Global Distribution of Perfluorooctane Sulfonate in Wildlife

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Here we report, for the first time, on the global distribution of perfluorooctanesulfonate (PFOS), a fluorinated organic contaminant. PFOS was measured in the tissues of wildlife. including, fish, birds, and marine mammals. Some of the species studied include bald eagles, polar bears, albatrosses, and various species of seals. Samples were collected from urbanized areas in North America, especially the Great Lakes region and coastal marine areas and rivers, and Europe. Samples were also collected from a number of more remote, less urbanized locations such as the Arctic and the North Pacific Oceans. The results demonstrated that PFOS is widespread in the environment. Concentrations of PFOS in animals from relatively more populated and industrialized regions, such as the North American Great Lakes, Baltic Sea, and Mediterranean Sea, were greater than those in animals from remote marine locations. Fisheating, predatory animals such as mink and bald eagles contained concentrations of PFOS that were greater than the concentrations in their diets. This suggests that PFOS can bioaccumulate to higher trophic levels of the food chain. Currently available data indicate that the concentrations of PFOS in wildlife are less than those required to cause adverse effects in laboratory animals.

Introduction

Research investigating the environmental fate of halogenated compounds has focused largely on chlorinated and brominated compounds. Fluorinated organic compounds (FOCs) have received less attention because their measurement is more difficult. Because they have surface-active properties, FOCs are more difficult to separate from tissues than the more classical neutral residues that have been studied. Also, because they are nonvolatile and have relatively high molecular weights, they require specialized liquid chromatography/mass spectrometry methods for analysis. Because much of the FOCs were incorporated into polymers, they are perceived as more biologically inert and therefore, less likely to have an impact on human health or the environment.

FOCs have been manufactured for over 50 years. FOCs are used as refrigerants, surfactants, and polymers, and as components of pharmaceuticals, fire retardants, lubricants, adhesives, cosmetics, paper coatings, and insecticides (1). Because of their many useful properties, since the 1970s there has been a steady increase in the use of FOCs for a variety of industrial applications.

Although partially fluorinated hydrocarbons may undergo chemical breakdown at functional group bonds (2), given



FIGURE 1. Structures of target fluorinated organic compounds.

the high energy of the carbon-fluorine bond (known to be the strongest of covalent bonds), some perfluorinated compounds are very stable in the environment (3). Even though FOCs could be persistent, the magnitude and extent of distribution of FOCs in the environment was unknown. However, the occurrence of FOCs in human blood sera was reported as early as 1968 (4). Further reports of FOCs in human sera from various locations in the U.S., South America, and Japan appeared over the next several decades (5-8). These reports would indicate a relatively widespread distribution of these materials in human sera and would suggest the potential for distribution in the environment. However, no compound-specific information on the extent of the distribution of FOCs in the environment or in wildlife was available. This was due, in part, to the lack of a practical compound-specific method of analysis that could be applied to a wide range of sample types with sufficient sensitivity to measure ecologically relevant concentrations of specific compounds. Recent development of a compound-specific method for the analysis of some FOCs using high-performance liquid chromatography-negative ion electrospray tandem mass spectrometry (9) permitted the survey of four FOCs in liver and blood plasma of wildlife on a global scale.

The number of synthetic FOCs has increased over the past few years. Among them, sulfonyl-based fluorochemicals have been produced and used for over 40 years. Perfluoroctanesulfonyl fluoride (POSF) is the basic building block of a series of perfluorinated alkyl sulfonates that are used as surface protectors in carpets, leather, paper and packaging, fabric, and upholstery. It has been hypothesized that, during their life cycles, POSF and some POSF-based products could ultimately degrade to other fluorochemicals including perfluoroctane sulfonate (*10*) (PFOS; Figure 1).

The studies on which we report here are part of a larger, ongoing, comprehensive program of investigation of global distribution and fate of PFOS in the environment. The objectives of this study were to elucidate the current environmental distribution of PFOS by use of biomonitoring and to evaluate bioaccumulation and biomagnification potential in aquatic and terrestrial food webs. Tissues of a variety of wildlife species, living near urban areas and in more remote areas, including the Arctic and the Antarctic, were studied to monitor for the presence of PFOS. In addition, species from different trophic levels with different feeding

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FIGURE 2. Sampling locations of wildlife to monitor fluorinated organic compounds.

strategies were selected to provide some insight into potential environmental pathways.

Materials and Methods

Tissues of various species of aquatic mammals, birds, fish, and amphibians were collected as part of earlier monitoring studies conducted by Michigan State University, East Lansing, Michigan. Samples were collected under permits from state and federal agencies. Tissues of most individuals of aquatic mammals were obtained from stranded dead animals. Samples were also obtained from archived tissues from federal and state agencies. Samples collected in the 1990s were used. Samples were obtained to represent various geographical locations including the Arctic and the Antarctic Oceans (Figure 2).

Concentrations of PFOS and other FOCs in liver, egg yolk, muscle, or blood plasma were determined by use of highperformance liquid chromatography (HPLC) with electrospray tandem mass spectrometry (9). The PFOS used as standards and for matrix spikes was purchased as the potassium salt (Fluka, Milwaukee, WI). The purity of the PFOS standard was 86%. The reported concentrations were not corrected for the purity. The internal standard, 1H,1H,2H,2Hperfluorooctane sulfonate (THPFOS) was purchased from ICN (Costa Mesa, CA). One mL of sera, 5 μ L of internal standard (250 ng), 1 mL of 0.5 M tetrabutylammonium hydrogen sulfate (TBA) solution, and 2 mL of 0.25 M sodium carbonate buffer (adjusted to pH 10) were added to a 15-mL polypropylene tube for extraction. After the solution was thoroughly mixed, 5 mL of methyl-tert-butyl ether (MTBE) was added, and the mixture was shaken for 20 min. The organic and aqueous layers were separated by centrifugation, and an exact volume of MTBE (4 mL) was removed from the solution. The aqueous mixture was rinsed with MTBE and separated twice; all rinses were combined in a second polypropylene tube. The solvent was allowed to evaporate under nitrogen before being reconstituted in 1 mL of methanol. The extract was vortex mixed for 30 s and passed through a $0.2 - \mu m$ nylon mesh filter into an autosampler vial. For the extraction of liver samples, a liver homogenate of 1 g of liver in 5 mL of purified (milli-Q) water was prepared. A 1-mL aliquot of the homogenate was added to a polypropylene tube, and the sample was extracted according to the procedure described above.

Analyte separation was performed using a Hewlett-Packard HP1100 liquid chromatograph modified with low dead-volume internal tubing. Extract (10 μ L) was injected onto a 50 \times 2 mm (5 μ m) Keystone Betasil C₁₈ column with a 2 mM ammonium acetate/methanol mobile phase starting at 45% methanol. At a flow rate of 300 μ L/min, the gradient was increased to 90% methanol before reverting to the original conditions after 9 min. Column temperature was maintained at 25 °C. The HPLC system was interfaced to a Micromass (Beverly, MA) Ultima or Quattro II atmospheric pressure ionization tandem mass spectrometer operated in the electrospray negative ion mode. In all cases, the capillary was held between 1.6 and 3.2 kV. Cone voltage was 20-60 v and collision energy was 30-45 v. The source temperature was maintained at 250 °C. Instrumental parameters were optimized to transmit the [M-H], [M-K], or [M-NH₄] ion for analytes. When possible, multiple daughter ions were monitored, but quantitation was based on a single product ion. In the electrospray tandem mass spectrometry (ESMSMS) system, the 499 $Da \rightarrow 80$ Da transition can be optimized to provide a stronger signal than the 499 $Da \rightarrow 99 Da$ transition of PFOS. However, in the analysis of tissue samples collected from some species of animals, an unidentified interferent was present in the 499 $Da \rightarrow 80$ Da transition. Although this interferent was rarely observed, to ensure complete selectivity, quantitation was based on the 499 $Da \rightarrow 99 Da$ transition. To further ensure that no interferent compromised the quantitation of PFOS, the transitions 499 Da \rightarrow 80 and 499 → 99 were compared. If a quantitative deviation of >20%was observed, a third transition, 499 Da \rightarrow 130 was also monitored. In all cases, the validity of the 499 Da \rightarrow 99 transition was confirmed by one of the other transitions. Samples were evaluated versus an averaged 1/x weighted, unextracted external calibration curve. Because of the variety of matrices analyzed, with respect to both species and tissues, and because of evolving analytical methods, the limit of quantitation (LOQ) was variable. Data quality assurance and quality control protocols included matrix spike, surrogate spike, laboratory blank, surrogate matrix blanks, and continuing calibration verification. Recoveries of target analytes spiked to rabbit sera or liver and passed through the analytical procedure ranged from 85 to 101% (9). Concentrations of target analytes were not corrected for recoveries of the surrogate standard or matrix spike recoveries.

TABLE 1. Ranges of Concentrations of PFOS (ng/g, wet wt, for Liver and Muscle Tissues and ng/mL for Blood Plasma) in Wildlife; Mean Concentrations Are Given in Parentheses

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| Dotal SkuaTerra Nova Bay, Antarcucaplasma2< 1 = 1, 4black-tailed gullHokkaido, Japanplasma242 = 12 (6)double-crested cormorantLake Winnepeg, Canadaegg yolk4130 = 320 (210)ring-billed gullLake Winnepeg, Canadaegg yolk3<35 = 150 | nerring gui | Lake Huron, Great Lakes | piasma | 2 | 00-79(73) | |
| Diakratiled guilHokkatolo, Japanplasma242-12 (6)double-crested cormorantLake Winnepeg, Canadaegg yolk4130-320 (210)ring-billed gullLake Winnepeg, Canadaegg yolk3<35-150 | polar skua | Terra Nova Bay, Antarctica | piasma | 2 | < 1 - 1.4 | |
| double-created cormonantLake Winnepeg, Canadaegg yolk4130-320 (210)ring-billed gullLake Huron, Great Lakesegg yolk3<35-150 | black-talled gull | Hokkaldo, Japan | plasma | 24 | 2-12(6) | |
| Ing-billed guilLake Huron, Great Lakesegg yolk3< 35 - 150Laysan and black-footed albatrossesMidway Atoll, North Pacificliver9< 35 | double-crested cormorant | Lake winnepeg, Canada | egg yolk | 4 | 130-320 (210) | |
| Laysan and black-footed albatrossesMidway Atoli, North Pacificliver9< 35common loonNorth Carolina, USAliver835–690 (290)brown pelicanItalyliver1233–470 (96)black-tailed gullKorealiver1570–500 (170)Laysan and black-footed albatrossesMidway Atoli, North Pacificplasma39–26 (18)double-crested cormorantLake Huron, Great Lakesplasma2280–450 (370)bald eagleMidway Atoli, North Pacificplasma2280–450 (370)bald eagleMidway Atoli, North Pacificplasma261–2570 (360)Laysan and black-footed albatrossesMidway Atoli, North Pacificplasma103–39 (9)Laysan and black-footed albatrossesMidway Atoli, North Pacificplasma2150–380 (260)brown troutMichigan waters, USAliver633–170 (110)lake whitefishMichigan waters, USAliver10<17–26 | ring-billed guil | Lake Huron, Great Lakes | egg yolk | 3 | < 35-150 | |
| Common loonNorth Carolina, USAIlver835–690 (290)brown pelicanMississippi, USAliver2290–620 (460)common cormorantItalyliver1233–470 (96)black-tailed gullKorealiver1570–500 (170)Laysan and black-footed albatrossesMidway Atoll, North Pacificplasma39–26 (18)double-crested cormorantLake Huron, Great Lakesplasma2280–450 (370)bald eagleMidway Atoll, North Pacificplasma2280–450 (370)bald eagleMidway Atoll, North Pacificplasma261–2570 (360)Laysan and black-footed albatrossesMidway Atoll, North Pacificplasma103–39 (9)Fishlake whitefishMichigan waters, USAeggs2150–380 (260)brown troutMichigan waters, USAeggs349–75 (64)Chinook salmonMichigan waters, USAliver533–81 (67)brown troutMichigan waters, USAliver10<17–26 | Laysan and black-footed albatrosses | Marth Que line 110 | liver | 9 | < 35 | |
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| double-crested cormorantLake Huron, Great Lakesplasma3110-430 (260)herring gullLake Huron, Great Lakesplasma2280-450 (370)bald eagleMidwestern USAplasma261-2570 (360)Laysan and black-footed albatrossesMidway Atoll, North Pacificplasma103-39 (9)Fishlake whitefishMichigan waters, USAeggs349-75 (64)brown troutMichigan waters, USAeggs349-75 (64)Chinook salmonMichigan waters, USAliver533-81 (67)brown troutMichigan waters, USAliver533-81 (67)yellow-fin tunaNorthern North Pacific Oceanliver12<7 | Laysan and black-footed albatrosses | Midway Atoll, North Pacific | plasma | 3 | 9-26 (18) | |
| herring gullLake Huron, Great Lakesplasma2280–450 (370)bald eagleMidwestern USAplasma261–2570 (360)Laysan and black-footed albatrossesMidway Atoll, North Pacificplasma103–39 (9)Fishlake whitefishMichigan waters, USAeggs2150–380 (260)brown troutMichigan waters, USAeggs349–75 (64)Chinook salmonMichigan waters, USAliver633–170 (110)lake whitefishMichigan waters, USAliver533–81 (67)brown troutMichigan waters, USAliver10<17–26 | double-crested cormorant | Lake Huron, Great Lakes | plasma | 3 | 110-430 (260) | |
| bald eagleMidwestern USAplasma261–25/0 (360)Laysan and black-footed albatrossesMidway Atoll, North Pacificplasma103–39 (9)Fishlake whitefishMichigan waters, USAeggs2150–380 (260)brown troutMichigan waters, USAeggs349–75 (64)Chinook salmonMichigan waters, USAliver633–170 (110)lake whitefishMichigan waters, USAliver533–81 (67)brown troutMichigan waters, USAliver10<17–26 | herring gull | Lake Huron, Great Lakes | plasma | 2 | 280-450 (370) | |
| Laysan and black-tooted albatrossesMidway Atoll, North Pacificplasma103–39 (9)Fishlake whitefishMichigan waters, USAeggs2150–380 (260)brown troutMichigan waters, USAeggs349–75 (64)Chinook salmonMichigan waters, USAliver633–170 (110)lake whitefishMichigan waters, USAliver533–81 (67)brown troutMichigan waters, USAliver10<17–26 | bald eagle | Midwestern USA | plasma | 26 | 1-25/0 (360) | |
| Fishlake whitefishMichigan waters, USAeggs2150–380 (260)brown troutMichigan waters, USAeggs349–75 (64)Chinook salmonMichigan waters, USAliver633–170 (110)lake whitefishMichigan waters, USAliver533–81 (67)brown troutMichigan waters, USAliver10<17–26 | Laysan and black-footed albatrosses | Midway Atoll, North Pacific | plasma | 10 | 3-39 (9) | |
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| brown troutMichigan waters, USAeggs349–75 (64)Chinook salmonMichigan waters, USAliver633–170 (110)lake whitefishMichigan waters, USAliver533–81 (67)brown troutMichigan waters, USAliver10<17–26 | lake whitefish | Michigan waters, USA | eggs | 2 | 150-380 (260) | |
| Chinook salmonMichigan waters, USAliver633–170 (110)lake whitefishMichigan waters, USAliver533–81 (67)brown troutMichigan waters, USAliver10<17–26 | brown trout | Michigan waters, USA | eggs | 3 | 49-75 (64) | |
| lake whitefishMichigan waters, USAliver533–81 (67)brown troutMichigan waters, USAliver10<17–26 | Chinook salmon | Michigan waters, USA | liver | 6 | 33-170 (110) | |
| brown troutMichigan waters, USAliver10<17-26yellow-fin tunaNorthern North Pacific Oceanliver12<7 | lake whitefish | Michigan waters, USA | liver | 5 | 33–81 (67) | |
| yellow-fin tunaNorthern North Pacific Oceanliver12<7blue-fin tunaMediterranean Sealiver821–87 (48)carpSaginaw Bay, Michigan, USAmuscle1060–300 (120)Chinook salmonMichigan waters, USAmuscle67–190 (110)lake whitefishMichigan waters, USAmuscle597–170 (130)brown troutMichigan waters, USAmuscle10<6–46 | brown trout | Michigan waters, USA | liver | 10 | <17-26 | |
| blue-fin tunaMediterranean Sealiver821–87 (48)carpSaginaw Bay, Michigan, USAmuscle1060–300 (120)Chinook salmonMichigan waters, USAmuscle67–190 (110)lake whitefishMichigan waters, USAmuscle597–170 (130)brown troutMichigan waters, USAmuscle10<6–46 | yellow-fin tuna | Northern North Pacific Ocean | liver | 12 | <7 | |
| carpSaginaw Bay, Michigan, USAmuscle1060-300 (120)Chinook salmonMichigan waters, USAmuscle67-190 (110)Iake whitefishMichigan waters, USAmuscle597-170 (130)brown troutMichigan waters, USAmuscle10<6-46 | blue-fin tuna | Mediterranean Sea | liver | 8 | 21-87 (48) | |
| Chinook salmon Michigan waters, USA muscle 6 7-190 (110) Iake whitefish Michigan waters, USA muscle 5 97-170 (130) brown trout Michigan waters, USA muscle 10 <6-46 | carp | Saginaw Bay, Michigan, USA | muscle | 10 | 60-300 (120) | |
| Iake whitefish Michigan waters, USA muscle 5 97–170 (130) brown trout Michigan waters, USA muscle 10 <6–46 | Chinook salmon | Michigan waters, USA | muscle | 6 | 7–190 (110) | |
| brown trout Michigan waters, USA muscle 10 <6-46 Turtles and Frogs yellow-blotched map turtle Mississippi, USA liver 6 39-700 (190) green frogs Southwest Michigan, USA liver 4 <35-290 spapping turtle Lake St Clair, Michigan, USA plasma 5 1-170 (72) | lake whitefish | Michigan waters, USA | muscle | 5 | 97–170 (130) | |
| Turtles and Frogs yellow-blotched map turtle Mississippi, USA liver 6 39–700 (190) green frogs Southwest Michigan, USA liver 4 <35–290 | brown trout | Michigan waters, USA | muscle | 10 | <6-46 | |
| yellow-blotched map turtleMississippi, USAliver639-700 (190)green frogsSouthwest Michigan, USAliver4<35-290 | Turtles and Frogs | | | | | |
| green frogs Southwest Michigan, USA liver 4 <35–290 snapping turtle Lake St. Clair, Michigan, USA plasma 5 1–170 (72) | yellow-blotched map turtle | Mississippi, USA | liver | 6 | 39-700 (190) | |
| spapping turtle Lake St. Clair, Michigan, USA plasma 5 1–170 (72) | green frogs | Southwest Michigan, USA | liver | 4 | <35-290 | |
| | snapping turtle | Lake St. Clair, Michigan, USA | plasma | 5 | 1-170 (72) | |

Results and Discussion

Samples were scanned for four FOCs: PFOS, perfluorooctane sulfonylamide (PFOSA), perfluorohexane sulfonate (PFHS), and perfluorooctanoate (PFOA). However, all concentrations of PFHS were less than the LOQ (LOQ range = 1 to 86 ng/g, wet wt) and only a few samples contained PFOA or PFOSA at levels greater than the LOQ of 2.5 to 180 ng/g, wet wt, and 1 to 38 ng/g, wet wt, respectively. Therefore, only the results for PFOS are presented (Table 1).

When a method detection limit of 1 ng/g, wet wt, was attained, PFOS was detectable in most samples including those from remote marine regions. Concentrations of PFOS in the blood of ringed and gray seals from the Canadian and Norwegian Arctic were in the range of 3 to 50 ng/mL. PFOS concentrations were 2-10 times greater (14–230 ng/mL) in seals from relatively more urbanized locations, such as the

Baltic Sea, than those from the Arctic Ocean. Blood plasma of Laysan and black-footed albatrosses collected from remote oceanic locations, such as Midway Atoll in the North Pacific Ocean, contained concentrations of PFOS ranging from 3 to 26 ng/mL. PFOS concentrations in the blood of cormorants and herring gulls from the Great Lakes were approximately 10-fold greater than those in albatrosses from Midway Atoll. Although the liver of yellow-fin tuna from the northern North Pacific did not contain quantifiable concentrations of PFOS (<7 ng/g, wet wt), blue-fin tuna from the Mediterranean Sea contained up to 87 ng/g, wet wt, PFOS in the liver. Livers of polar bears from Alaska contained concentrations of PFOS between 180 and 680 ng/g, wet wt. Although PFOS is distributed in remote marine environments including polar regions, concentrations of PFOS in these regions were several fold less than those from relatively more industrialized

locations such as the Baltic Sea and the Great Lakes.

Among various bird species analyzed, blood plasma of bald eagles from the midwestern U.S. contained PFOS concentrations of up to 2570 ng/mL (mean: 360). All plasma samples were obtained from bald eagle chicks less than 200 days old. Other fish-eating water birds such as common loons and brown pelicans contained quantifiable concentrations of PFOS in the liver. All the bird tissues from Canada, Italy, Japan, and Korea contained quantifiable concentrations of PFOS. PFOS concentrations in the blood of black-tailed gulls (2-12 ng/mL) from Hokkaido in Japan were less than those found in albatrosses from Midway Atoll. Occurrence of PFOS in these birds suggests that their potential source of exposure is the fish diet. Tissues of fish from the Great Lakes and the Mediterranean Sea contained measurable concentrations of PFOS. The highest PFOS concentrations observed in fish (300 ng/g, wet wt) were in the muscle of carp from Saginaw Bay, Michigan.

Mink from the midwestern U.S. contained significant concentrations of PFOS in their livers (970-3680 ng/g, wet wt). Mink are opportunistic predators but do eat fish as part of their diet. When mink were fed carp from Saginaw Bay, Michigan, containing an average concentration of 120 ng PFOS/g (wet wt), under laboratory conditions, the estimated biomagnification factor based on the concentrations of PFOS in livers of mink was approximately 22.

Although the results of this study have demonstrated for the first time that PFOS is widely distributed on a global scale and can be persistent and bioaccumulative in various food chains, little information is available on possible toxic effects of PFOS to wildlife. PFOS and related perfluorinated compounds have been shown to affect cell-cell communication, membrane transport and process of energy generation, and proxisome proliferation (11, 12). Currently available data (unpublished reports to U.S. EPA; Docket No. FYI-0500-01378) indicate that concentrations of PFOS in wildlife are less than those required to cause significant adverse effects in laboratory animals. However, as more species-specific information becomes available, a more refined assessment of the risks to wildlife will be possible.

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