Environ. Sci. Technol. **2003**, *37,* 5545−5550

Perfluorinated Chemicals Infiltrate Ocean Waters: Link between Exposure Levels and Stable Isotope Ratios in Marine Mammals

KRISTIN INNEKE VAN DE VIJVER,*,† PHILIPPE TONY HOFF,† KRISHNA DAS,‡ WALTER VAN DONGEN,§ EDDY LOUIS ESMANS,§ THIERRY JAUNIAUX,|| JEAN-MARIE BOUQUEGNEAU,‡ RONNY BLUST,† AND WIM DE COEN†

Department of Biology, University of Antwerp, Groenenborgerlaan 171, 2020 Antwerpen, Belgium, MARE Center, Laboratory for Oceanology, Liège University, B6, B-4000, Liège, Belgium, Nucleoside Research and Mass Spectrometry Unit, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium, and Department of Pathology, Faculty of Veterinary Medicine, Liège University, B43, B-4000 Liège, Belgium

This is the first study to report on concentrations of perfluorinated organochemicals (FOCs) in marine mammals stranded along the southern North Sea coast in relation to stable nitrogen and carbon isotope ratios (δ^{15}N and δ^{13}C). The presence of FOCs in top predators such as marine mammals would indicate a potential biomagnification of these compounds and their widespread occurrence. Liver and kidney tissues of nine marine mammal species have been sampled. Among all the measured FOCs compounds, PFOS (perfluorooctane sulfonate) was predominant in terms of concentration. The highest PFOS concentrations were found in the liver of harbor seal compared to white-beaked dolphin, harbor porpoise, gray seal, sperm whale, white-sided dolphin, striped dolphin, fin whale, and hooded seal. PFOS concentrations differed significantly between sexes and age classes in harbor porpoises. Stable isotope measurements (δ^{13}C and δ^{15}N) were used in this study to describe the behavior of contaminants in food webs. We found a significant ($p <$ 0.05) linear relationship between PFOS concentrations in livers of harbor porpoises and both muscle δ^{13}C and δ^{15}N measurements. Harbor and gray seals and white-beaked dolphin, which displayed the highest trophic position, contained the highest PFOS levels, while offshore feeders such as sperm whales, fin whales, striped dolphin, and white-sided dolphin showed lower PFOS concentrations than inshore species.

Introduction

The global marine ecosystem is continuously under pressure due to expanding anthropogenic activities and the development and release of new chemicals. High contaminant burdens in animals from higher trophic levels have led to a need for more information on the occurrence, distribution, and fate of several hazardous compounds. 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. High concentrations of persistent compounds in marine mammals are frequently reported in the literature (*1*−*4*). Polychlorinated biphenyls (PCBs), organochlorine pesticides (such as DDT and its derivates), and polybrominated diphenyl ethers (PBDEs) are just a few chemicals occurring in tissues of marine mammals.

To understand the fate of contaminants and their impact on the marine ecosystem, it is essential to gain knowledge on the trophic relationships within this ecosystem. In the past, trophic position was mainly assessed using food composition analysis. However, as gut content analyses give only indirect information on trophic interactions, stable nitrogen and carbon isotope measurements have recently been used in order to quantify trophic status and dietary overlap of marine organisms (*5*, *6*). Nitrogen isotope ratios (^{15}N/^{14}N) are a good indicator of the trophic position of an animal. On the other hand, carbon isotope ratios (^{13}C/^{12}C) provide information on inshore versus offshore sources of prey in the marine environment (*6*). The stable isotope ratios show a stepwise enrichment from one trophic level to another due to a preferential excretion (^{14}N) and respiration (^{12}C) of the lighter isotope leading to high values in top predators. Stable nitrogen isotope ratios exhibit a 3−5‰ enrichment value in the heavy isotope from prey to predator, while carbon isotope ratios show a 1‰ enrichment (*5*, *7*, *8*).

Furthermore, stable isotope analysis is often used to provide a continuous variable with which to assess both trophic level and trophic transfer of contaminants in aquatic food webs (*9*, *10*). Persistent hydrophobic organic components tend to bioaccumulate and biomagnify along the food chain. Strong relationships have already been described between stable isotopes and a number of organochemicals, such as PCBS and DDTs, to explain the differences in contaminant concentrations among species and to establish the biomagnification factors of these chemicals (*11*−*13*). Recently, it was suggested that many aquatic wildlife species and especially top predators such as marine mammals contain high levels of anthropogenic perfluorinated acids and related perfluorinated compounds with concentrations increasing near shores and in estuaries (*14*). Perfluorooctane sulfonate (PFOS) seems to be the predominant compound, and Kannan et al. (*15*) demonstrated its accumulation potential through the food web. Perfluorinated chemicals are highly persistent and show little to no biodegradation (*16*). They are used as refrigerants, surfactants, and components of pharmaceuticals, flame-retardants, cosmetics, and insecticides. Several of these applications involve long-lived goods which will serve as sources for possible environmental contamination for many years. Unlike the "conventional" persistent organic pollutants (POPs), fluorinated chemicals do not accumulate in the blubber, but due to their surfactant-like structure, they bind to blood proteins and accumulate in the liver of exposed organisms.

The objective of this study was to determine the current concentrations of fluorochemicals in marine mammals of West Europe. For the latter purpose, liver and kidney tissues

* Corresponding author phone: +32 3 265 33 50; fax: +32 3 265 34 97; e-mail: inneke.vandevijver@ua.ac.be.
† Department of Biology, University of Antwerp.
‡ MARE Center, Liège University.
§ Nucleoside Research and Mass Spectrometry Unit, University of Antwerp.
|| Faculty of Veterinary Medicine, Liège University.

10.1021/es0345975 CCC: $25.00
Published on Web 11/04/2003

of nine species of marine mammals stranded on the Belgian, French, and Dutch North Sea coast between 1994 and 2000 were collected and different perfluorinated chemicals were measured. To explain the differences in PFOS concentrations between the various organisms and to evaluate the potential of PFOS to be biomagnified along the food chain, we investigated the behavior of PFOS and other FOCs within higher trophic levels using previously described $\delta^{13}C$ and $\delta^{15}N$ measurements in marine mammal muscles (*17*).

Materials and Methods

Marine Mammal Sampling. Between 1995 and 2000, tissue samples were taken from various marine mammals stranded along the Belgian, French, and Dutch North Sea coasts. The tissues of 48 harbor porpoise (*Phocoena phocoena*), 23 harbor seal (*Phoca vitulina*), six gray seals (*Halichoerus grypus*), 1 hooded seal (*Cytophora cristata*), 8 white-beaked-dolphins (*Lagenorhynchus albirostris*), 2 white-sided dolphins (*Lagenorhynchus acutus*), 2 striped dolphins (*Stenella coerulealba*), 7 sperm whales (*Physeter macrocephalus*), and 3 fin whales (*Balaenoptera physalus*) were collected. Liver and kidney tissues were sampled and stored at −20 °C until further analysis. From one harbor porpoise it was possible to collect the placenta. Age categories were determined based on body length: neonatal (≤ 90 cm), juvenile (90−130 cm), and adult (>130 cm). Details about the necropsy and general body condition of all marine mammals from this study have been presented elsewhere (*18*−*20*).

PFOS Analysis. Liver and kidney tissue extracts were analyzed using high-performance liquid chromatography combined with electrospray tandem mass spectrometry (LC-MS/MS) as described by Hansen et al. (*21*) with some minor modifications described by Hoff et al. (*22*).

One gram of soft tissue was homogenized on ice with an MSE 150W ultrasonic disintegrator (MSE Scientific instruments, Sussex, U.K.) with 3 mL of Milli-Q water (Millipore, Belgium). Afterward, 500 μL of homogenate, 10 μL of internal standard, 1H,1H,2H,2H-perfluorooctane sulfonic acid (Sigma-Aldrich Chemical Co., Milwaukee, WI), 1 mL of 0.5 M tetrabutylammonium hydrogen sulfate solution (TBAS; adjusted to pH 10), and 2 mL of 0.25 M sodium carbonate buffer were thoroughly mixed. Five milliliters of methyl-*tert*-butyl ether (MBTE) 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 of methanol and filtered trough a 0.2 μm nylon mesh filter.

Perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorooctane sulfonate (PFOS), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUA), and perfluorododecanoic acid (PFDOA) were measured in liver samples. In kidney tissue, only PFOS was measured. Briefly, HPLC was done on a CapLC system (Waters, Millford, MA) connected to a Quattro II triple-quadrupole mass spectrometer (Micromass, Manchester, U.K.). Aliquots of 5 μL of extract were loaded on an Optiguard C18 precolumn (10 mm × 1 mm i.d., Alltech, Sercolab, Belgium) followed by a Betasil C18 column (50 mm × 1 mm i.d., Keystone Scientific, Bellefonte, PA) at a flow rate of 40 μL/min. The mobile phase was 2 mM NH_4OAc/methanol, starting at 10% methanol and increasing until 90% in 8 min. After 10 min, initial conditions were resumed. All components were measured under negative electrospray ionization using the following transitions 413→369 (PFOA), 463→419 (PFNA), 499→99 (PFOS), 513→469 (PFDA), 563→519 (PFUA), and 613→569 (PFDOA). The internal standard, 1H,1H,2H,2H-perfluorooctane sulfonate (Sigma-Aldrich Chemical Co.) was measured under the same conditions (427→81). No other standards were included. The dwell time was 0.1 s. The ES-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} mmHg (Ar). These settings were the same for all measurements. The limit of quantitation (LOQ) of PFOS was 10 ng/g wet weight (wet wt), whereas for the other measured compounds it varied from 10 to 110 ng/g wet wt. Data quality assurance and quality control protocols include matrix spike, laboratory blanks, and continuing calibration verification. Recoveries of PFOS (Sigma-Aldrich Chemical Co.) spiked into harbor porpoise liver tissue and passed through the analytical procedure varied from 61% to 110%. The PFOS standard was 98% pure. Concentrations were evaluated versus an unextracted standard curve and were not corrected for the recoveries or for the purity of the PFOS standard. The repeatability and reproducibility were 86% and 88% respectively.

Stable Isotopes. Muscle tissues were analyzed for stable isotope measurements. Organisms may vary in their concentrations of lipids. As lipids have been shown to be depleted in ^{13}C relative to the diet (*23*), they were extracted from muscle samples using repeated rinses with 2:1 chloroform:methanol prior to analysis. After drying at 50 °C (48 h), samples were ground into a homogeneous powder. Carbon dioxide and nitrogen gas were analyzed as decribed previously (*17*) on a V. G. Optima (Micromass) IR-MS coupled to a N−C−S elemental analyzer (Carlo Erba) for automated analyses. Routine measurements are precise to 0.3‰ for both 13-carbon and 15-nitrogen. Stable isotope ratios were expressed in δ notation according to the following

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000$$

where X is ^{13}C or ^{15}N and R is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Carbon and nitrogen ratios are expressed relative to the v-PDB (Vienna Peedee Belemnite) standard and to atmospheric nitrogen, respectively. Reference materials were IAEA-N1 ($\delta^{15}N = +0.4 \pm 0.2$ ‰) and IAEA CH-6 (sucrose) ($\delta^{13}C = -10.4 \pm 0.2$ ‰).

Statistical Analysis. Tissue concentrations were tested to fit a normal distribution using Kolmogorov−Smirnov 1-sample tests. Because of a lack of normality in the distribution of the PFOS concentration of some species, a nonparametric Kruskal−Wallis analysis of variance was used for the statistical comparison of PFOS concentrations between species. A Dunn's multiple comparisons test was performed as post-hoc criterion. The significant difference level was decided as $p < 0.05$. To investigate the influence of age and sex, a nonparametric Mann−Whitney U-Test was applied. The comparison between liver and kidney concentrations for the various species was done by the paired T test or the Wilcoxon matched pairs signed test, depending on the normality of the data. To correlate the data of the PFOS concentrations and the stable isotopes measured in the different species, a Spearman rank correlation was used. All analyses were performed using the software package Statistica (Statsoft Inc., Tulsa, OK).

Results and Discussion

Concentrations of Perfluorinated Chemicals. At present, these results are the first to report concentrations of perfluorinated compounds in marine mammals stranded at the Belgian, Dutch, and French North Sea coast. Of all perfluorinated chemicals measured, PFOS was the predominant compound in the tissues analyzed (Tables 1 and 2). PFOS was found in livers of the 48 harbor porpoises and the 2 striped dolphins as well as in livers and kidneys of all white-beaked dolphins and gray seals analyzed. Some individuals contained concentrations under the detection limit of 10 ng/g wet wt. PFOS concentrations in livers of white-beaked dolphins (132 ± 149 ng/g wet wt) from the North Sea were

TABLE 1. Concentrations of PFOS in Liver and Kidney Tissue (ng/g wet wt) of Cetaceans Stranded along the Southern North Sea Coast

species	tissue	*n*	range of PFOS concentration (mean concentrations are given in parentheses)	no. of individuals below LOQ (<10 ng/g wet wt)
Phocoena phocoena (harbor porpoise)	liver	48	12−395 (93)	0
	kidney	43	<10−821	6
Phoca vitulina (harbor seal)	liver	24	<10−532	2
	kidney	22	<10−489	1
Halichoerus grypus (grey seal)	liver	6	11−233 (88)	0
	kidney	6	23−167 (81)	0
Physeter macrocephalus (sperm whale)	liver	6	19−52 (36)	0
	kidney	5	12	4
Lagenorhynchus albirostris (white-beaked dolphin)	liver	7	14−443 (132)	0
	kidney	7	13−290 (87)	0
Lagenorhynchus acutus (white-sided dolphin)	liver	2	<10−26	1
	kidney	1	18	0
Stenella coerulealba (striped dolphin)	liver	2	11	0
	kidney	2	<10	2
Cytophora cristata (hooded seal)	liver	1	<10	1
	kidney	1	<10	1
Balaenoptera physalus (fin whale)	liver	1	<10	1

TABLE 2. Concentrations of Perfluorinated Organic Compounds in Liver (ng/g wet wt) of Different Individual Marine Mammals

species	PFOS	PFNA	PFDA	PFUA
Phocoena phocoena	336	<90	<50	110
Phocoena phocoena	59	<90	<50	<30
Physeter macrocephalus	30	240	<50	50
Lagenorhynchus acutus	26	<90	<50	60
Lagenorhynchus albirostris	443	480	110	150
Lagenorhynchus albirostris	105	<90	120	140
Lagenorhynchus albirostris	38	<90	<50	<30
Lagenorhynchus albirostris	190	<90	90	50
Lagenorhynchus albirostris	79	<90	<50	<30
Lagenorhynchus albirostris	14	<90	<50	<30

found to be in the same range than those of striped and bottlenose dolphins from the coastal waters of Florida as reported earlier by Kannan et al. (*14*). No detectable concentration of PFOS was found in the placental tissue of one of the harbor porpoises.

The highest hepatic PFOs level were measured in the harbor seal followed by white-beaked dolphin > harbor porpoise > gray seal > sperm whale > white-sided dolphin > striped dolphin > fin whale/hooded seal. However, no significant ($p > 0.05$) differences in liver concentrations between the species could be detected. Kidney concentrations in harbor seals were significantly higher than those of harbor porpoises ($p < 0.05$). Although mean concentrations in liver were higher than in kidney of all species analyzed, the highest PFOS level was detected in kidney tissue of a harbor porpoise (821 ng/g wet wt).

While PFOS was detected in most of the animals, some of the other perfluorinated chemicals were only quantified in a few species. The measured perfluorocarboxylates were generally present at detectable concentrations in those individuals with the highest PFOS concentrations. However, most of the animals had values below the detection limit. Perfluorooctanoic acid (PFOA) and perfluorododecanoic acid (PFDOA) were not detected above the detection limit of 110 and 10 ng/g wet wt, respectively, in any of the species. Table 2 gives an overview of the species and individuals in which we could detect some of the FOCs. In livers of different marine mammal species, PFDA (<50−120 ng/g wet wt) and PFUA (<30−150 ng/g wet wt) concentrations were up to 4-fold lower than the PFOS concentrations. Concentrations of PFNA were above the detection limit for the sperm whale and the white-beaked dolphin (90 ng/g wet wt) and were higher than PFOS concentrations measured in the same animals. Previous studies on the occurrence of perfluorinated chemicals in wood mice (*Apodemus sylvaticus*) (*24*) and common shiner (*Notropus cornutus*) (*25*) showed PFNA, PFDA, and PFUA concentrations which were in the same range as perfluorocarboxylate concentrations shown in this study. However, in contrast to the present study, PFNA concentrations in liver tissue of wood mice (<90−270 ng/g wet wt) were lower than the measured PFOS concentrations (470−178 550 ng/g wet wt).

PFOS Concentrations in Relation to Sex and Age. There is a significant difference in mean liver and kidney concentrations between the different age classes of harbor porpoises. Despite the fact that the number of juveniles analyzed in this study was higher than adults, juvenile porpoises contained higher concentrations than adult organisms (Figure 1a; $p < 0.05$). Kannan et al. (*14*) reported no significant relationship between age and PFOS concentrations in livers of marine mammals, although it was reported that concentrations in adults tend to be higher than in subadults and juveniles. An age-dependent decreasing trend was reported in another recently published study on PFOS concentrations in marine mammals originating from the Mediterranean region (*26*). Although the age determination in the present study was based on body length and not on cementum layers, which makes the determination more accurate, these results might be an indication that age has an influence on PFOS concentrations.

Mean PFOS levels in female porpoise livers were significantly higher than those in male porpoises (Figure 1b; $p < 0.05$). However, within the different age categories, no gender-specific relationship could be detected.

The same trend is visible in liver concentrations of harbor seals, where female samples contained higher concentrations than males ($p > 0.05$). However, stable isotope values did not differ between sexes (*17*). In contrast with our findings are the significant differences between males and females reported in gray seals from the Baltic Sea, with males having higher concentrations of PFOS in their livers and blood than females (*26*).

The present results clearly show the difference between the accumulation pattern of PFOS and that of other persistent organochlorine chemicals such as PCBs and DDTs in marine mammals. In general, adult females contained 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 (*27*). This forms a major excretory

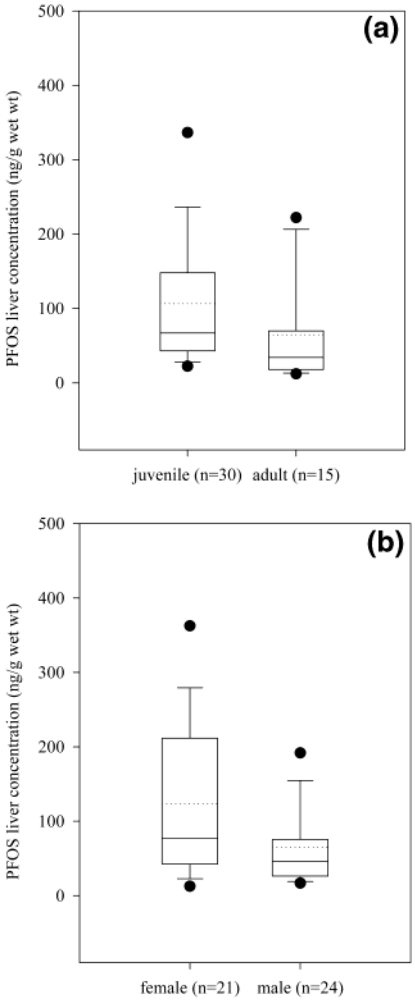

FIGURE 1. Liver perfluorooctane sulfonate (PFOS) concentrations in harbor porpoises as a function of age class (a) and sex (b). The straight line is the median, and the dotted line represents the mean. The 25th and 75th percentiles define the boxes. The wiskers represent the 10th and 90th percentiles, while the dots represent the 5th and 95th percentiles. There is a significant difference between the mean of the adult and the mean of the juvenile group ($p < 0.05$), and there is a significant difference between the mean of the male and the mean of the female group ($p < 0.05$).

route for females in both cetaceans and pinnipeds. As PFOS does not accumulate in lipid-rich tissues, such as milk and blubber, but binds to specific proteins in liver, kidney, and blood plasma, a similar transfer from mother to calf is not obvious. The fact that females have higher concentrations than males might be an indication that for FOCs this main excretory route is not followed.

An additional possible explanation for the results found in this study is provided by the data from stable isotope analysis. Adult female porpoises fed at a higher trophic level than adult males, while juvenile porpoises display no differences between sexes (*17*). The low δ^{15}N signature (and δ^{13}C) of the males suggest that they obviously fed on more offshore prey with a low δ^{15}N signature such as the adult herrings while females and juveniles might stay closer to shallow waters (*17*). The higher PFOS levels in female porpoises might be linked to both their higher trophic position (*17*) and their more coastal distribution (*28, 29*).

In this way, females can accumulate more PFOS through the food web as measured in the present study. Female porpoises are usually larger than males, confirming the observed difference in trophic level and possibly explaining the difference in PFOS concentrations between both genders.

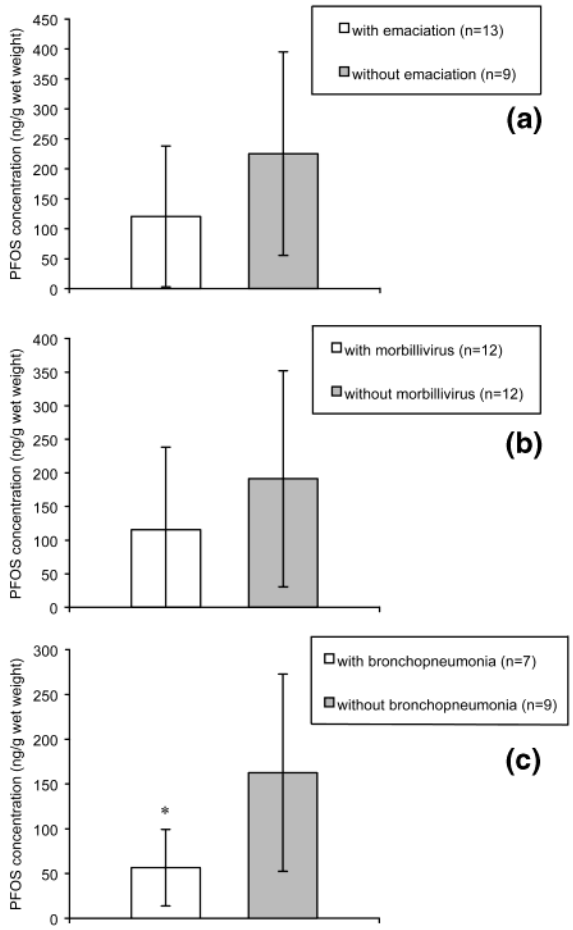

FIGURE 2. Difference in mean PFOS concentration and standard deviations in livers of harbor seals (*Phoca vitulina*) between animals (a) with or without emaciation, (b) with and without morbillivirus, and (c) with and without bronchopneumonia. The asterisk above the bars indicates that the means are significantly (<0.05) different between the animals with and without bronchopneumonia.

General Body Condition. PFOS liver concentrations were examined in relation to the general body condition of harbor porpoises and harbor seals. All animals were investigated for histopathological, immunohistochemical, bacteriological, parasitological, and virological lesions (*18*−*20*). The main gross findings were severe emaciation, acute pneumonia, severe parasitosis, and haemorrhagic enteritis. No link could be established between the PFOS levels and the necropsy results for harbor porpoises. However, in harbor seals, individuals with a rather good body condition had higher PFOS concentrations in their liver (Figure 2). The mean PFOS concentration (60 ± 40 ng/g wet wt) of harbor seals with lesions in the respiratory system was significantly lower ($p < 0.05$) than the mean PFOS concentration (160 ± 110 ng/g wet wt) of animals without bronchopneumonia. As our data are the first trying to link pathological phenomena and FOC pollution levels, care must be exercised to draw final conclusions on the overall impact of these contaminants on the overall health of marine mammals.

Stable Isotope Analysis. We observed a significant relationship when correlating the PFOS concentrations of marine mammals with the results of the stable isotope analysis. The $^{15}N/^{14}N$ ratio in muscle tissue was strongly associated with PFOS concentrations in livers of marine mammals ($r = 0.60$, $p < 0.0001$; Figure 3) with animals that displayed the highest trophic positions having the highest PFOS levels. A relationship has only been realized for harbor seal, gray seals, harbor porpoise, and white-beaked dolphin

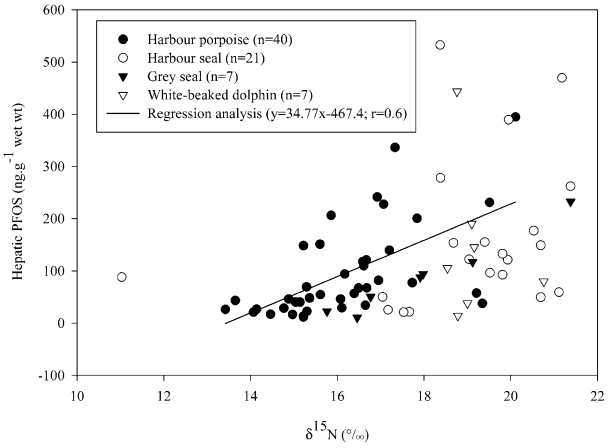

FIGURE 3. Regression analysis between δ^{15}N in muscle tissue and PFOS concentrations in livers of four species of marine mammals ($n = 85$, $r = 0.6$, $p < 0.0001$).

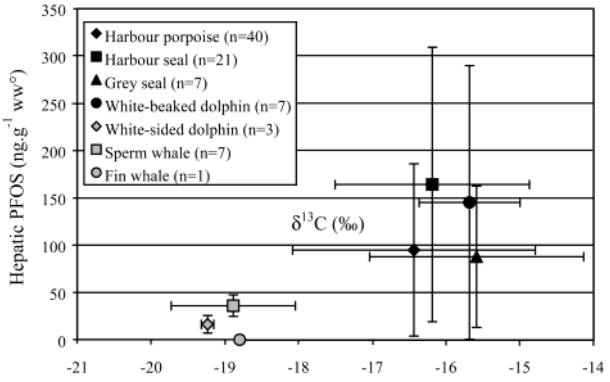

FIGURE 4. PFOS concentrations and stable isotopes. δ^{13}C mean values versus mean liver PFOS concentrations in marine mammals from the southern North Sea. On the basis of the stable isotope δ^{13}C, the species are divided into two groups: inshore (black dots) and offshore (grey dots) species.

as their carbon signature suggests that they belong to the Southern North Sea food web (*17*). Indeed, δ^{15}N of marine predator tissues is determined initially by the isotopic composition of the baseline phyto- and zooplankton sources, technically measured in the particulate organic matter (POM). As a result, part of the δ^{15}N interspecific variation in marine mammals might be related to coastal versus offshore δ^{15}N signature of the primary producers. Indeed, some species such as the striped dolphin are typically oceanic while others, such as the harbor porpoise, are more coastal (*30*). Previous studies have examined the relationship between a variety of chemical compounds and the trophic position, defined by δ ^{15}N values (*12*, *13*, *31*). Several food webs that were investigated contained marine mammals as top predators, next to fish and marine birds. Usually the whole ecosystem was evaluated, beginning with producers (plankton) to primary and secondary consumers (mostly crustaceans and fish). This way the potential of the toxic compounds to be biomagnified in different trophic levels could be quantified. However, it is impossible to estimate a biomagnification factor using our results because we only have data (PFOS levels and δ ^{15}N values) on the top predators of different food chains of the southern North Sea. No detailed information is yet available about the specific prey species of these marine mammals within the southern North Sea area (*17*, *32*). The data of the present study being preliminary, however, support the observation that PFOS has a potential for biomagnification. As more information on the whole food web with prey/predator data becomes available, a more refined assessment will be possible.

δ^{13}C is more useful to indicate the origin of carbon sources than as an indicator of the trophic level. The general pattern of inshore, benthos linked food webs being more enriched in ^{13}C compared with offshore, pelagic food webs presents a potentially useful tool. For example, δ^{13}C values are typically higher in coastal or benthic food webs than in offshore food webs (*34*). White-sided dolphin, sperm whale, and fin whales were 13-C depleted compared to more coastal species such as the harbor seal, gray seal, harbor porpoise, and white-beaked dolphin, suggesting that they might feed more offshore (*17*). The different feeding ecology of these two groups of species (offshore vs inshore) is likely to contribute to the lower PFOS concentrations measured in the offshore species, namely, the sperm whale, fin whale, and white-sided dolphin (Figure 4). It is reasonable to expect lower concentrations in marine mammals living in deeper and offshore waters, further away from direct pollution sources. However, PFOS levels up to 50 ng/g wet wt in sperm whales which feed mainly on abyssal cephalopods, but also on bottom-dwelling organisms, suggest that fluorinated organic compounds have not only reached remote Arctic regions (*15*, *16*) but also deeper water layers. Furthermore, given the biomagnification potential of PFOS, these results confirm the idea that even small marine species living on the seabed, like crabs and fish, must already be exposed to this class of chemicals (*34*).

Acknowledgments

Thanks to Jan Tavernier and Jan Haelters for logistical coordination. This study was financially supported by FWO-Flanders, Brussels, Belgium, and tissue sampling by the Belgian office for Scientific, Technical and Cultural Affairs (contract MN/DD/50). K.D. received grants from the "Fonds pour la Recherche dans l'Agriculture et l'Industrie" (F.R.I.A) and Marie-Curie Fellowship. The anonymous reviewers are gratefully acknowledged for helping improve the quality of the manuscript. This paper is a MARE Publication No. 028.

Literature Cited

(1) Boon, J. P.; Oostingh, I.; van der Meer, J.; Hillebrand, M. T. J. *Eur. J. Pharmacol.* **1994**, *270*, 237−251.
(2) Reijnders, P. J. H. *Sci. Total Environ.* **1994**, *154*, 229−236.
(3) Holsbeek, L.; Joiris, C. R.; Debacker, V.; Ali, I. B.; Roose, P.; Nellissen, J. P.; Gobert, S.; Bouquegneau, J. M.; Bossicart, M. *Mar. Pollut. Bull.* **1999**, *38*, 304−313.
(4) Aguilar, A.; Borrell, A.; Reijnders, P. J. H. *Mar. Environ. Res.* **2002**, *53*, 425−452.
(5) De Niro, M. J.; Epstein, S. *Geochim. Cosmochim. Acta* **1981**, *45*, 341−351.
(6) Kelly, J. F. *Can. J. Zool.* **2000**, *78*, 1−27.
(7) De Niro, M. J.; Epstein, S. *Geochim. Cosmochim. Acta* **1978**, *42*, 495−506.
(8) Hobson, K. A.; Welch, H. E. *Mar. Ecol. Prog. Ser.* **1992**, *84*, 9−18.
(9) Kidd, K. A.; Schindler, D. W.; Hesslein, R. H.; Muir, D. C. G. *Sci. Total Environ.* **1995**, *160/161*, 381−390.
(10) Das, K.; Lepoint, G.; Loizeau, V.; Debacker, V.; Dauby, P.; Bouquegneau, J. M. *Mar. Pollut. Bull.* **2000**, *40*, 102−109.
(11) Broman, D.; Naf, C.; Rolff, C.; Zebuhr, Y.; Fry, B.; Hobbie, J. *Environ. Toxicol. Chem.* **1992**, *11*, 331−345.
(12) Jarman, W. M.; Hobson, K. A.; Sydeman, W. J.; Bacon, C. E.; McLaren, E. B. *Environ. Sci. Technol.* **1996**, *30*, 654−660.
(13) Fisk, A. T.; Hobson, K. A.; Norstrom, R. J. *Environ. Sci. Technol.* **2001**, *35*, 732−738.
(14) Kannan, K.; Koistinen, J.; Beckmen, K.; Evans, T.; Gorzelany, J. F.; Hansen, K. J.; Jones, P. D., Helle, E.; Nyman, M.; Giesy, J. P. *Environ. Sci. Technol.* **2001**, *35*, 1593−1598.
(15) Giesy, J. P.; Kannan, K. *Environ. Sci. Technol.* **2001**, *35*, 1339−1342.
(16) Key, B. D.; Howell, R. D.; Criddle, C. S. *Environ. Sci. Technol.* **1998**, *32*, 2283−2287.
(17) Das, K.; Lepoint, G.; Leroy, Y.; Bouquegneau, J. M. *Mar. Ecol.-Prog. Ser.*, in press.
(18) Jauniaux, T.; Brosens, L.; Jacquinet, E.; Lambrigts, D.; Addink, M.; Smeenk, C.; Coignoul, F. *J. Wild Dis.* **1998**, *34*, 99−109.

(19) Jauniaux, T.; Boseret, G.; Desmecht, M.; Haelters, J.; Manteca, C.; Tavernier, J.; Van Gompel, J.; Coignoul, F. *Vet. Rec.* **2001**, *148*, 587−591.
(20) Jauniaux, T.; Petitjean, D.; Brenez, C.; Borrens, M.; Haelters, J.; Tavernier, J.; Coignoul, F. *J. Comp. Pathol.* **2002**, *126*, 243−253.
(21) Hansen, K. J.; Clemen, L. A.; Ellefson, M. E.; Johnson, H. O. *Environ. Sci. Technol.* **2001**, *35*, 766−770.
(22) Hoff, P. T.; Van de Vijver, K.; Van Dongen, W.; Esmans, E. L.; Blust, R.; de Coen, W. M. *Environ. Toxicol. Chem*. **2003**, *22*, 608−614.
(23) Tieszen, L. L.; Boutton, T. W.; Tesdahl, K. G.; Slade, N. A. *Oecologia* **1983**, *57*, 32−37.
(24) Hoff, P. T.; Scheirs, J.; Van de Vijver, K.; Van Dongen, W.; Esmans, E. L.; Blust, R.; de Coen, W. M. *Environm. Health Perspect*. Submitted for publication.
(25) Moody, C. A.; Martin, J. W.; Kwan, W. C.; Muir, D. C. G.; Mabury, S. A *Environ. Sci. Technol.* **2002**, *36*, 545−551.
(26) Kannan, K.; Corsolini, S.; Falandysz, J.; Oehme, G.; Focardi, S.; Giesy, J. P. *Environ. Sci. Technol.* **2002**, *36*, 3210−3216.
(27) Norstrom, R. J.; Muir, D. C. G. *Sci. Total Environ*. **1994**, *154*, 107−128.
(28) Smith, G. J. D.; Gaskin, D. E. *Ophelia* **1983**, *22*, 1−13.
(29) Kinze, C. C. In *Special Issue 15: Gillnets and cetaceans;* Perrin, W. F., et al.; Eds.; International Whaling Commission: Cambridge, 1994; pp 183−187.
(30) Evans, P. G. H. *The Natural history of whales and dolphins;* Academic Press Harcourt Brace & Company Publishers: London, 1987; p 343.
(31) Hop, H.; Borga, K.; Gabrielsen, G. W.; Kleivane, L.; Skaare, J. U. *Environ. Sci. Technol.* **2002**, *36*, 2589−2597.
(32) Lick, R. R. 1991. In *European research on Cetaceans 5;* Proceedings of the fifth conference of the European Cetacean Society, Sandefjord, Norway Feb 21−23, 1991.
(33) Hobson, K. A. *Oecologia* **1999**, *120*, 314−326.
(34) Van de Vijver, K. I.; Hoff, P. T.; Van Dongen, W.; Esmans, E. L.; Blust, R.; De Coen, W. M. *Environ. Toxicol. Chem*. **2003**, *22*, 2037−2041.

Received for review June 13, 2003. Revised manuscript received September 23, 2003. Accepted September 24, 2003.

ES0345975