PERFLUOROOCTANE SULFONIC ACID IN BIB (TRISOPTERUS LUSCUS) AND PLAICE (PLEURONECTES PLATESSA) FROM THE WESTERN SCHELDT AND THE BELGIAN NORTH SEA: DISTRIBUTION AND BIOCHEMICAL EFFECTS

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(Received 3 June 2002; Accepted 6 September 2002)

Abstract—A biomonitoring campaign was conducted in the Belgian North Sea and in the Western Scheldt (The Netherlands) with the primary goal to assess perfluorooctane sulfonic acid (PFOS) contamination and distribution in different biota. This study covers the results obtained for bib (Trisopterus luscus) and plaice (Pleuronectes platessa) and includes the assessment of some stress-related biochemical endpoints. Analysis of liver and muscle PFOS concentrations of both species provided evidence for the existence of a PFOS pollution gradient along the Western Scheldt with higher levels at the upstream locations and a lower degree of PFOS pollution at the marine locations. Cellular necrosis was studied by measuring aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels in the serum. Serum ALT but not serum AST was shown to correlate positively with the PFOS liver concentration in bib ($r = 0.44, p < 0.05$), indicating that PFOS might contribute to the induction of hepatic damage in bib in the area of study. Analysis of total carbohydrate, lipid, and protein content of bib liver tissue revealed a positive correlation between the protein content and the PFOS liver concentration ($r = 0.55, p < 0.01$). Whether this is due to induction of compensatory mechanisms, detoxification, or repair processes remains unclear.

Keywords—Perfluorooctane sulfonic acid Western Scheldt Aminotransferase Protein

INTRODUCTION

The widespread character of perfluorooctane sulfonic acid (PFOS) in wildlife was only recently demonstrated by Giesy and Kannan [1]. Other reports confirmed the presence of PFOS in marine mammals [2] and fish-eating water birds [3]. The PFOS tissue concentrations in aquatic organisms and their predators were mainly described for species in the United States, Canada, and the Pacific region, and there is a lack of data on the degree of PFOS pollution and distribution in aquatic wildlife tissues in Western Europe.

In this context, the knowledge of PFOS tissue burdens and distribution in wildlife species in the North Sea and the Western Scheldt are of major interest considering the high ecological value of the Western Scheldt as a nursery [4–7] and the reported biologically relevant effects on wildlife as a result of pollution [8–10]. Furthermore, it is known that the Belgian part of the North Sea is under the continuous threat of anthropogenic pollutants such as heavy metals [11] and polychlorinated biphenyls [12]. The importance of assessing coastal and estuarine ecosystems for PFOS exposure is emphasized by the observation that PFOS concentrations in animal tissues from more densely populated and industrialized locations is higher than for animals at more remote marine locations [1].

The degree of PFOS pollution in the Western Scheldt estuary (The Netherlands) is of particular interest because an important fluorochemical production plant is located in Antwerp (Belgium), a city upstream of the Western Scheldt estuary. Next to PFOS discharges into this estuary, other fluorochemicals that have been discharged may transform metabolically to PFOS as an end-stage metabolite and might possibly cause increased PFOS levels in tissues of the estuarine and marine fauna [13].

Although PFOS is not characterized very well on a toxicological level, a substantial number of effects have been documented. PFOS was shown to have membrane-related effects in vitro studies such as increase of membrane fluidity [14] and inhibition of gap junction intercellular communication [15].

In vivo experiments showed that PFOS affects lipid metabolism in rodents [16–18]. Furthermore, PFOS induces reduced maternal body weight gain and feed consumption, increased abortions, and reduced fetal weights in rabbits [19].

Recently, a short term in vivo exposure study with the common carp (Cyprinus carpio) showed that PFOS induced an increase in serum aspartate aminotransferase and alanine aminotransferase levels indicative for leakage of hepatocytes and possibly liver necrosis (P. Hoff et al., Antwerp University, Antwerp, Belgium, unpublished data).

The first aim of the present study was to determine PFOS concentrations in muscle and liver of two common fish species (bib and plaice) at various locations on the Belgian continental shelf and in the Western Scheldt in order to obtain preliminary information about the severity of PFOS pollution and its distribution in this ecosystem. Analysis of the relation between the PFOS liver concentrations and biological endpoints such as fork length and serum activity of ALT and AST allowed a preliminary effect evaluation of PFOS on fish in an estuarine/marine environment. The overall effect of PFOS on the major components of the energy metabolism were investigated by

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assessing correlations between liver PFOS burdens and liver total protein, lipid, and carbohydrate content.

MATERIALS AND METHODS

Sampling

Fishes were sampled with a 3-m beam trawl with fine-meshed nets (6 × 6 mm) in the Belgian part of the North Sea and the Western Scheldt in October and November 2001 using the research vessel Zeeleeuw. Trawling was carried out with the tide with a speed of 1.5 to 2 knots for about 30 min. The locations at which trawling was initiated were location 1 (Zeebrugge coastal area, 51°22’N, 03°16’E), location 2 (east of the Spijkerplaat, 51°25’N, 03°36’E), location 3 (east of Terneuzen, 51°21’N, 03°45’E), and location 4 (Hanssweert, 51°25’N, 04°00’E) for bib and location 5 (Nieuwpoort coastal area, 51°09’N, 02°40’E), location 6 (Nieuwpoort marine area, 51°24’N, 02°38’E), location 7 (Zeebrugge marine area, 51°32’N, 02°55’E), and location 8 (West of Terneuzen, 51°20’N, 03°51’E) for plaice. The distance from these sites to Antwerp was calculated as the shortest distance over water (Fig. 1). The number of fish caught at each location ranged between 4 and 8 for bib and 4 and 7 for plaice. The captured fishes were kept in a tank with aerated seawater until they were killed by a blow on the head. Blood was taken immediately after killing via caudal puncture. Serum was prepared on board by centrifugation at room temperature (4,000 rpm, 5 min) and collection of the supernatant. Serum, liver, and muscle tissue was stored on-board at −20°C until further analysis.

Determination of PFOS concentrations

The PFOS concentrations in the liver and muscle tissue of the animals were measured using liquid chromatography/electrospray tandem mass spectrometry according to Giesy and Kannan [1]. The HPLC was done on a CapLC system (Waters, Millford, MA, USA) connected to a Quattro II triple quadrupole mass spectrometer (Micromass, Manchester, UK). Aliquots of 5 μl were loaded on an Optiguard C18 precolumn (10 mm × 1 mm i.d.; Alltech, Sercalob, Belgium). The analysis was performed on a Betasil® C18 column (50 mm × 1 mm i.d.; Hypersil-Keystone Scientific, Bellefonte, PA, USA) at a flow rate of 40 μl/min. The mobile phase was 2 mM NH4OAc (A)/CH3OH (B). A gradient elution was used starting at 45% B and going to 90% B in 3 min. After 5 min, initial conditions were resumed. The PFOS was measured under (−) electrospray ionization using single-reactant monitoring ([SRM] m/z 499 → 99). The internal standard (1H, 1H, 2H, 2H-perfluorooctane sulfonic acid) was measured under the same conditions ([SRM] 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 × 10−3 mm Hg (Ar).

Determination of serum aminotransferase activities

Serum alanine aminotransferase and aspartate aminotransferase activities were determined by the spectrophotometric methods described in Bergmeyer et al. [20] and Bergmeyer et al. [21] respectively. The Bio-Rad protein assay (Bio-Rad, Munich, Germany) was used for assessing the total serum protein concentration.

Determination of lipid, carbohydrate, and protein concentrations in the liver

Liver samples were homogenized on ice with an MSE 150 Watt ultrasonic disintegrator (MSE Scientific Instruments, Sussex, UK). Lipid and carbohydrate determination was carried out according to De Coen and Janssen [22]. The Bio-Rad protein assay (Bio-Rad) was used for assessing the total liver protein concentration.

Statistical analysis

The mean intraspecies bib liver PFOS concentrations for each location were compared with one-way analysis of variance and Tukey’s test as post hoc criterion. Homogeneity of variance was confirmed with Bartlett’s test. The nonparametric Kruskal–Wallis test was applied with Dunn’s test as post hoc criterion at a significance level of p = 0.05 for comparison of the mean plaice PFOS liver concentrations, the serum ALT and AST activity, and the liver protein content at the sampling locations. To investigate the associations between the PFOS liver content and the distance from Antwerp; the serum ALT and AST activity; the fork length; the liver protein, carbohydrate, and lipid content, Pearson product-moment correlation analysis was used because the normality of these variables was confirmed with Kolmogorov–Smirnov’s test. The same methodology was used for investigation of the association between the distance of the sampling locations from Antwerp and the AST and ALT activities and for analysis of the correlation between the serum aminotransferase activities.

RESULTS

The serum ALT and AST activity and the liver protein contents for plaice and bib are plotted in Figures 2 and 3, respectively. For plaice, no significant differences between the locations were observed, although the mean serum ALT activity in the Western Scheldt (location 8) was higher than those at the marine locations. The mean liver protein content was lower at location 8. For bib, increased values of both aminotransferase activities and the liver protein content were observed along the Westerscheldt axis. The mean serum AST activity and the mean liver protein content at location 4 were significantly higher than those at location 1, while the mean ALT activity at location 4 was significantly higher than at location 2.

The PFOS concentrations measured in liver of plaice and bib are shown in Figures 4 and 5, respectively. All liver concentrations were above the detection limit (10 ng/g wet wt). For plaice liver, no significant differences in mean PFOS liver content between sampling locations were recorded (Fig. 4). At the only estuarine sampling location where plaice was caught, three spec-
imens out of four had extremely high liver concentrations, i.e., 1,286 ng/g wet weight, 1,744 ng/g wet weight, and 7,760 ng/g wet weight, accounting for the high variation at that site (Fig. 4).

Figure 4 also shows that the mean and median marine liver concentrations in plaice from the North Sea were lower than the mean and median at the estuarine location (location 8).

In bib, the mean PFOS concentration at location 4 was found to be significantly higher than the mean at location 1. As can be seen in Table 1, a significant negative correlation was found between the distance from the sampling locations to Antwerp and the PFOS liver content in bib. The mean PFOS concentrations in bib liver at the Western Scheldt locations were always higher than those at location 1, the only marine sampling location where bib was sampled (Fig. 5).

A clear reduction in the percentage of plaice with PFOS muscle concentrations below the detection limit was observed when the estuarine sampling location (location 8) was compared with the marine locations (locations 5, 6, and 7), as can be seen in Table 2. For plaice, the maximal marine muscle concentrations were 2.22 to 6.25 times lower than the highest estuarine muscle concentration determined for that species.

For bib, an increasing trend was observed for the minimum and maximum PFOS muscle concentrations when sampling was done at locations closer to Antwerp (Table 3). The ratios in Table 3 show that the various maximal estuarine muscle concentrations in bib are between 2.3 and 3.7 times higher than the maximal marine concentration measured.

The liver PFOS content of bib was significantly correlated with the liver protein content and the serum ALT activity (Table 1). The PFOS content of bib liver was not found to be significantly associated with the liver lipid and carbohydrate content, and no significant correlation was found between the liver PFOS content and the serum AST activity. Furthermore, a significant negative correlation between the liver PFOS content and the fork length of bib was shown. For plaice, none of these endpoints were found to correlate with the liver PFOS content. The serum AST and ALT activity in bib were both significantly correlated with the distance toward the city of Antwerp. The serum activities of ALT and AST were shown to correlate significantly (Table 1).

DISCUSSION

In the present study, we report for the first time on the exposure levels of PFOS in the Western Scheldt and the Bel-
Table 1. Results of the correlation analysis between the liver perfluorooctane sulfonic acid (PFOS) content and the endpoints investigated and between some endpoints*  

<table>
<thead>
<tr>
<th>Correlation</th>
<th>Bib (n = 21)</th>
<th>Plaice (n = 22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFOS liver concentrations versus distance toward Antwerp</td>
<td>*r = −0.61</td>
<td>ND</td>
</tr>
<tr>
<td>Serum AST activity versus distance toward Antwerp</td>
<td>***p = 0.004</td>
<td>ND</td>
</tr>
<tr>
<td>PFOS liver concentrations versus serum ALT activity</td>
<td>*p = 0.047</td>
<td>p = 0.85</td>
</tr>
<tr>
<td>PFOS liver concentration versus serum AST activity</td>
<td>p = 0.155</td>
<td>p = 0.62</td>
</tr>
<tr>
<td>PFOS liver concentrations versus liver lipid content</td>
<td>r = 0.24</td>
<td>r = −0.027</td>
</tr>
<tr>
<td>PFOS liver concentrations versus liver carbohydrate content</td>
<td>p = 0.28</td>
<td>p = 0.91</td>
</tr>
<tr>
<td>PFOS liver concentrations versus liver protein content</td>
<td>r = −0.24</td>
<td>r = −0.034</td>
</tr>
<tr>
<td>PFOS liver concentrations versus fork length</td>
<td>r = 0.55</td>
<td>r = −0.081</td>
</tr>
<tr>
<td>Serum ALT activity versus serum AST activity</td>
<td>***p = 0.008</td>
<td>p = 0.72</td>
</tr>
</tbody>
</table>

*Pearson product-moment correlation, *p < 0.05, **p < 0.01, ***p < 0.005. ND = not determined; 
 r = correlation coefficient; p = p value; n = number of fish; AST = aspartate aminotransferase; ALT = alanine aminotransferase.

gian North Sea. The significant positive correlation observed between the bib liver PFOS content and the proximity of the sampling locations to Antwerp suggests discharge in the Western Scheldt upstream from location 4. The increasing trends observed for the minimum and maximum muscle concentrations in bib caught closer to Antwerp illustrate furthermore that PFOS might indeed be present as a gradient along the Western Scheldt, decreasing toward the sea. This distribution pattern was previously shown for several metals and volatile chlorinated compounds in the Scheldt [11,23,24]. Interestingly, the presence of these compounds in the ecosystem is also reflected in the tissue concentrations of aquatic biota, as was shown by De Wolf et al. [25]. These authors observed a concentration gradient for a number of metals in the soft body tissues of the periwinkle (Littorina littorea) paralleling their Western Scheldt pollution gradient. However, this is the first time that such a pattern is documented for PFOS.

Although the PFOS tissue concentrations might reflect the distribution of PFOS in the Western Scheldt, a tissue dilution effect could contribute, however, to the decreasing trends for liver and muscle PFOS concentrations observed in bib when these fish were caught further away from Antwerp. Indeed, not only the PFOS levels increased at the various sampling sites but also the fork length of the fish decreased closer to the city of Antwerp. As a consequence of this type of dilution, PFOS could be more concentrated in smaller tissue volumes as a mere consequence of the lower degree of diffusion in a smaller volume of tissue compared with a larger one. Watanabe et al. [26] suggested that tissue dilution probably accounted for the decrease of polychlorinated biphenyl and hexachlorocyclohexane isomer levels in blubber of immature Caspian seals when the animals had larger body lengths. Solé et al. [27] found lower concentrations of several organic pollutants in muscle tissue of deep-sea fish with larger sizes and suggested that tissue dilution might be responsible for this phenomenon. Seasonal variation in eelpout (Zoarces viviparus) liver mercury burden results largely from the dilution of similar burdens by a seasonally growing and shrinking liver, illustrating the possible impact of tissue dilution on concentrations of toxicants in fish tissues [28]. Tissue dilution as a result of changes in nutritional status can also greatly influence tissue concentrations, as was illustrated for cadmium exposure of dogwhelks (Nucella lapillus) [29]. Because the liver PFOS concentration has the tendency to be higher in fishes with a
smaller fork length, as suggested by the significant correlation between both endpoints, tissue dilution might contribute to the explanation of the PFOS gradient observed.

Differences in diet of small and large bib might also affect PFOS tissue burdens, leading to higher PFOS concentrations in smaller fish. Studies on the feeding patterns of bib, however, suggest that a difference in feeding habits is probably not an important factor. Hamerlynck and Hostens [30] reported that the feeding pattern of small bib in a coastal area of the south-west Netherlands changed to a regime of almost exclusively shrimp and small fish when they were about 100 mm in length. In the mesohaline zone of the Western Scheldt estuary, Hostens and Mees [7] found that bib showed a diet shift at 50 and 130 mm. Because the smallest bib in the present study measured 130 mm, it can be reasonably assumed that the nutritional habits of the examined fish were similar.

The high PFOS liver concentrations found in plaice caught at the inflow of the canal Ghent-Terneuzen (1,286, 1,744, and 7,760 ng/g wet wt) suggests that substantial amounts of PFOS are discharged via this canal. Other studies show that organic pollutants might be present at relatively high concentrations at Terneuzen. Steen et al. [31] showed that this canal is a source for inflow of several pesticides into the Western Scheldt. Polychlorinated biphenyl levels in the blood of common terns (Sterna hirundo) were reported to be significantly elevated at Terneuzen compared with two other reference sites [32]. Because Terneuzen is the most inland location at which plaice was caught, however, these high PFOS concentrations might also reflect PFOS inflow in the Western Scheldt from locations that are situated further upstream. The much higher percentage of plaice with muscle PFOS concentrations below the detection limit at the marine sampling locations also suggests that the marine locations are less polluted, which is probably a consequence of dilution of PFOS in the North Sea. This supports the data on bib liver and muscle PFOS burdens that also suggest higher PFOS concentrations in tissues at locations further upstream in the Western Scheldt.

Giesy and Kannan [1] reported PFOS concentrations in the livers of 41 fish from various species and sampling locations ranging from <7 to 170 ng/g wet weight, while the PFOS tissue concentrations in the Western Scheldt in the present study ranged from 11 to 217 ng/g wet weight liver in bib and from 107 to 7,760 ng/g wet weight in plaice. The measured muscle tissue concentrations in the Western Scheldt ranged between <10 and 111 ng/g wet weight for bib and <10 and 87 ng/g wet weight for plaice, while concentrations between <6 and 300 ng/g wet weight have been reported for fish muscle tissue [1]. The highest Western Scheldt liver PFOS concentrations in plaice were 1,286, 1,744, and 7,760 ng/g wet weight, higher than any fish liver concentration reported before. The maximum concentration in plaice liver (7,760 ng/g wet wt) is about two times higher than the maximum concentration ever reported for an animal tissue (3,680 ng/g wet wt), and the three highest liver concentrations are between 7.5 and 46 times higher than the maximum PFOS concentration in fish liver documented so far [1]. Although there are differences between the fish species of the present study and those studied by Giesy and Kannan [1], the PFOS liver concentrations of both fish species presented here show that the PFOS pollution level in the Scheldt estuary can be considered among the highest ever reported. Additional research should be conducted to assess the full impact of this pollution problem.

In a previous study (P. Hoff et al., unpublished data) significant dose-dependent increases of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were observed in the serum of common carp (Cyprinus carpio) exposed to PFOS suggesting PFOS-mediated induction of cellular necrosis.

In the present study, a significant correlation was observed between the bib liver PFOS content and the serum ALT levels. Because the serum AST activity was significantly correlated with the proximity to Antwerp but not with the liver PFOS content in bib, it could be possible that tissue necrosis-inducing compounds different from PFOS are present as a gradient along the longitudinal axis of the Western Scheldt. Because both AST and ALT are significantly correlated in this study, it might be that these unspecified compounds are also (partly) responsible for the significant correlation observed between the serum ALT activity and the proximity to Antwerp. Therefore, the significant association between the serum ALT activity and the liver PFOS content in bib, which also has a gradient-like profile along the Western Scheldt, might not have an unambiguous interpretation, especially because an increase of serum AST and ALT activity might not (exclusively) be linked to toxicant exposure but might be influenced by environmental factors varying along the Western Scheldt as well (e.g., salinity).

The finding that the PFOS content in bib liver did not correlate with the serum AST level but that a correlation was observed with the serum ALT activity can be due to a less pronounced PFOS-mediated increase of serum AST compared with serum ALT in the field. This relatively weak increase of serum AST activity compared with serum ALT activity was
PFOS biomonitoring in an estuarine and marine ecosystem

observed in carp exposed to PFOS under laboratory conditions (P. Hoff et al., unpublished data).

A second biochemical effect reported was the significant linear correlation between the PFOS content and the bib liver protein content. Increases of protein content in fish liver as a result of toxicant exposure have been reported before and can be concurrent with the induction of detoxification mechanisms [33]. In addition, increased protein synthesis in fish subjected to a toxic challenge might be linked to chronic repair processes [34]. Compensatory induction of protein synthesis as a result of toxicant-mediated inhibition is also possible. Although differences in dietary habits between sampling locations could also affect the liver protein content, this is not very likely, as discussed earlier.

Although a significant correlation was found between the liver PFOS content and the serum ALT activity and between the liver PFOS content and the liver protein content in bib, such a correlation was not found in plaice. This could be due to a lower sensitivity of this species to PFOS toxicity and differences in uptake, elimination, or metabolism. Most important, it should be considered that the sampling locations for plaice were different than those for bib, suggesting different environmental conditions for the two fish species. Four out of five plaice sampling locations were marine locations, while for bib, only one sampling location out of four was situated in the coastal zone. Furthermore, differences in feeding pattern of bib and plaice have been described [7,35] and could account for differences in response of the two species investigated.

CONCLUSIONS

The current results suggest the existence of a gradient of PFOS exposure along the Western Scheldt estuary. A general decrease in PFOS contamination was observed downstream and results in a pollution level of the Belgian part of the North Sea that is lower than that of the Western Scheldt. On the biological level, the PFOS liver content in bib was found to be positively correlated with the liver protein content, the serum ALT activity, and the fork length. Assessment of confounding factors such as tissue dilution, salinity, and other pollutants would allow a more profound interpretation of the observations made and thus lead to a better understanding of the hazard in aquatic ecosystems linked to PFOS.

REFERENCES


