Perfluorooctanesulfonate and Related Fluorochemicals in Several Organisms Including Humans from Italy

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Introduction

Perfluorooctane sulfonate (PFOS) is a persistent organic pollutant, extremely resistant to environmental degradation and is ubiquitous in the environment. Traditional monitoring studies for persistent chemicals failed to identify this contaminant for a long time because of its unique physicochemical properties and its tendency to bind to proteins instead of accumulating in fatty tissues. PFOS is known to be toxic in laboratory animals (rats, mice, monkeys) at levels close to the range already found in organisms and people [1]. PFOS has been commercially produced by an electrochemical fluorination process for over 40 years[2]. Perfluorooctane sulfonylfluoride (POSF; $C_8F_{17}SO_2F$) is used as a building block for further reactions that produce several other sulfonated fluorinated compounds, including perfluorooctane sulfonate ($C_8F_{17}SO_3^-$)[3-4] and other precursor molecules such as n-ethyl or n-methyl perfluorooctanesulfonamidoethanol. POSF-based fluorochemicals have been used in a wide variety of industrial and consumer products, including protective coatings for carpets and apparel, paper coatings, insecticide formulations, and surfactants[4]. These compounds repel water and oil, reduce surface tension, catalyze oligomerization and polymerization, and maintain their properties under extreme conditions. Depending upon the specific functional derivatization or the degree of polymerization, POSF-based chemicals may degrade or metabolize to PFOS[3, 5], which is known to be the final metabolite of POSF-based fluorochemicals[3]. PFOS is stable, chemically inert, and non-reactive and has the potential to bioaccumulate[6-8]. It has been found in polar bears from the Arctic[8-9], albatross and other fish-eating water birds in the mid-Pacific[10], and aquatic organisms[11] and people[12] world-wide. PFOS and other perfluorinated chemicals such as perfluorooctanesulfonamide (PFOSA), perfluorohexanesulfonate (PFHxS), and perfluorooctanoate (PFOA) have been detected in human blood[12] . In this study, concentrations of PFOS, FOSA (or PFOSA), PFHxS, and PFOA in marine mammals including bottlenose dolphins (*Tursiops truncatus*), striped dolphins (*Stenella coeruleoalba*), common dolphins (*Delphinus delphi*), fin whales (*Balenoptera physalus*), long-finned pilot whales (*Globicephala melas*); in fishes such as northern bluefin tuna (*Thunnus thynnus*), swordfish (*Xiphias gladius*); and in common cormorants (*Phalacrocorax carbo*) collected from Italian coast of the Mediterranean Sea[11] and in blood of the general population from Italy are reported.

Materials and Methods

Samples. Blood samples of bottlenose dolphins were collected by bleeding (10 mL) captive animals born in delphinariums. In the delphinarium, these dolphins were fed mackerel and herring caught in the Mediterranean Sea and capelin from the North Sea. Livers of common, bottlenose and striped dolphins were collected during mass mortality events along the Italian coast in 1991. All the dolphins were found stranded, dead along the Adriatic and Thyrrenian Seas. The livers of striped and bottlenose dolphins were freeze-dried prior to analysis. Tissues of long finned pilot whale and fin whales were taken from animals stranded in the Thyrrhenian Sea. Liver and blood samples of sexually mature (fork length > 110 cm) bluefin tuna were collected in Palizzi, southern coast of Italy. Liver and blood were taken from mature swordfish, which were caught by harpooning in the Ionian - Thyrrenian Seas. Livers of cormorant (*Phalacrocorax carbo*) were collected from the birds that were originally sacrificed in 1997 by the Department of Sanitation-Division of Rearing and Zootechnical Resources due to sanitary regulations. Age class and sex of cormorants were recorded when available. Human blood samples were collected from the local hospitals in Siena, Italy. Samples were void of personal identifiers. Details regarding donor's city of residence, sampling date, age, and gender were available. Serum was obtained from whole blood after clotting. All of the samples were kept at -20°C until analysis.

Analyses. PFOS, PFHxS, PFOA, and PFOSA were extracted using an ion-pairing extraction procedure and were determined by use of an high-performance liquid chromatograph (HPLC) with an electrospray tandem mass spectrometer (ES-MS/MS)[12] interfaced to a Micromass® (Beverly, MA) Quattro II atmospheric pressure ionization tandem mass spectrometer operated in the electrospray negative mode (for details on instrumental parameters see Kannan et al.[11]). Both HPLC-MS/MS and HPLC-MS analyses were performed for some samples, to compare and confirm the results. Details of preparation of tissues, reagents, and standards have been described earlier[12]. Analyte separation was performed using a Hewlett-Packard HP1100 HPLC (for details see Kannan et al.[11]).

Potassium salts of PFOS (86.4%), PFHxS (99.9%), and PFOA (98%) and PFOSA (95%) were provided by the 3M Company, St. Paul, MN. 1H,1H,2H,2H-perfluorooctanesulfonate (THPFOS; ICN, Costa Mesa, CA) and/or perfluorobutanesulfonate (PFBS; 99% purity, The 3M Company, St. Paul, MN) were used as internal standards and were spiked into blood samples prior to the addition of reagents for extraction. Recoveries of PFBS ranged from 75 to 120%. Reported concentrations were not corrected for the recoveries. Solvents, blood collection tubes, and method and matrix blanks were checked for the presence of the perfluorinated compounds analyzed in this study. Blanks contained PFOA and PFOS at concentrations less than 1 pg. Attempts were made to reduce the background levels of contamination in procedural blanks. The limit of quantitation (LOQ) was determined based on the linear range of the calibration curve prepared at a concentration range of 0.5 to 100 ng/mL. Concentrations in samples that were at least 3-fold greater than the lowest acceptable standard concentration were considered to be valid. A curve point was deemed acceptable if 1) it was back-calculated to be within 30% of the theoretical value when evaluated versus the 1/x weighted curve, and 2) the peak area of the standard was at least 3 times greater than that in the blank. Concentration/dilution factors are included in the calculation of the LOQ. The LOQs for PFOS, PFHxS, PFOA, and PFOSA varied from 1 to 1.3, 1 to 1.3, 3 to 20, and 1.3 to 6 ng/mL, respectively.

Results and Discussion

Mediterranean Sea organisms. PFOS was the most predominant fluorochemical in the tissues analyzed (Tables 1-2). PFOS was found in blood of captive bottlenose dolphins at concentrations ranging from 42 to 210 ng/mL (Table 1). The greatest PFOS concentration found in the liver of a common dolphin was 940 ng/g wet wt; muscle tissue from the same individual contained a PFOS concentration that was 12-fold less than that in liver (Table 1). Four of five livers of bottlenose dolphins collected from the Adriatic and Thyrrenian Seas contained quantifiable concentrations of PFOS. The mean±SD concentration of PFOS in livers of striped dolphins was 26±9 ng/g wet wt. Concentrations of PFOS in livers of bottlenose and striped dolphins were less than those found in cetaceans from the coastal waters of Florida[9]. Nevertheless, the concentration of PFOS measured in common dolphin liver was similar to those reported for dolphins from the Florida coast. Among the other fluorochemicals measured, FOSA was a prominent compound in livers of dolphins and whales.

Livers of most of the cetaceans (except striped dolphin) contained quantifiable concentrations of FOSA (Table 1). The greatest FOSA concentration was found in the liver of a common dolphin (878 ng/g wet wt). Occurrence of FOSA in marine mammals from the Mediterranean region indicates the presence of specific and current sources. PFOA and PFHxS were found in blood of a few individuals of bottlenose dolphins at concentrations ranging from <2.5 to 6.1 ng/mL. PFHxS was detected in a striped dolphin and swordfish liver at concentrations of 6.8 and 10 ng/g, wet wt, respectively.

Concentrations of PFOS in cormorant livers collected from Cabras Lagoon in Sardinia ranged from 32 to 150 ng/g wet wt (mean: 61 ng/g) (Table 1). Mean PFOS concentrations in juvenile birds were not significantly different from those in adults ($p < 0.05$). This is similar for bald eagles collected from the midwestern U.S.[13]. In general, PFOS concentrations in cormorants were similar to or less than those found in cormorants and other fish-eating water birds collected from the North American Great Lakes[13]. PFOA was consistently found in all the livers of cormorants at concentrations ranging from 29 to 450 ng/g wet wt.

Concentrations of PFOS in blood of bluefin tuna and swordfish ranged from 27 to 52 (mean: 40) and 4 to 21 ng/mL (mean: 10), respectively (Table 1). The PFOS concentration in livers of bluefin tuna was 21-87 ng/g wet wt (mean: 47), greater than that determined in swordfish (<1-13 ng/g; mean: 7) (Table 1). The ratios of concentrations of PFOS in liver to blood of bluefin tuna and swordfish were 0.85 and 1.4, respectively. These ratios are 7-12-fold less than those calculated for polar bears from Alaska[9]. Although the PFOS concentrations in bottlenose dolphins blood were 4 to 14-fold greater than those in bluefin tuna and swordfish, blood-to-liver ratios of PFOS were less in dolphins than in fishes. This suggests that the distribution of PFOS between liver and blood in fishes is different than in mammals. Concentrations of FOSA in the bluefin tuna blood (mean: 15 ng/mL) were 2 to 4-fold less than those of PFOS (40 ng/mL), which was different from that observed in bottlenose dolphins. FOSA concentrations in swordfish blood was 1.5-fold greater than that of PFOS. FOSA was not found in the fish livers at the quantitation limit of 38 ng/g, wet wt.

Human blood samples. Mean, median, and range of PFOS, PFHxS, PFOA, and PFOSA concentrations in serum samples are shown in Table 2; their concentrations were 4.4 ng/mL, 1.3 ng/mL, <3 ng/mL and 1.7 ng/mL respectively in female and 4.3 ng/mL, 1.7 ng/mL, <3 ng/mL and 1.8 ng/mL respectively in male donors. PFOS was found in most of the samples collected (87.5%

in female and 90.5% in male). PFHxS and PFOSA were found in the blood of Italian donors. Samples did not contain PFOA, at a detection limit of 3 ng/mL. In general, no significant difference ($p>0.05$) in the concentration of either PFOS or PFOA was found between the sexes.

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Table 1: Perfluorochemical concentrations (ng/g wet wt) in Mediterranean organisms and human blood from Italy (ND = Not determined; range is in brackets; a: values were converted from dry weight basis to wet weight assuming a moisture content of 75%; b: M = male, F = female. Length of fish and dolphins represents fork length).

Species/tissue	Location; date	PFOS	FOSA	PFOA	PFHxS	Sample details[b]
cormorant liver	Cabras Lagoon (Sardinian Sea); 1997	43	<38	41	<7	M, adult, 2.4 kg
		57	<38	100	<7	F, adult, 2.2 kg
		49	<38	51	<7	F, adult, 2.3 kg
		98	<38	90	<7	F, adult, 2.33 kg
		34	<38	30	<7	F, adult, 2.7 kg
		150	<38	140	<7	F, adult, 2.2 kg
		91	89	84	<7	F, juvenile, 2 kg
		47	<38	450	<7	F, juvenile, 1.4 kg
		50	<38	47	<7	F, juvenile, 1.96 kg
		43	<38	45	<7	M, juvenile, 2 kg
		32	<38	30	<7	M, juvenile, 2.4 kg
		34	<38	29	<7	M, juvenile, 2.4 kg
bottlenose dolphin liver[a]	Marina Grosseto (N Thyrrenian Sea); 1991	45	55	<36	<7	
	Livorno (N Thyrrenian Sea); 1991	75	90	<72	<7	240cm
	Croatia (N Adriatic Sea); 1992	42.5	32.5	<72	<7	M, 288 cm
	Lecce (S Adriatic Sea); 1991	108	32.5	<72	<7	M, 235 cm
	Lido Camaiore (N Thyrrenian Sea); 1991	<1.4	30	<72	<7	279cm
	Mar Tirreno; NA	110	139	<38	<19	NA
bottlenose dolphin blood (n=4)	Riccione (Adriatic Sea); 1997	143 (42-210)	223 (190-270)	3.1 (<2.5-3.8)	4.5 (<1-6.1)	3 M, 1 F; 2-19 yrs
striped dolphin liver[a]	Lecce (S Adriatic Sea); 1991	40	<38	<72	<7	M, 201 cm
	Viareggio (N Thyrrenian Sea); 1991	22.3	<38	<72	<7	F, 200 cm
	Lecce (S Adriatic Sea); 1991	23.5	<38	<72	<7	F
	Lecce (S Adriatic	16.3	<38	<72	6.8	F, 201 cm

	Sea): 1991					
common dolphin muscle	Giglio Is, N Thyrrenian Sea; 1998	77	142	<38	<19	F, 203 cm
common dolphin liver	Giglio Is, N Thyrrenian Sea; 1998	940	878	<38	<19	F, 203 cm
fin whale muscle	Livorno, (N Thyrrenian Sea); 1998	<19	<19	<38	<19	M, 13.8 m, 13.7 tons
long-finned pilot whale muscle	Elba Is, N Thyrrenian Sea; 1996	52	48	<38	<19	160 cm, pup, suckling
long-finned pilot whale liver	Elba Is, N Thyrrenian Sea; 1996	270	50	<38	<19	160 cm, pup, suckling
swordfish liver	St Messina (Ionian-Thyrrenian Sea); 1999	3	<38	<36	10	150 cm, 44 kg
		5	<38	<36	<7	156cm, 45 kg
		8	<38	<36	<7	140 cm, 53 kg
		<1	<38	<36	<7	161 cm, 61 kg
		13	<38	<36	<7	F, 70 kg
swordfish blood (n = 7)	St Messina (Ionian-Thyrrenian Sea); 1999	7.2 (4-14)	15 (1.1-28)	<2.5	<1	3 M, 3 F, 1 ND; 107-190; 15-83 kg
tuna liver	Reggio Calabria (Ionian Sea); 1999	35	<38	<36	<7	M, 250 cm,
		43	<38	<72	<7	156 cm
	Palizzi; 1999	87	<38	<36	<7	M, 147cm, 54 kg
		57	<38	<72	<7	M, 157cm, 61 kg
		49	<38	<36	<7	M, 158cm, 51 kg
		21	<38	<36	<7	139 cm
		56	<38	<36	<7	M, 149 cm, 46 kg
		25	<38	<72	<7	152 cm, 56 kg
tuna blood (n = 6)	Palizzi; 1999	40 (27-52)	15 (13-19)	<2.5	<1	4 M, 2 ND; 113-158 cm fork length; 25-61 kg
human blood (n = 50)	Siena; 2001	See Table 2				42 M, 8 F; 39 (20-59) yrs;

Table 2: Perfluorochemical concentrations (ng/mL) in human blood samples from Italy.

	n	PFOS	PFHS	PFOA	PFOSA
female mean	8	4.4	1.3	<3	1.7
female median		3.5	1.3	<3	1.7
min		2.5	1.3	<3	1.3
max		8.0	1.4	<3	1.7
% detect		87.5	37.5	0	12.5
male mean	42	4.3	1.7	<3	1.8
male median		4.2	1.7	<3	1.6
min		1.0	1.3	<3	1.5
max		10.3	2.1	<3	2.3
% detect		90.5	33	0	9.5