of the corresponding shorter, even-chain-length PFCA. Thus, PFUA (1.1 ng/g wet wt) was higher than PFDA (0.52 ng/g wet wt) and PFTrA (1.7 ng/g wet wt) was found having a higher concentration than PFDoA (0.55 ng/g wet wt).

Another study conducted on marine fish from the Canadian Arctic reported on different PFAS in Arctic cod (*Boreogadus saida*) and deepwater redfish (*Sebastes mentella*) collected from Davis Strait in 2000 and 2001 (65). PFOSA could not been detected in any of the fish samples. PFOS concentrations in cod and redfish ranged from 0.3 to 4.7 and from <0.06 to 6.3 ng/g wet wt, respectively. PFOA was detected in only a single Arctic cod (0.47 ng/g wet wt) and was about 3-fold less abundant than the average PFOS concentrations. PFOS and PFOA (<0.2-5.3 ng/g wet wt) concentrations were similar in redfish, although PFOA was quantifiable in only 2 out of 7 samples. *N*-EtPFOSA ranged in Arctic cod from 9.6 to 144.6 ng/g, but was not detected in deepwater redfish.

A study on fish from different coastal regions of Japan investigated PFAS concentrations in blood and liver tissue (63). PFOS concentrations ranged from 1 to 834 ng/mL in blood and from 3 to 7900 ng/g wet wt in liver tissue. PFOS could be detected in all samples, although concentrations varied depending on species and location. PFHxS was detected in about 33 % of all samples at concentrations severalfold less than those of PFOS. PFBS was found in any of the samples. PFHS was detected in 33% of the fish analyzed.

Extreme high PFOS concentrations (14-7760 ng/g wet wt) were also measured in liver tissue of plaice (*Pleuronectes platessa*) sampled at various locations on the Belgian continental shelf and in the Western Scheldt (10). In this study, both muscle and liver samples were taken from plaice and bib (*Trisopterus luscus*). All liver samples contained quantifiable concentrations of PFOS (11-218 ng/g wet wt in bib). PFOS concentrations in muscle ranged from <10 to 87 ng/g wet wt in plaice and from <10 to 111 ng/g wet wt in bib. For plaice, no significant difference in liver concentrations between locations could be established. However, fish from the only estuarine location had an extreme high PFOS pollution burden, with a mean concentration of 2725 ng/g wet wt. For bib, a significant correlation was found between the distance from the sampling site to Antwerp and the PFOS liver content, with the highest values found in fish close to the harbour of Antwerp. This PFOS pollution gradient suggests discharge in the Western Scheldt upstream from Hansweert. However, a tissue dilution effect could also contribute to the decreasing trends for liver and muscle PFOS levels observed in bib as the fork length of the fish decreased

closer to the city of Antwerp. As a consequence, higher PFOS concentrations could be detected in smaller fish.

1.3.6. Seabirds

Perfluorinated alkylated substances have been found in seabirds across the world: from Antarctica to the Arctic, from the United States, over Europe, to Japan and Korea. In the section below, concentrations ranges for most of the species are given, grouped for the different regions. The wide variability in PFAS concentrations is likely related to interspecies differences like diet choices, migration habits, exposure and differences in metabolic capabilities, but also to both spatial and temporal (not all birds were caught in the same period) differences. Overall, lower PFAS concentrations are found in birds from remote and/or offshore areas, further away from direct pollution sources like the Arctic, the Antarctic and open sea (7, 14, 65, 74, 79) and in birds (or eggs) collected several years ago (54,77).

Only two plasma samples of polar skua from Antarctica were analysed for PFOS (7). Concentrations were <1 and 1.4 ng/mL. In comparison, low PFOS concentrations were detected in liver samples collected in the Canadian Arctic from the common loon (*Gavia immer*) (11-26 ng/g wet wt), the northern fulmar (*Fulmarus glacialis*) (1-1.5 ng/g wet wt) and the black guillemot (*Cepphus grylle*) (not detected) (Martin et al., 2004), and in liver of the black-legged kittiwake (1.2-20 ng/g wet wt) and the glaucus gull (9.9-33.3 ng/g wet wt) (Tomy et al., 2004). PFOA could only be quantified in one of the glaucus gulls (0.33 ng/g wet wt). Other PFAS could only be detected in some of the common loon samples: FOSA (2.0-13 ng/g wet wt), PFDA (<0.5-0.55 ng/g wet wt), PFUA (<0.5-2.2 ng/g wet wt), PFDoA (<0.5-0.74 ng/g wet wt) and PFTrA (<0.5-1.5 ng/g wet wt). Glaucus gull liver samples from the marginal ice zone in the Barents Sea contained PFOS levels around 180 ng/g wet wt (78), considerably higher than the glaucus gull liver PFOS concentrations from the Canadian Arctic. PFHxS, PFHxA, PFNA and PFDA levels varied between 0.15 and 7.6 ng/g wet wt. PFOA was below the detection limit of 1.28 ng/g wet wt.

Bossi et al. (74) reported on perfluorochemicals in black guillemots (*Cepphus grylle*). PFOS concentrations ranged between 13 and 16 ng/g wet wt in liver tissue of birds from west Greenland, and a pooled liver fish sample from east Greenland contained 14 ng PFOS/g wet wt. Male Northern fulmars (*Fulmarus glacialis*) from the Faroe Islands contained slightly lower PFOS concentrations (24 ng/g wet wt) than female birds (29 ng/g wet wt). However, it should be noted here that male birds were also slightly heavier (731-948g) than females (471-754g), suggesting a tissue dilution effect. PFOSA, PFOA and PFHxS could not be detected in any of the samples. Fulmars from the Faroe Islands seem to be more contaminated with PFOS than fulmars from the Canadian Arctic, however, the birds of the Arctic were sampled in 1993, while the ones of the Faroe Island were caught between 1998 and 1999, indicating that there might be an influence of both spatial and temporal differences.

Several fish-eating birds from the United States, including Laysan albatross (*Diomedea immutabilis*) and black-footed albatross (*Diomedea nigripes*) from the North Pacific Ocean, were investigated for PFOS (71). For birds of the Great Lakes Region, only whole blood, plasma and/or egg yolk was analysed. Plasma of bald eagles (*Haliaeetus leucocephalus*) showed the highest PFOS concentrations (up to 2220 ng/mL in a 50-day old nestling), followed by plasma of herring gull (*Larus argentatus*) (up to 391 ng/mL) and plasma of double crested cormorant (*Phalacrocorax auritus*) (up to 372 ng/mL). In general, the ratios of concentrations of PFOS in plasma to that in whole blood varied from 2 to 5. The presence of PFOS into the blood of nestling may indicate exposure from eggs proteins and diet, as also egg yolk of double crested cormorants contained PFOS (21-220 ng/g wet wt). Although the data were limited, no significant difference in the blood concentrations of PFOS between male and female eagles were described. PFOS was not detected in livers or kidney of the two albatross species from the North Pacific Ocean. However, sera contained concentrations, ranging from 3.0 to 34 ng/mL. These results are adding further suggestive evidence that PFOS levels are lower in birds from remote regions.

In the Sardinean Sea in Italy, liver tissue of common cormorants (*Phalacrocorax carbo*) contained PFOS concentrations between 32 and 150 ng/g wet wt (54). Surprisingly and in contrast to most of the other research studies, PFOA was present in concentrations ranging from 29 to 450 ng/g wet wt. Levels of both PFHxS and PFOSA were not quantifiable. The same study also investigated white-tailed eagles (*Haliaeetus albicilla*) collected near the coast of Poland and east Germany between 1979 and 2000. PFOS levels in livers ranged between <3.9 and 127 ng/g wet wt. These concentrations were severalfold less than those found in bald eagles from the US (see above). There was a significant increase in PFOS concentrations in sea eagle livers with time. In 1979, mean PFOS concentration was 22 ng/g wet wt, whereas in 1999, mean PFOS concentration was 45 ng/g wet wt. No significant correlation between PFOS and age or gender of birds was found. FOSA, PFHxS and PFOA were not detected in livers of white-tailed eagles. The fact that PFOA was detected at relatively high concentrations in cormorants from Italy suggests a region-specific distribution of the different fluorochemicals.

The development of concentrations of PFAS in birds from the marine environment over time was also described by Holmström et al. (77). They have analysed archived guillemot eggs (*Uria aalge*) from 1968 to 2003. PFOS was found in all samples analysed and a clear increase in PFOS concentrations was seen during the time period studies, from 25 ng/g wet wt in 1968 to 614 ng/g wet wt in 2003. There was a statistically significant increasing trend of, on average, 7 to 11% per year. As none of the European PFOS-producing facilities was located within the Baltic Sea drainage basin, the PFOS found in the Baltic marine environment most likely originates from industrial and consumer use in the surrounding countries of the Baltic Sea and/or from PFOS or PFOS precursors by air. The PFOS concentration in eggs from 2003 was comparable with levels in 1996. In between, a peak in PFOS concentrations was observed with levels up to 1324 ng/g wet wt in 1997. The decrease following these maximum values, is not likely to be a consequence of reductions in emissions, as the PFOS phase-out did not begin until 2000. As the guillemot is a fish-eating bird, a more plausible explanation, suggested by the authors, could be that the peak coincides with a period of time that the birds had an higher intake of herring, which was suffering from a dip in condition factor and low fat content, and thus a higher accumulation of PFOS during these years.

Livers from 6 bird species collected in Japan [sea gull (*Larus crassirostris*), spot-billed duck (*Anas Poecilorhyncha*), black-headed gull (*Larus ridibundus*), black-eared kite (*Milvus lineatus*), grey heron (*Ardea cinerea*), common cormorant (*Phalacrocorax carbo*)] and from 9 bird species from Korea [sea gull (*Larus crassirostris*), common gull (*Larus canus*), black-necked grebe (*Podiceps nigricollis*), common tern (*Sterna hirundo*), great knot (*Calidris tenuirostris*), greenshank (*Tringa nebularia*), herring gull (*Larus argentatus*), sanderling (*Crocethia alba*), little egret (*Egretta garzetta*)] were analysed (Kannan et al., 2002c). PFOS was found in 85% of the samples in levels up to 650 ng/g wet wt and concentrations within the ranges reported from the US and Europe. PFOA and PFHxS were found in 5 to 10% of the samples analysed in concentrations up to 21 and 34 ng/g wet wt respectively. FOSA was only detected in common cormorants (*Phalacrocorax carbo*) from one single location in levels up to 215 ng/g wet wt.

1.3.7. Marine mammals

The global marine ecosystem is continuously under pressure due to expanding anthropogenic activities and the development and release of new chemicals. Marine mammals occupy the highest trophic positions in the marine food web and may therefore be more affected by pollutants in comparison to other animals. Different research studies have focussed on perfluorinated alkylated compounds in marine mammals like seals, dolphins, whales and polar bears, from a wide range of geographical regions: from remote regions such as the Canadian and Norwegian Arctic, to more industrialized, coastal areas like Florida and the Baltic Sea. Like in birds, a lot of the variability of PFAS concentrations between species is due to interspecies differences (feeding habits, diet, metabolism, migration routes, trophic level) and temporal and regional differences. However, where possible, a comparison will be made between regions for the same animal (e.g. polar bear).

One of the first research papers on PFOS in marine mammals described PFOS concentrations in 247 tissue samples from 15 species of marine mammals, originating from the west and east coast of the US, Alaskan coastal waters, the Canadian and Norwegian Arctic, and the Baltic Sea (68). PFOS could be detected in most of the samples, including

those from marine remote regions. Concentrations in the liver of cetaceans were in the order of bottlenose dolphin (*Tursiops truncates*) (48.2-1520 ng/g wet wt) > striped dolphin (*Stenella coeruleoalba*) (36.6-388 ng/g wet wt) > spinner dolphin (*Stenella clymene*) (78.8-168 ng/g wet wt) > rough-toothed dolphin (*Steno bredanensis*) (42.8-65.6 ng/g wet wt) > pygmy sperm whale (*Kogia breviceps*) (6.6-23 ng/g wet wt). As pygmy sperm whales are more oceanic feeders, the exposure to PFAS is expected to be minimal, in contrast to the near-shore feeding habits of bottlenose dolphins. Concentrations of PFOS in livers of pinnipeds (sea lion, elephant seal, harbour seal and norther fur seal) ranged between <5 and 133 ng/g wet wt. These low concentrations (on average 20-fold less than in bottlenose dolphins) may suggest lesser exposure and/or a greater ability to metabolize and excrete this specific class of compounds, possibly through annual moulting, as compared to cetaceans. Otters (*Enhydra lutris nereis*) from inland waters (otters are top predators of riverine food chains) contained considerably higher PFOS concentrations (33.6-994 ng/g wet wt) than those found in sea otters from coastal waters (<5-14.3 ng/g wet wt). These results suggest greater exposure in inland waters, most likely due to primary sources of PFAS (consumer products, fire fighting foams,…).

PFOS was detected in all blood samples of ringed seals (*Phoca hispida*) from the Northern Baltic Sea (mean up to 242 ± 142 ng/mL), the Norwegian Arctic (10.1 ± 2.7 ng/mL) and in blood of grey seals (*Halichoerus grypus*) from the Canadian Arctic (27.7 ± 11 ng/mL) (68). As a general observation, the study noted that PFOS did not increase with age of marine mammals, unlike certain other persistent pollutants such as PCBs. Further, samples of successive years indicated a temporal trend, as blood samples collected in 1998 contained significantly higher concentrations of PFOS than samples from 1996. Concentrations of PFOS in livers of grey seals from the Baltic Sea ranged from 140 to 360 ng/g wet wt (54). PFOS concentrations in livers of ringed seals from the same region were significantly greater (130-1100 ng/g wet wt). As in the study of Kannan et al. (68), no influence of age was seen on the concentrations of PFOS in liver tissue of both ringed and grey seal. In addition, there was no significant difference in the concentration of PFOS between sexes of ringed seals. Male grey seals, however, contained significantly higher PFOS values than females. The difference between ringed and grey seals could be explained by specific reproductive parameters. Earlier parturition (reproduction from ages

3 to 5) may explain the lower PFOS concentrations in female grey seals than in ringed seals (reproduction from about 6 years old). FOSA and PFOA were rarely detected in the seals (2 of the 52 animals). PFHxS was not detected at all. In another study (14), livers from ringed seals in the Canadian Arctic showed PFOS at concentrations of 8.6 to 37 ng/g wet wt. The dominant perfluorocarboxylate detected in these samples, was PFNA (2.4-8.8 ng/g wet wt), followed by PFUA (1.4-5.9 ng/g wet wt). FOSA was detected at concentrations of <0.5 to 5.5 ng/g wet wt. Ringed seals from east Greenland contained hepatic PFOS concentrations of 52 to 67 ng/g wet wt, and these levels were higher than the concentration in ringed seals from central west Greenland (<10-13 ng/g wet wt) and from northwest Greenland (27 ng/g wet wt) (74). PFOA, PFOSA and PFHxS concentrations were not quantifiable. The highest east Greenland concentrations were about 20 times lower than the highest concentration found in liver of ringed seals from the Baltic Sea (130-1100 ng/g wet wt) (54), however, they were 2 times higher than hepatic concentrations in ringed seals from the Canadian Arctic (up to 37 ng PFOS/g wet wt). Not only spatial trends, but also temporal trends, were observed in a study by Bossi et al. (79). Therefore, archived liver samples of ringed seal were used. A regression analysis of PFOS, PFDA and PFUA concentrations showed a significant increase of concentrations over time, in ringed seals from both east and west Greenland.

Polar bear (*Ursus maritimus*) livers from the Canadian Arctic (Hudson Bay) showed the highest concentration of each perfluorinated acid among all animals analysed in the study of Martin et al. (14; see also section 1.3.5.). As the concentration of PFOS exceeded 1 μg/g wet wt in all polar bear livers (range: 1700 - >4000 ng/g wet wt), PFOS was suggested to be the most prominent individual organohalogen contaminant detected in polar bears (compared to e.g. PCBs, chlordane components and hexachlorocyclohexane). PFOA levels were from 2.9 to 8.6 ng/g wet wt. The concentrations of individual PFCA homologues in polar bears ranged from <0.5 to 230 ng/g wet wt, with PFNA representing the highest concentrations. Concentrations generally decreased with increasing perfluorinated chain length. Liver from polar bears from Alaskan waters (68) contained 10-fold lower PFOS concentrations (175-678 ng/g wet wt), probably because the sampling location of the polar bears in the study of Kannan et al. (68) was at a higher latitude and thus, probably further away from regional sources of PFOS. Martin et al. (14) also commented that it can be

generalised from the data that were collected, that mammals feeding at higher trophic levels have higher concentrations of PFOS than mammals feeding at lower trophic levels. On this basis, a polar bear is at the top of the food chain and would therefore be expected to contain comparatively high levels of PFOS.

Two studies described PFAS concentrations in polar bears from east Greenland (69, 70, 74). PFOS concentrations of two pooled samples (5 individuals each) were 1245 and 1325 ng/g wet wt (74). However, concentrations in 29 polar bears analysed in the study of Smithwick et al. (69,70) ranged from 911 to 6340 ng/g wet wt, with a mean concentration of 2140 ng/g wet wt. These PFOS concentrations were amongst the highest ever measured in marine mammals. No clear explanation could be given for this difference in concentrations, as both studies were done between 1999 and 2001, at around the same altitude and with no clear difference in age or gender between the two sampling groups. Hepatic PFOS concentrations ranged from 263-2410 ng/g wet wt in the High Arctic to 2000-3770 ng/g wet wt in the South Hudson Bay. Alaskan polar bears (435-1480 ng/g wet wt) were significantly less contaminated than polar bears from all other regions. The higher concentrations in South Hudson Bay and East Greenland samples are probably due to the proximity to sources in Eastern North America and Europe. PFNA was the predominant of all carboxylates with concentrations up to 540 ng/g wet wt in polar bears from the North West Territories. PFOA, PFNA, PFDA, PFHxS and PFOSA were more or less evenly distributed over the different regions, while the PFCAs with chain lengths greater than 10 carbons showed similar geographical trends to PFOS. There were no significant differences between males and females for any of the perfluorinated compounds analysed. This is consistent with the fact that PFAS are retained via enterohepatic recirculation and this process is similar to both males and females. In addition, PFAS do not accumulate in fatty tissue, as other organohalogenated chemicals do, and thus, they are not expected to be excreted via lactation, a process that could result in a lower contaminant burden in older females. A significant increase in PFOS levels with age was described in four of the seven locations. The lack of a correlation at the other sampling sites may be due to the limited sample size, but also to differences in metabolism, diet and/or habitat.

Tomy et al. (65) described PFAS concentrations in beluga (*Delphinapterus leucas*), narwhal (*Monodon monoceros*) and walrus (*Odobenus rosmarus*) liver samples from the eastern Arctic. For PFOS, concentrations were 1.4-3.6 ng/g wet wt in walrus, 5.4-17.7 ng/g wet wt in narwhal and 9.8-33.2 ng/g wet wt in beluga. Walrus had nd-0.7 ng PFOA/g wet wt, narwhals contained 0.7-1.1 ng PFOA/g wet wt and beluga had the highest PFOA concentrations, ranging from 1.0 to 2.8 ng/g wet wt. Walrus liver concentrations of both PFOS and PFOA were statistically lower than those of narwhal and beluga. *N*-EtPFOSA was highest in beluga (0.1-11.7 ng/g wet wt), but also detectable in narwhal (0.5-6.9 ng/g wet wt). PFOSA concentrations were in some cases higher than PFOS concentrations, ranging from 6.8-10.9 ng/g wet wt in narwhal and from 3.9-48.4 ng/g in beluga. On average, *N*-EtPFOSA concentrations were 5-fold less than those of PFOSA. The authors make the suggestion that belugas have a greater ability to biotransform *N*-EtPFOSA as compared to narwhal as they both fed at a similar trophic level and likely have similar exposure to these compounds.

A screening of the European Nordic environment (47; see also section 1.3.4.) included three marine mammal species, namely grey seal, minke whale (*Balaenopterus acutorostrata*) and pilot whale (*Globicephala melas*). Grey seals from Denmark (ca. 550-990 ng PFOS/g wet wt) and Sweden (ca. 250-520 ng PFOS/g wet wt) were highest contaminated and characterised by dominant PFOS concentrations. A clear PFOS pollution gradient could be observed from the most northern Baltic region to Sweden/Denmark. Minke whales, a species that has a lower trophic position than pilot whales, contained overall relatively low PFAS levels. In comparison, minke whales from Greenland contained similar PFOS concentrations (<10 ng/g wet wt) (74). In pilot whales from the Faroe Islands, PFOSA exceeded in two case the PFOS levels (up to 300 ng PFOSA/g wet wt). Although lower concentrations were described by Bossi et al. (74) in pilot whales from the same region, a similar distribution of PFOSA (43-62 ng/g wet wt) and PFOS (28-65 ng/g wet wt) was seen. A liver sample of a suckling pilot whale pup from the Thyrrenian Sea contained 270 ng PFOS/g wet wt and 50 ng PFOA/g wet wt (54).

Also in marine mammals from the Italian coast of the Mediterranean Sea, PFOS was the most predominant fluorochemical in the tissues analysed (54). Hepatic PFOS concentrations were found in striped dolphins (16.3-40 ng/g wet wt), a common dolphin (*Delphinus delphi*) (940 ng/g wet wt) and bottlenose dolphins (<1.4-110 ng/g wet wt). PFOSA was detected in liver of bottlenose dolphin (30-139 ng/g wet wt), but not in striped dolphins.

The occurrence of PFOSA in different marine mammals is indicative of the presence of specific sources. PFOSA is an intermediate in the production of several PFAS and, probably of more importance in the accumulation process in marine mammals, a metabolic product in mammals of *N*-EtPFOSA (sulfuramid), a well known insecticide.

1.4. Toxicity and mode of action

Notwithstanding the global occurrence and distribution of PFAS in the aquatic environment, the toxicological information on these chemicals is very incomplete and insufficient to assess the impact and the hazard for man and biota. The last decade, a diversity of adverse effects have been reported in organisms exposed to various PFAS. Although most of the effects occur only at levels higher than those encountered in the environment until now, there are exceptions as some species appear to be markedly more sensitive than others. Furthermore, there still exists a critical data-gap on the precise details on the mechanistic molecular understanding of the mode of action and on the potency of toxic effects of PFAS. Moreover, most of the research studies are focussing on PFOS, PFOA and a small number of other PFAS. In addition, most of the toxicity data gathered so far deals with mammalian model species, and toxicity experiments were preferentially performed under controlled laboratory conditions. About 50% of the approximately 45 studies on toxicity testing of PFOS and related chemicals, available through *Web of Science* (on 21/12/2005), handles on terrestrial mammals (mouse, rat, monkey and guinea pig). Information on the impact of these chemicals on marine ecosystems is rather scarce. Below, a short overview of some of the most documented effects in different test species, is given.

Research studies have reported that some PFAS, like PFOS and PFOA, have potent hepatic peroxisome proliferating capacities in rats, mice and freshwater fish, a phenomenon that has been intimately correlated with hepatocellular carcinomas in rats and mice (80-83). Peroxisomes are very small, single-membrane organelles (0.5 μm diameter) present in nearly all eukaryotic cells, which contain several oxidative enzymes and which may produce hydrogen peroxide (H_2O_2). They are also involved in β-oxidation of fatty acids, synthesis of bile acids and cholesterol and, metabolism of amino acids and purines (30). As a consequence, peroxisome proliferation may lead to increase in liver weight, decrease in body weight, lowering of serum cholesterol and induction of peroxisomal β-oxidation, microsomal l-acylglycerophosphocholine acyltransferase and cytosolic long-chain-acyl-CoA hydrolase (84-86). Exposure of rats to PFDA seemed to be more toxic to hepatocytes than PFOA exposure and resulted in additional effects related to phospholipids metabolism (87). Effects on the oxidation of palmitoyl CoA and on the activity of catalase are generally indicating peroxisomal proliferation. This phenomenon is, as far as it's known for rats and mice, indissolubly connected with induction of cytochrome P-450 ω-hydroxylation and some drug metabolizing enzyme activities such as aminopyrine demethylase and ethoxycoumarin deethylase (81, 88-90).

Membrane-related effects were demonstrated in *in vitro* studies such as effects on the membrane permeability and an increase of membrane fluidity in fish leukocytes, (91) and inhibition of gap junction intercellular communication (92). It has been hypothesis that the inhibition of gap junction intercellular communication (GJIC), a process which is necessary for normal cell growth and functioning, is associated with tumour promotion. Hu et al. (92) used a rat liver epithelial cell line and a dolphin kidney epithelial cell line. The dolphin cell line was used in a first effort to develop a marine mammalian model for testing the effect of PFAS. PFOS, PFOSA and PFHxA were found to inhibit GJIC in a dose-dependent manner. The inhibition occurred rapidly and was reversible. A study of Upham et al. (93) showed that PFOA and PFDA also inhibited GJIC and that the inhibitory potency of PFCAs depends on the length of the carbon chain, as chain lengths of less than 5 or more than 16 did not had any effect on GJIC, which is consistent with the fact that Hu et al. (92) did not detect any inhibition of GJIC by PFBS.

Membrane disturbance was also detected in bacterial, cellular receptor-reporter assays, which were used to unravel the toxicological mechanisms of perfluorinated compounds (94). Thirteen different bacterial transgenic strains were tested to evaluate the cellular toxicity and mode of action of perfluorinated sulfonic acids and carboxylic acids with varying chain length. The effects studied included oxidative stress, heat shock response, DNA damage, DNA adduct formation and membrane disturbance. PFOS and its related compounds are causing multiple effects: membrane disturbance, oxidative stress and DNA damage. It seems that the observed effects depend on the chain length of the hydrophobic tail.

The reproductive and developmental toxicity of PFAS, and predominantly of PFOS, have been investigated in several organisms such as nematode worms (*Caenorhabditis elegans*), freshwater invertebrates like *Daphnia sp.*, birds, rodents and monkeys. Overall, the fecundity was strongly disrupted. In worms, a significant decrease in the number of eggs and worms was detected in the fourth generation after exposure to concentrations of 10 pM (5.38ng/L) PFOS and 1 nM PFNA (95). Acute water exposure to PFOS concentrations between 31.1 and 169 mg/L, resulted in immobility and a reduction in mean number of young in the brood of *Daphnia* (96). A dietary dose of 10 mg/kg PFOS resulted in an increased incidence of small testes in adult male birds. An overall effect on chick health, described as "failure to thrive", was reported in bobtail quail (*Colinus virghinianus*) (97). In rodents, dose-dependent deleterious effects were seen in newborns. When pregnant dams were exposed to high doses of PFOS (5-10 mg/kg), nearly all pups did not survive the first day of postnatal life, and only few pups reached puberty. However, survival improved with lower PFOS exposure (98). A similar observation was made by Butenhoff et al. (99), when exposing female rats to PFOA during pregnancy. However, apparently, maternal exposure of rats to PFBS did not produce any adverse effects on embryo/fetal development. Postnatal deaths and developmental problems were also reported in PFOS exposed monkeys in addition to weight losses, convulsions and changes in liver enzymes (21).

The scarce knowledge on toxicity data points out that there is an urgent need for the mechanistic molecular understanding of the mode of action of these chemicals.

Toxicogenomic-based methods are potentially well suited to meet this challenge by measuring specific changes in gene expression profiles as a result of exposure to environmental contaminants. Gene expression data can be useful in identifying affected pathways and possible mode of actions. However, once genes or pathways have been identified, changes in more toxicologically relevant parameters such as proteins or substrates should be measured subsequently. Until now, only few studies have focussed on genes responsive to PFOS or related chemicals, using relatively new techniques like microarray technologies (100-103). Hu et al. (100,101) used the Affymetrix rat genome U34A gene chip to identify alterations in gene expression. The main conclusion was that PFOS alters gene expression profiles, both *in vivo* and *in vitro*. Genes which were up regulated after PFOS exposure were involved in fatty acid metabolism and hormone regulation, or encoding for cytochrome P450s. Genes which were suppressed by PFOS play a role in signal transduction pathways and neurosystem regulation.

In a preliminary study (102), a microarray analysis suggested that PFOS could elicit up regulation of some carp hepatic genes involved in a number of physiological processes such as liver fatty acid binding protein, chymotrypsinogen, toxin-1 and serum lectin. However, these results could not be confirmed by Real Time-PCR, concluding that further work should be done to unravel the molecular mode of action of PFOS in juvenile carp. A very recent study on fluorotelomer alcohols showed estrogen-like properties of these compounds as revealed by MCF-7 breast cancer cell proliferation (103). FTOHs seemed to have a similar xeno-estrogen character as seen with nonylphenol, a very well known endocrine disruptor.

In general, authors suppose that effects detected in laboratory studies do not a pose a direct threat to wildlife or humans, as concentrations used in these studies are (still) several times higher than those found in the environment. However, ecotoxicological studies performed on mice, birds and fish from a Belgian nature reserve in the vicinity of a fluorochemical plant, have described PFOS-mediated stress responses (e.g. increase in relative liver weight, increased microsomal lipid peroxidation, elevated serum aminotransferase activity and increased hematocrit values) (11-13). Furthermore, very high concentrations of PFOS have been reported in the environment following accidental spills of PFOS (39). This

brings into concern possible adverse impacts on aquatic organisms like midge which seemed to be influenced already at concentrations of 50 µg/L (104). Moreover, effects on the neuroendocrine system of rats, namely disruption of the regularity of the estrous cycle and increased serum corticosterone levels, were observed at PFOS exposure concentrations lower than levels present in wildlife and similar to levels in occupationally exposed humans (105).

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