EXTRACTABLE ORGANIC FLUORINES, PERFLUOROOCTANE SULFONATES AND OTHER FLUOROCHEMICALS IN INDO-PACIFIC HUMBPACK DOLPHIN AND FINLESS PORPOISE IN HONG KONG, CHINA

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Abstract

Recent investigations have demonstrated that PFCs are present in the coastal waters of Hong Kong and the Pearl River Delta (PRD). Since PFCs have been found to be bioaccummulative in the local biota, there is thus an increasing interest in studying the environmental concentrations and potential ecological risks of these compounds. Liver samples of the Indo-Pacific humpback dolphin and the finless porpoise, the two resident species in Hong Kong, were analyzed for PFC contaminations. The dolphin and porpoise tissues had similar PFOS concentrations (65.7 and 96.6 ng/g wet wt, respectively) that are comparable to those reported in other cetacean species from other countries. The PFCs in the finless porpoise were largely made up of PFOS (82%) and, to a lesser extent, of PFUnDA (9%), PFDA (4%), and PFNA (3%). However, the PFCs in the dolphins consisted of 65% PFOS, 18% PFOSA and smaller concentrations of PFUnDA (9%), PFDA (2%), PFNA (2%), and PFHpA (2%). Different habitats and food habits might account for these different composition profiles. Low PFC/EOF ratio (10-15%) in dolphin liver samples suggested that large portion of the extractable organic fluorine remained unknown.

Introduction

Marine mammals (dolphins and porpoises) are of great conservation value in Hong Kong. The Indo-Pacific humpback dolphin (*Sousa chinensis*) and the finless porpoise (*Neophocaena phocaenoides*) are the two resident species.[1] Although there is still no precise quantitative information as to whether the local dolphin and porpoise populations are declining, recent studies have revealed that environmental pollution is a major threat to the health of these species. Studies on stranded cetaceans have shown that these animals accumulate relatively high levels of metals, organochlorines,[2,3] and polybrominated diphenyl ethers (PBDEs).[4,5]

Perfluorinated compounds (PFCs) are a group of chemicals that has attracted increasing attention in recent years. PFCs have been manufactured for more than 50 years, and are widely used in industry, particularly in the manufacture of electronic and textile products. Studies on the global distribution of PFCs have detected perfluorooctane sulfonate (PFOS) in the tissues of humans and wildlife, including fish, birds, and marine mammals.[6] Recent investigations have demonstrated that PFCs are present in the coastal waters of Hong Kong and the Pearl River Delta (PRD).[7,8] The information that is available indicates PFOS to be persistent and toxic,

and to cause cellular dysfunction[9]. Another widespread PFC, perfluorooctanoic acid (PFOA), has been identified as a suspect carcinogen[10]. Although PFC concentrations in the coastal waters of the PRD have been found to be lower than those from other countries, they have been found to be bioaccummulative in the local biota.[11] There is thus an increasing interest in studying the environmental concentrations and potential ecological risks of these compounds. In the present study, PFCs and extractable organic fluorine were quantified in dolphin and porpoise liver samples.

Materials and Methods

Liver samples of Indo-Pacific humpback dolphins and finless porpoises were collected from stranded animals by the Agricultural, Fisheries and Conservation Department in Hong Kong between 2003 and 2007. The liver samples were stored in polypropylene (PP) plastic bags at -20^{o}C in the laboratory at the City University until analysis. Around 1 g of liver sample was homogenized in 5 mL of MilliQ, and 1 mL of the homogenate was used for PFC extraction. Individual PFCs were extracted using an ion-pairing method and were reduced to 1 mL. Blank and recovery tests were conducted on each batch of samples. To quantify EOF, 0.5 mL of the sample extract was subjected to combustion ion chromatography (CIC), and 0.5mL of the sample extract underwent envicarb and solid phase extraction (SPE) cleanup. The concentrations of perfluorinated sulfonates (PFOS, perfluorohexane sulfonate – PFHxS, perfluorobutane sulfonate – PFBS), perfluorooctanesulfonamide (PFOSA), and perfluorinated carboxylates (Perfluorohexanoic acid – PFHxA, perfluoroheptanoic acid – PFHpA, PFOA, perfluorononanoic acid – PFNA, perfluorodecanoic acid – PFDA, perfluoroundecanoic acid – PFUnDA, and perfluorododecanoic acid – PFDoDA, perfluorotetradecanoic acid – PFTeDA, perfluorohexadecanoic acid – PFHxDA and perfluorooctadecanoic acid – PFOcDA), fluorotelomer carboxylate (8:2 FTCA), and fluorotelomer unsaturated carboxylate (8:2 FTUCA) were determined by HPLC-MS/MS. The separation of the analytes was performed by using an Agilent HP1100 liquid chromatograph (Agilent, Palo Alto, CA) that was interfaced with a Micromass Quattro Ultima Pt mass spectrometer (Waters Corp., Milford, MA) and operated in the electro-spray negative mode. A 10-µL aliquot of extract was injected onto a Keystone Betasil C18 column (2.1 mm i.d. x 50 mm length, 5 µm, 100Å pore size, endcapped) with 2 mM of ammonium acetate and methanol as the mobile phases. The details of the procedure for the LC-MS/MS are reported elsewhere.[12] EOF concentrations were determined using CIC with a method that involves the modification of the traditional CIC by the combination of an automated combustion unit (AQF-100 (type AIST), Dia Instruments Co. Ltd. Japan) and an ion chromatography system (ICS-000 (type AIST), Dionex Co. Ltd., Japan). The sample extract was set on a silica boat and placed in a furnace at 900–1000°C. The combustion of the sample in the furnace converted the organofluorines and inorganic fluoride into hydrogen fluoride (HF), which was then absorbed into sodium hydroxide solution (0.2 mmol/L). The concentration of F^- in the solution was analyzed using ion chromatography. Sodium fluoride (99% purity; Wako Pure Chemical Industries, Tokyo, Japan) was used as the

standard for quantification. The analytical procedures for ion chromatography are described elsewhere.[1,2] All of the solutions were prepared in Milli-Q water with a fluoride concentration of <0.025 µg/L.

Results and Discussion

The concentrations of PFOS, PFHxS, PFOSA, PFDoDA, PFUnDA, PFDA, PFNA, PFOA, and PFHpA, were measured. The levels of PFBS and PFHxA, FTUCA, and FTCA were all below LOQ. The blank and recovery test results and PFC concentrations are summarized in Table 1. PFOS was found to be the dominant PFC in dolphins, followed by PFOSA, PFUnDA, PFDA, and PFNA. PFOS was also found to be the dominant PFC in porpoises, followed by PFUnDA, PFDA, and PFNA.

The dolphin and porpoise tissues had similar PFOS concentrations (65.7 and 96.6 ng/g wet wt, respectively) that are comparable to those reported in other cetacean species from other countries (14.8-489 ng/g wet wt.[13], 33.1-210 ng/g wet wt.[14]). Van de Vijver et al.[14] reported that over 80% of the PFCs in harbor porpoises consisted of PFOS and, to a lesser extent, PFNA, PFDA, PFUnDA, and PFDoDA. Similarly, in this study, the PFCs in the finless porpoise were largely made up of PFOS (82%) and, to a lesser extent, of PFUnDA (9%), PFDA (4%), and PFNA (3%). However, the PFCs in the dolphins consisted of 65% PFOS, 18% PFOSA and smaller concentrations of PFUnDA (9%), PFDA (2%), PFNA (2%), and PFHpA (2%).

There are two possible reasons for the discrepancies in the PFC composition profiles between the dolphins and porpoises. Although dolphins and porpoises both live in Hong Kong waters, they occupy different areas and there is only a small overlap between their habitats.[15] Dolphins inhabit the western waters of Hong Kong, which receive discharge from the Pearl River, whereas porpoises reside in the southern and eastern waters of Hong Kong, which are largely oceanic. This is reflected in the different feeding habits of the two species. Dolphins mainly feed on estuarine fish such as croakers and mullets, whereas porpoises prefer to feed on squid and shrimp.[15,16] It is conceivable that these different feeding habits may at least in part account for the observed discrepancies in the PFC composition profiles. Our results demonstrate that marine mammals in Hong Kong bioaccummulate PFCs in their tissues to concentrations that are much higher than those in the surrounding environment.[7,8]

Two dolphin liver samples were subjected to EOF analysis. Female dolphin had a higher amount of EOF and PFCs which suggested that there might be a gender-specific bioaccumulation in PFCs and EOF. However, further investigation is needed to test this hypothesis due to the limited sample size in the present study. Low PFC/EOF ratio suggested that large amount of unknown organic fluorines were present in the dolphin liver samples. Different PFC/EOF ratios among human blood in China[17], US[18], and Japan[18] (40-90%) and dolphin liver[19] in Hong Kong (10-15%) samples were observed.

To our knowledge, this is the first report on PFCs in marine mammals in the PRD. Further investigations should be initiated to identify the sources of contamination, and to evaluate the health risk to marine mammals of exposure to PFCs in south China.

Reference

1. Parsons ECM *Environ. Conser.* 1999; 26: 30-40.
2. Minh TB, Nakata H, Watanabe M, Tanabe S, Miyazaki N, Jefferson TA, Prudente M, Subramanian A *Arch. Environ. Con Toxicol.* 2000; 39: 398-410.
3. Minh TB, Watanabe M, Nakata H, Tanabe S, Jefferson TA *Mar. Poll. Bull.* 1999; 39: 383-392.
4. Ramu K, Kajiwara N, Tanabe S, Lam PKS, Jefferson TA *Mar. Poll. Bull.* 2005; 51:669-676.
5. Kajiwara N, Kamikawa S, Ramu K, Ueno D, Yamada TK, Subramanian A, Lam PKS, Jefferson TA, Prudente M, Chung KH, Tanabe S *Chemosphere* 2006; 64: 287-295.
6. Giesy JP, Kannan K *Environ. Sci. Technol.* 2002 ; 36:146A-152A.
7. So MK, Taniyasu S, Yamashita N, Giesy JP, Zheng J, Fang Z, Im SH, Lam PKS *Environ. Sci. Technol.* 2004; 38: 4056-4063.
8. So MK, Miyake Y, Yeung WY, Ho YM, Taniyasu S, Rostkowski P, Yamashita N, Zhou BS, Shi XJ, Wang JX, Giesy JP, Yu H, Lam PKS *Chemosphere* (In press).
9. Hu W., Jones PD, DeCoen W, Newsted, JL, Giesy JP. *Environ. Toxicol. Pharmacol.* 2005 ; 19 : 153-160
10. USEPA: SABSO: SAB Review of EPA's Draft Risk Assessment of Potential Human Health Effects Associated with PFOA and Its Salts, January 20, 200601-30-2006 Available at: http://www.epa.gov/sab/pdf/2006_0120_final_draft_pfoa_report.pdf
11. Sinclair E, Mayack DT, Kenneth R, Yamashita N, Kannan K *Arch. Environ. Con. Toxicol.* 2006; 50: 398-410.
12. Taniyasu S, Kannan K, So MK, Gulkowska A, Sinclair E, Okazawa T, Yamashita N *J Chromatogr A* 2005; 1093: 89-97.
13. Kannan K, Koistinen J, Beckmen K, Evans T, Gorzelany JF, Hansen KJ, Jones PD, Helle E, Nyman M, Giesy JP *Environ. Sci. Technol.* 2001; 5: 159-1598.
14. Van de Vijver KI, Holsbeek L, Das K, Blust R, Joiris C, De Coen W. *Environ. Sci. Technol.* 2007 ; 41: 315-320.
15. Jefferson TA *Wildlife Monographs* 2000; 14: 1-65.
16. Hung CL, Xu Y, Lam JC, Jefferson TA, Hung SK, Yeung LW, Lam MH, O'toole DK, Lam PK *Chemosphere* 2006; 63: 845-852.
17. Yeung LWY, Miyake Y, Taniyasu S, Wang Y, So MK, Yu H, Yamashita N, Lam PKS Extractable organic fluorines, perfluorinated compounds in Chinese blood samples *Organohalogen Compounds*, 2007*(Submitted).*
18. Miyake Y, Yamashita N, So MK, Rostkowski P, Taniyasu S, Lam PK, Kannan, K *J. Chromatogr. A* (In press).
19. Yeung LWY, Miyake Y, Taniyasu S, Lam PKS, Kannan K, Guruge KS, Yamashita N Persistent perfluorinated chemicals and total fluorine in the blood of wild rat; new approach to estimate human exposure *Organohalogen Compounds*, 2007*(Submitted).*

Table 1. Blank, recoveries and concentrations of PFC in dolphin and porpoise liver samples (ng/g wet wt.).

		PFOS	PFHxS	PFOSA	PFDoDA	PFUnDA	PFDA	PFNA	PFOA	PFHpA	Total PFC
	Blank	<0.02	<0.02	<0.01	<0.004	<0.004	<0.01	<0.002	<0.028	<0.002	
	Recovery	87	74	71	65	83	90	92	76	80	
	S.D.	3	9	1	1	10	3	6	8	8	
Indo-Pacific Humpback dolphin	Mean	65.7	0.232	18.6	0.693	9.45	2.17	2.01	0.517	1.51	101
n=3	S.D.	38.5	0.186	3.85	0.40	5.57	1.412	0.734	0.219	1.45	46.8
	Min	25.0	<0.02	13.7	0.26	3.51	0.884	1.02	0.20	0.533	57.7
	Max	117	439.5	24.0	1.21	16.3	4.29	2.96	0.71	3.61	163
Finless porpoise	Mean	96.6	0.12	0.802	0.78	10.7	4.75	3.63	0.372	0.0968	118
n=3	S.D.	49.2	0.167	0.506	0.0827	2.1	1.69	1.10	0.135	0.133	52.2
	Min	56.1	<0.02	0.53	0.647	7.86	3.23	2.56	0.262	<0.02	73.4
	Max	179	0.343	1.36	0.868	13.7	7.47	5.16	0.521	0.245	205

Table 2. Extractable organic fluorine (EOF) and perfluorinated compounds in dolphin liver samples (ng-F/g wet wt.)

			(ng-F/g wet wt.)		
Sample No	Age class	Sex	EOF	PFC	PFC/EOF %
SC01	Adult	F	1084.3	112.9	10
SC02	Adult	M	533.4	78.0	15