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EXPOSURE PATTERNS OF PERFLUOROOCTANE SULFONATE IN AQUATIC INVERTEBRATES FROM THE WESTERN SCHELDT ESTUARY AND THE SOUTHERN NORTH SEA

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Abstract—Over the past decades little research has been conducted on the environmental behavior and effects of fluorinated organochemicals (FOCs). Recently it has been reported that perfluorooctane sulfonic acid (PFOS) is occurring worldwide. Little is known about the PFOS levels in organisms originating from the southern North Sea and the Western Scheldt estuary. In this study, we determined, for the first time, the PFOS-exposure levels in *Crangon crangon*, *Carcinus maenas*, and *Asterias rubens* from these ecosystems. Concentrations on a wet-weight basis in soft tissues of shrimp, crab, and starfish ranged from 19 to 520 ng/g, from 24 to 877 ng/g, and from 9 to 176 ng/g, respectively. These results show the existence of a PFOS pollution gradient in organisms along the Western Scheldt estuary, with the highest concentrations near Antwerp. The range of PFOS levels in shrimp and crab are slightly higher in coastal regions compared with sampling sites in open water. This study shows widespread distribution of PFOS in the Belgian and Dutch marine and estuarine environment at rather high concentrations.

Keywords—Perfluorooctane sulfonic acid Aquatic invertebrates Western Scheldt estuary North Sea Pollution gradient

INTRODUCTION

Like many other estuarine areas, the Western Scheldt estuary, situated southwest of The Netherlands and north of Belgium, is polluted. A significant input of pollutants to the North Sea comes from the extension of the Scheldt discharge plume. The plume contains polybrominated diphenyl ethers, polychlorinated biphenyls (PCBs), heavy metals, pesticides, and volatile organic compounds [1–4]. Earlier studies of this system reported on the distribution and effects of halogenated organochemicals and other contaminants on fish [5,6], shrimp [7], crab [8], and starfish [9,10].

Due to their environmental persistence and tendency to bioaccumulate, organochlorinated compounds have been a topic of several research studies. More recently, the importance of fluorinated organic compounds (FOCs) in the environment has been recognized [11,12]. Fluorinated organic compounds are globally distributed in various wildlife species. In particular, samples from the Mediterranean and Baltic Sea and the Pacific region have revealed elevated concentrations of perfluorinated sulfonic acid (PFOS) [13]. Perfluorinated compounds such as the sulfonic acids, carboxylic acids, and the sulfonamides have unique physico-chemical properties (such as extreme electronegativity of the fluorine atoms, very strong C-F bond, and surfactant-like structure), which make them commercially useful as refrigerants, surfactants, components of pharmaceuticals, flame-retardants, cosmetics, and insecticides [14]. Several of these applications involve long-lived goods that will serve as sources for possible environmental contamination for many years. Adverse effects of FOCs have been reported in studies with rodents and fish [15–18]. As investigations continued, a major FOCs producer (3M, Maplewood, MN, USA) announced in 2000 that it was phasing out the production of perfluorooctane sulfonyl–based compounds [19].

This study was conducted to evaluate the magnitude of exposure in invertebrate biota from the Western Scheldt estuary and North Sea. One of the largest fluorochemical production plants is located near Antwerp and is thought to be a potential source of PFOS in the Western Scheldt. During sampling campaigns in October and November 2001, we collected starfish (*Asterias rubens*), crab (*Carcinus maenas*), and shrimp (*Crangon crangon*). Data for two fish species (*Pleuronectes platessa* and *Trisopterus luscus*) collected during these campaigns are reported elsewhere [20]. The results from these studies provide initial insight into chemical organo-fluor contamination levels in the marine and estuarine environment in Belgium and The Netherlands. Furthermore, this study establishes the basis for further studies on the distribution patterns of PFOS in other marine and estuarine ecosystems.

MATERIALS AND METHODS

Sampling

During two field campaigns in October and November 2001, tissues of various invertebrate species were collected on the Belgian continental shelf and in the Western Scheldt estuary. Samples were taken from the RV De Zeeleeuw (VLIZ, Flanders Marine Institute, Oostende, Belgium) using a 3-m beam trawl equipped with a fine-meshed (6 × 6 mm) net. The duration of the hauls was about 30 min and ship speed relative to the bottom when trawling was 1.5 to 2 knots. Eight sampling locations were selected randomly along the coastline and in the estuary (Fig. 1). In the southern part of the North Sea,

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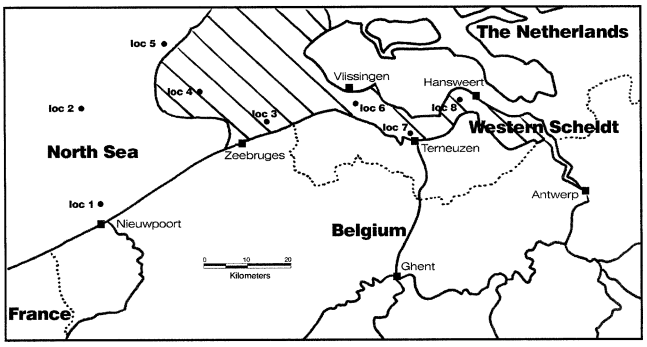

Fig. 1. Area of study and sampling sites and the possible outflow of pollutants from the Western Scheldt estuary in the Belgian Coastal Zone (after Delhez et al. [25]). The marked zone indicates the river plume of pollutants.

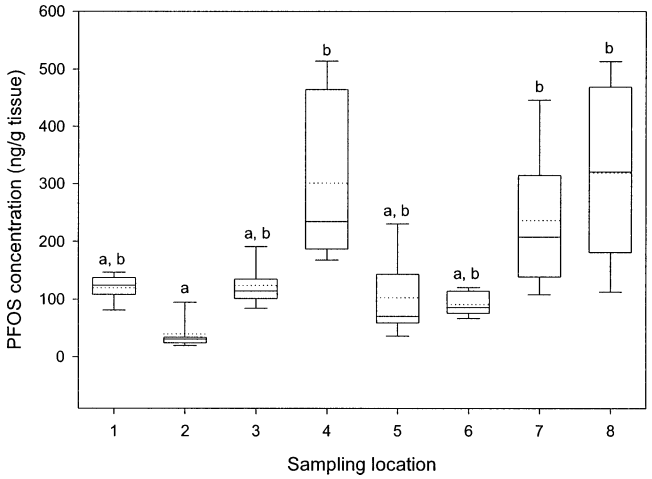

Fig. 2. Perfluorooctane sulfonic acid (PFOS) concentrations in *Crangon crangon* at eight sampling locations (see Fig. 1). The solid line is the median and the dotted line represents the mean. The fifth and 95th percentiles define the boxes. The whiskers represent 10th and 90th percentiles. The number of shrimp was 6 in all cases except for locations 2 and 5, where $n = 4$. Different letters above the bars indicate that the means are significantly different among sampling locations ($p < 0.05$).

samples were taken before Nieuwpoort and Zeebruges. Three locations were selected in the Western Scheldt: east of the Spijkerplaat and east of Terneuzen and Hansweert. At each location, we collected crabs and shrimp. Starfish were collected only at two locations (2 and 4) in the North Sea. The organisms were stored on board at −20°C until analysis. The distance from these sites to the harbor of Antwerp was calculated using the shortest distance over water.

Chemical analysis

Concentrations of PFOS in whole body tissues of all of the animals were determined using high-performance liquid chromatography combined with electrospray tandem mass spectrometry (LC-MS/MS) as described by Giesy and Kannan [11], with minor modifications. One gram of soft tissue was homogenized on ice with an MSE 150 W ultrasonic disintegrator (MSE Scientific Instruments, Sussex, UK) with 3 ml Milli-Q water (Millipore®, Brussels, Belgium). Afterward, 500 μl of homogenate; 10 μl of internal standard; 1H, 1H, 2H, and 2H-perfluorooctane sulfonic acid (Sigma-Aldrich Chemical, Milwaukee, WI, USA); 1 ml of 0.5 M tetrabutylammonium hydrogen sulfate solution (adjusted to pH 10); and 2 ml of 0.25 M sodium carbonate buffer were thoroughly mixed. Five milliliters of methyl-*tert*-butyl ether was added and the mixture was shaken for 2 h at 20°C (250 rpm). The organic and aqueous layers were separated by centrifugation, and 5.45 ml was removed from the aqueous layer. After evaporating the solvent under a stream of N_2, the extract was resuspended in 0.5 ml methanol and filtered trough a 0.2 μm nylon mesh filter. We analyzed the samples by high-performance liquid chromatography (CapLC system; Waters, Millford, MA, USA) connected to a Quattro II triple quadrupole mass spectrometer (Micromass, Manchester, UK) operated in the electrospray negative mode. Five-microliter aliquots of extracts were injected onto an Optiguard C18 precolumn (Alltech, Sercolab, Belgium), followed by a Keystone Betasil C18 column (50 × 1 mm internal diameter, Hypersil-Keystone Scientific, Bellefonte, PA, USA). The flow rate was 40 μl/min. The mobile phase was 2 mM ammonium acetate/methanol. A gradient solution was used starting at 45% methanol and increasing until 90% in 3 min. The PFOS was measured under (−) electrospray ionization using single-reactant monitoring (m/z 499→99). The internal standard was measured under the same conditions (single reactant monitoring m/z 427→81). The dwell time was 0.1 s. The electrospray-capillary voltage was set at −3.5 kV and the cone voltage was 24 V. The source temperature was 80°C. The pressure in the collision cell was 3.3×10^{-5} mm Hg (Ar).

Determination of total carbohydrate, lipid, and protein content

After homogenization of the soft tissues, lipid and carbohydrate concentrations were determined as described by De Coen and Janssen [21]. Total protein concentrations in tissue homogenates were measured using the Bio-Rad Protein Assay (Bio-Rad, Munich, Germany).

Statistical analyses

Values are presented as mean concentrations ± standard error. A nonparametric Kruskall-Wallis ANOVA was used to compare mean concentrations among sampling sites, as data were inspected and found to have nonhomogeneous variances, which could not be made to approximate normal by transformation. A Dunn's test was used as a post hoc criterion to determine if the differences among the sites were statistically significant ($p = 0.05$). We used a Pearson product-moment correlation analysis to assess the correlations between the shortest distance over water to the harbor of Antwerp and the various PFOS concentrations in the different species. The same statistical test was used to analyze the associations between the total carbohydrate, lipid, and protein content and the PFOS levels.

RESULTS

Perfluorooctane sulfonic acid levels were detected in all samples analyzed. The results obtained by analysis of the whole body, soft tissue of shrimp and crab are presented in Figures 2 and 3 and are shown as the minimal, maximal, mean, and median concentrations. Mean concentrations in starfish ranged from 16 ± 3 ng/g (location 2; $n = 6$) to 93 ± 34 ng/g (location 4; $n = 5$), with a maximal concentration of 176 ng/g determined at location 4. All concentrations are measured on a wet-weight basis. The PFOS concentrations in shrimp ranged from 19 to 520 ng/g. Significantly higher levels were

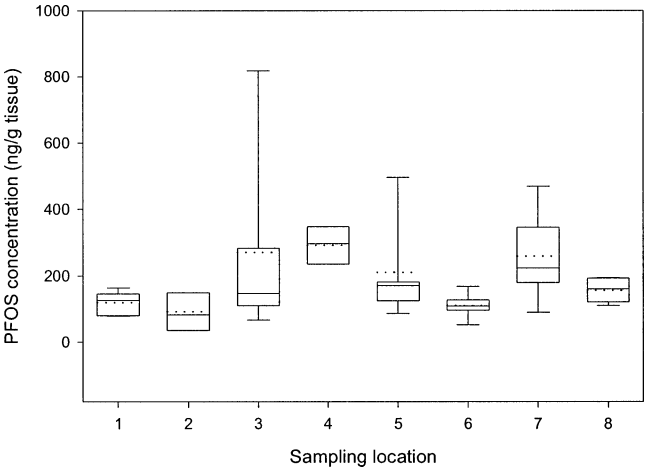

Fig. 3. Perfluorooctane sulfonic acid (PFOS) concentrations in *Carcinus maenas* at eight sampling locations (see Fig. 1). The solid line is the median and the dotted line represents the mean. The fifth and 95th percentiles define the boxes. The whiskers represent 10th and 90th percentiles. The numbers of crab was 6 in all cases except for location 4, where $n = 5$.

found in shrimp tissue from locations 4, 7, and 8 (301 ± 62, 237 ± 59, and 319 ± 70 ng/g, respectively) compared with location 2 (40 ± 13 ng/g; Fig. 2). No significant difference could be detected between the mean concentrations found at these three locations and at locations 1, 3, 5, and 6. In addition, a significantly positive correlation was found between the PFOS content in shrimp and the distance from the sampling locations in the Western Scheldt to the harbor of Antwerp, which is considered to be the most likely source of any possible local PFOS pollution ($r^2 = -0.99$; $p < 0.05$).

Although mean concentrations of PFOS in crab tissue (ranging from 93 ± 36 to 292 ± 45 ng/g) showed no significant difference when comparing the various sampling locations (Fig. 3); the highest concentration determined in crab from Zeebruges is 4.9 times higher than the maximal concentration in crab from Nieuwpoort (877 vs 180 ng/g).

On average, concentrations of PFOS were slightly higher in coastal regions compared with the open-water sampling locations for the three species. The mean PFOS concentration in shrimp and crab originating from the coast of Nieuwpoort were 120 ± 10 ng/g and 120 ± 15 ng/g, respectively, versus 40 ± 13 ng/g and 93 ± 36 ng/g in shrimp and crab collected at open sea. Although we could only measure PFOS concentrations in starfish from two marine locations, a similar result could be found. A significantly higher contaminant level ($p < 0.05$) was detected in starfish from location 4 compared with location 2.

When comparing the different species for locations 2 and 4, a significant difference was found between the mean concentration in crab and starfish from location 2. For location 4, both the mean concentrations in crab and shrimp were significantly different from the mean concentration in starfish ($p < 0.05$). No differences in PFOS levels between shrimp and crab could be detected for the various sampling sites.

Lipid, protein, and carbohydrate levels are frequently used as measurements for organismal energy reserves, and these parameters might be an indication of the health conditions of organisms. A significant positive correlation was found between the PFOS contaminant load in starfish and the total protein content ($r^2 = 0.74$, $p < 0.001$). Positive correlations between PFOS concentrations and total lipid content in shrimp ($r^2 = 0.17$, $p = 0.005$) and total carbohydrate content crab ($r^2 = 0.10$, $p < 0.05$) were also revealed. However, these correlations seemed to be highly circumstantial. No other correlations could be found between the measured biochemical parameters and the PFOS content.

DISCUSSION

This paper is among the first detailed studies on PFOS concentrations in invertebrates from the southern North Sea and Western Scheldt estuary. The results show the existence of a PFOS pollution gradient along the Western Scheldt, with the highest concentrations near the harbor of Antwerp. Elevated levels in shrimp and crab close to Antwerp can be explained by the presence of a major fluorochemical manufacturing facility near Antwerp. Although there might be an influence of large domestic and industrial wastewater discharges further upstream, there are no obvious potential sources of fluorochemicals in the river. In a previous study about trace levels of PFOS and PFOA in the Tennessee River (USA), Hansen et al. [22] indicated that effluent from a fluorochemical production plant may be one route of introduction to some fluorinated chemicals in the environment.

In contrast to shrimp, PFOS levels in crab tissue showed no significant correlation with the proximity of the sampling sites to Antwerp. However, the higher concentrations in crab caught at the inflow of the canal Ghent-Terneuzen suggest that this canal might contribute considerably to the observed PFOS levels in these ecosystems. Several applications of PFOS and related products exist in various types of industry situated in the industrialized region around the canal Ghent-Terneuzen. For example, paper mills might apply sulfonated fluorochemicals for surface treatment of paper products (i.e., to render them resistant to grease, water, and oil).

Different pollution gradients are known to exist along the Western Scheldt, with increasing levels of heavy metals and volatile compounds toward the canal of Ghent-Terneuzen and the harbor of Antwerp, suggesting high inputs of anthropogenic discharges into the aquatic ecosystem through this river system [2,23,24]. In a previous study about the Western Scheldt, a PFOS pollution gradient in liver of bib (*Trisopterus luscus*) and plaice (*Pleuronectes platessa*) has been suggested [20]. The PFOS muscle concentrations decreased when sampling was done closer to the North Sea.

Elevated concentrations of PFOS in shrimp, crab, and starfish from locations near the harbor of Zeebruges can be explained by the influence of the Western Scheldt upon the North Sea. A model of the Scheldt basin and the Belgian coastal zone (Sea Air Land Modelling Operational Network project) has been developed to describe the extension of the Scheldt plume and to investigate possible consequences of discharges of pollutants in the river. This model reveals a marked river plume extending along the Belgian coastal zone with strong offshore gradients [25]. This plume flows along the Belgian coast toward Zeebruges and Oostende with the highest pollution values at the center of the plume, which might explain the higher contaminant levels found at locations 3, 4, and 5 (Fig. 1). The fact that the river plume does not reach Nieuwpoort and its surroundings might explain why PFOS levels in organisms sampled near Nieuwpoort are lower than those from Zeebruges.

Coastal ship traffic around the harbor of Zeebruges also might influence the higher PFOS concentrations in inverte-

brates sampled at these locations. At present, it remains unclear which contribution the maritime transport will have on the PFOS distribution. Discharges of PFOS from the coastal zone into the aquatic environment might be the reason why concentrations near the coast are slightly higher than further into sea. A similar tendency was found in aquatic mammals from Oregon and Washington (USA) [12]. Levels of PFOS detected in organisms from inland and coastal waters were greater than those found in oceanic waters. Higher concentrations of PFOS in tissue can be expected near direct discharges, rather than at marine stations.

Differences in routes of exposure and/or uptake mechanism of PFOS could account for the observed differences in PFOS concentrations in soft tissues of the organisms we sampled. At present, no clear insight has been gathered on the most important routes of exposure. On the contrary, there have been clear contradictions in the literature. According to Hansen et al. [22], water concentrations of PFOS in the Tennessee River, near a fluorochemical manufacturing site, seem to be rather low (ppt). The authors believe that there are no physical depletion mechanisms such as volatilization or adsorption to soil or sediment. However, a report from RIZA (Institute for Inland Water Management and Waste Water Treatment, Lelystad, The Netherlands) about the effects of fire-fighting foams on the environment declares that adsorption to sediment is the most important mechanism for removal of fluorinated surfactants from water [26]. In our study, the elevated levels in shrimp and crab suggest at least some interaction of the chemical with the sediment and/or particulate phase. The concentrations measured in starfish are significantly lower than the concentrations in shrimp and/or crab from the sampling locations 2 and 4. Shrimp and crab are found on substrates such as sand and rocky ground. Starfish are more commonly associated with mussel beds or can be found among barnacles in rocky intertidal zones. Differences in routes of exposure to the sediment may influence accumulation patterns of PFOS in benthic organisms. The different feeding ecology of the species might be another factor that leads to elevated concentrations of PFOS in the organisms sampled. Mycids and amphipods appear to be the dominant prey of *Crangon crangon* [27]. The most common prey species of *Carcinus maenas* are molluscs, arthropods, and annelids [28]. Starfish feed on a wide variety of invertebrates, such as polychaete worms, crustaceans, molluscs, and other echinoderms. The PFOS concentrations in biota can be influenced by a different accumulation pattern in the various prey species and by differences in the metabolic pathways and/or the elimination processes of perfluorinated compounds. However, at present no specific information about elimination of PFOS for aquatic invertebrates is known.

Levels of PFOS in shrimp, crab, and starfish from this study are compared with body burden residues in different wildlife samples (Fig. 4) [11–14]. In our study, all measured concentrations fall into the range of previously detected levels. The PFOS levels in oysters (*Crassostrea virginica*) from the Gulf of Mexico and the Chesapeake Bay (USA) were as high as 100 ng/g wet weight [14], but it is difficult to compare these results with ours because in the oyster study small patches of contamination and known points of waste discharge were deliberately avoided. Maximum values of shrimp and crab exceed 500 ng/g PFOS and are among the highest measured PFOS concentrations in marine environmental samples. Moody et al. [29] reported total perfluoroalkanesulfonate concentrations in fish liver tissue from the highly polluted Etobicoke Creek (Toronto, ON, USA), which ranged from 2 to 73 μg/g. The PFOS was detected in each fish liver sample that was analyzed, and from all FOCs measured, PFOS was the predominant homologue. However, these extremely high values are more than likely attributed to the accidental release of fire retardant foam containing high concentrations of perfluorinated compounds into Etobicoke Creek.

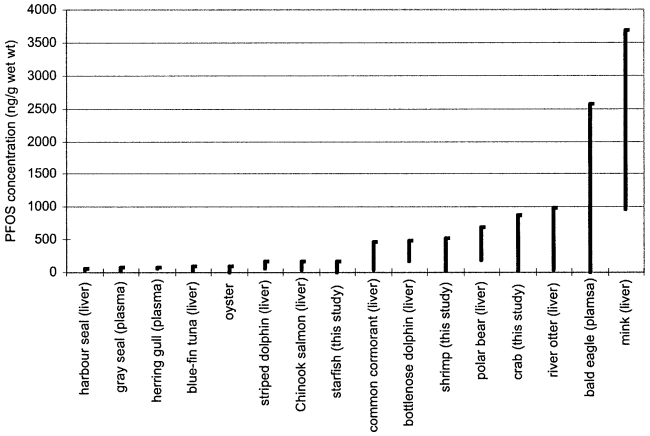

Fig. 4. Ranges in concentrations of perfluorooctane sulfonic acid (PFOS; ng/g wet wt) for different organisms. Values for organisms not mentioned in this study are from References [11–14].

To obtain data on the potential impact of perfluorooctane sulfonic acid, a variety of biochemical parameters were investigated. A positive relation was found between the protein content and the PFOS concentration in starfish. A similar correlation between PFOS concentrations and total protein content was reported in bib liver [20]. A possible explanation may be that exposure to xenobiotics might lead to an increase of the protein level and consequently have an influence on the induction of detoxification processes [30]. The correlations found between lipid content and PFOS concentrations in shrimp, and between the carbohydrates and pollutant levels in crab, have poor predictive capacity. This might be due to the relatively small sample set. More data are needed to get further insight into the working mechanisms and the impact of these compounds on the overall health of these organisms.

In summary, the results of this study revealed a gradient concentration of PFOS in organisms along the Western Scheldt estuary, with the highest concentrations nearest the harbor of Antwerp. The range of PFOS concentrations in soft tissues of shrimp and crab were slightly higher in coastal regions compared with sampling locations in open water. The higher levels of PFOS in organisms originating from Zeebruges might be explained by the influence of the industrialized Western Scheldt estuary.

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