PERSISTENT PERFLUORINATED CHEMICALS AND TOTAL FLUORINE IN THE BLOOD OF WILD RAT; NEW APPROACH TO ESTIMATE HUMAN EXPOSURE

Yeung LWY[1, 2], Miyake Y[1], Taniyasu S[1], Lam PKS[2], Kannan K[3], Guruge KS[4], Yamashita N[1]

[1]National Institute of Advanced Industrial Science and Technology (AIST), 16-1 Onogawa, Tsukuba, Ibaraki, Japan; [2]Department of Biology and Chemistry, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong; [3]New York State Department of Health and Department of Environmental Toxicology and Health, State University of New York, Empire State Plaza, PO Box 509, Albany, NY 12201-0509, USA. [4] Toxico-Biochemistry Section, National Institute of Animal Health (NIAH), Kannondai 3-1-5, Tsukuba, Ibaraki 305-0856, Japan

Abstract
The number of perfluorochemicals (PFCs) that are found in biological and environmental matrices is increasing as analytical standards and methods evolve. Perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) constitute only a fraction of the total of PFCs that are present in environmental and biological matrices. A robust method and approach is needed to evaluate the mass of fluorinated compounds in biological matrices. In this study, we determined the concentrations of PFCs and total fluorine (TF) in whole blood from wild rats and plasma from exposed rats. The PFCs as measured by HPLC-MS/MS accounted for 1.96–60.0% and 76.5–103% of the TF in the wild rats and exposed rats, respectively. These results suggest the existence of an as yet uncharacterized fluorine fraction in rat blood. Further studies are needed to characterize the aqueous fraction that contains inorganic fluorine and non-extractable forms of fluorine.

Introduction
A number of poly- and per-fluorinated compounds (PFCs) have been identified in the environment and biological samples. Whereas the number of fluorinated organic chemicals found in biota is increasing, it is not known whether any unidentified per-/poly-fluorinated compounds are present in human and wildlife tissues. A robust method and approach is needed to evaluate the mass of fluorinated compounds in biological matrices. We have developed a method to measure the total fluorine (TF) in human blood matrices using combustion ion chromatography with improved sensitivity for fluorine (CIC-F). Known PFCs account for 10 to 15% of the total F in the plasma of the general population and 80% of the total fluorine in occupationally exposed individuals. We applied the new method to several species of wild rat that were collected from Japan and to exposed rats. Blood samples from each specimen were subjected to individual PFC analysis using HPLC-MS/MS and total fluorine analysis by CIC. We estimated the contribution of individual PFCs in total fluorine in these tissues and discuss the results for known and unknown proportions of fluorinated compounds in wild and exposed rats.

Materials and Methods
Samples of whole rat blood were collected from 12 cities in 10 prefectures of Japan between 2004 and 2006. The samples were stored in polypropylene (PP) containers or vacutainers at -20 ˚C until analysis. Individual PFCs were extracted using an ion-pairing method, and were reduced to 1 mL. In addition, 0.5 mL of the sample extract was used to quantify the extractable organic fluorine by CIC, and another 0.5 mL underwent further envicarb and solid phase extraction (SPE) cleanup. The concentrations of perfluorinated sulfonates (PFOS, perfluorohexane sulfonate - PFHxS), 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) and other PFCs were determined by using 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 an electro-spray negative mode. The details of the procedure for the LC-MS/MS are reported elsewhere.[1]

The concentrations of TF in the blood samples were determined using CIC. The method involved the modification of the traditional combustion ion chromatography (CIC) by using a 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 blood sample 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 procedure for the ion chromatography is given in Miyake et al.[2, 3] All of the solutions were prepared in Milli-Q water with a fluoride concentration of < 0.025 μg/L.

Results and Discussion

Individual PFCs. The concentrations of PFOS, PFHxS, PFOSA, PFDoDA, PFUnDA, PFDA, PFNA, PFOA, PFHpA, and PFHxA were measured. The levels of PFBS and PFHxA, FTUCA, and FTCA were all below LOQ. The blank and recovery test results and the PFC concentrations are summarized in Table 1.

Table 1. Concentrations of individual PFCs in wild rat blood collected from 12 cities in 10 prefectures in Japan (ng/mL).

			PFOS	PFHxS	PFOSA	PFDoDA	PFUnDA	PFDA	PFNA	PFOA	Total PFC
Blank			<0.01	<0.01	<0.05	<0.02	<0.02	<0.05	<0.01	<0.03	
Recovery	**Mean**		89.9	89.0	65.0	81.8	89.1	104	103	97.1	
	S.D.		9.64	9.34	1.40	4.70	5.99	8.72	6.68	6.13	
Matrix-spike recovery	**Mean**		86.8	97.4	57.3	101	96.8	100	98.3	89.3	
	S.D.		0.873	5.44	9.71	6.96	2.52	2.07	4.43	4.60	
Hokkaido		**F**	123	0.306	13.0	0.756	4.94	1.90	1.48	0.141	158
Aomori		**M**	26.0	<0.02	2.16	0.556	3.54	1.25	6.71	0.335	41.7
		F	5.09	<0.02	2.79	0.431	2.79	0.929	0.139	0.0473	12.2
Ibaraki	**Mean**	**M**	9.13	0.564	2.45	1.68	8.36	6.38	25.5	6.57	60.8
	S.D. (n=2)		4.38	0.300	1.68	1.46	6.21	3.93	5.33	1.86	24.4
	Mean	**F**	8.14	0.0295	3.36	1.15	5.08	3.92	0.713	0.182	22.7
	S.D. (n=2)		0.891	0.00354	1.09	0.610	1.11	2.72	0.224	0.0339	3.85
Chiba		**M**	5.48	<0.02	6.59	0.388	4.54	0.820	0.670	0.0550	18.5
		F	19.0	<0.01	9.07	0.339	3.83	1.70	0.924	0.0620	34.9
Tokyo	**Mean**	**M**	6.68	0.210	2.36	0.237	4.76	1.49	9.87	1.62	27.2
	S.D. (n=2)		0.723	0.176	0.760	0.181	0.577	0.227	8.60	0.437	7.20
		F	14.1	<0.02	3.02	0.530	5.14	1.39	0.236	0.103	24.5
Kanagawa		**M**	27.0	0.282	3.91	0.162	1.39	2.07	4.02	0.789	39.7
		F	9.99	0.231	1.51	0.152	0.726	1.10	2.78	0.274	16.8
Shizuoka		**M**	38.1	0.450	4.32	1.20	8.69	2.42	13.2	2.60	71.1
		F	10.3	<0.02	6.10	1.09	6.31	2.10	0.540	0.0682	26.6
Osaka		**M**	0.831	<0.04	0.361	0.420	0.900	0.522	0.440	1.04	4.52
		F	7.78	<0.04	1.96	2.61	9.77	15.7	7.46	4.83	50.3
Fukuoka		**M**	9.90	<0.04	3.65	0.653	4.64	1.10	0.203	0.188	20.4
		F	2.42	<0.04	<0.2	0.110	0.797	0.439	0.374	0.194	4.33
Okinawa		**M**	3.56	0.135	<0.2	0.139	1.42	3.25	3.15	0.463	12.1
		F	3.44	0.241	0.149	0.378	2.00	2.04	4.27	0.519	1.10
Total	**Mean**		16.0	0.355	4.32	0.709	4.38	2.57	5.44	1.22	34.5
	S.D. (n=21)		25.9	0.293	4.99	0.703	3.06	2.96	8.06	2.05	34.0

Most of the earlier available biological monitoring data come from waterbirds, fish, and cetacean samples, but data from terrestrial animals are scarce. Wild rats feed on various food types, such as fish, fruit, grains, and leftovers from garbage, and thus, wild rat samples can be excellent indicators of PFC exposure in terrestrial mammals including humans. In general, PFOS was the dominant PFC type, but in some samples, such as those from Ibaraki (male), Osaka, Tokyo (male), Chiba (male), and Okinawa, PFNA, PFUnDA, and PFOSA were found to be the dominant types. The PFOS concentrations in the wild rats are comparable to those already reported in fish and waterbirds from Japan.[4, 5] Hokkaido had the highest PFOS concentration (123 ng/mL) and Osaka the lowest (0.831 ng/mL). These results suggest that the degree and sources of contamination differ across different regions of Japan. Differences in the PFOS and PFOA concentrations were observed between genders in some cities, which suggest gender-specific PFOS and PFOA bioaccumulation. This finding is consistent with other experimental studies that have found that male and female rats bioaccumulate PFOS and PFOA differently.[6]

Mass balance analysis of fluorine. Twenty-one samples of whole blood from wild rats in Japan and 20 samples of

plasma from rats that were exposed to PFOA in the laboratory were subjected to CIC analysis. The TF concentrations in the wild rat blood ranged from 44.1 to 173 ng F/mL, whereas in the exposed rat plasma they ranged from 46,800 to 111,000 ng F/mL. In terms of composition, 1.96–60.0% of the TF in the wild rat samples consisted of known PFCs, and the remaining portion was composed of known PFCs that could not be extracted using the present extraction method, inorganic fluorine, and unknown organofluorines. In the exposed rat samples, 77–103% of the TF was found to be PFOA. Plasma samples from the exposed rats showed that PFOA made a strong contribution to the TF. In addition, an increase in the concentration of PFCs in the wild rat blood and exposed rat plasma resulted in an increase in the PFC contribution to the TF. These results further suggest that the unknown fraction still accounts for a major proportion of the TF, and may contain several unidentified or non-extractable organic forms of fluorine. Similar studies reported different PFC/extractable organic fluorine (EOF) ratios in Chinese[7], Japanese and US human blood samples[3], and dolphin liver samples[8] which suggested that other fluorochemicals bioaccumulated to a different degree in various biological matrices. Further studies are needed to characterize this unknown fraction. The application of the mass balance approach to source materials (e.g., industrial products) and environmental and biological samples should provide valuable information on the extent of contamination by other unidentified fluorochemicals in the environment. As conclusion, clear variations of individual PFCs and unknown fluorinated chemicals among prefectures and gender-specific accumulation suggested the remark, namely wild rat that expected to be exposed by kinds of hazardous chemicals in garbage is potential indicator animal to estimate human exposure.

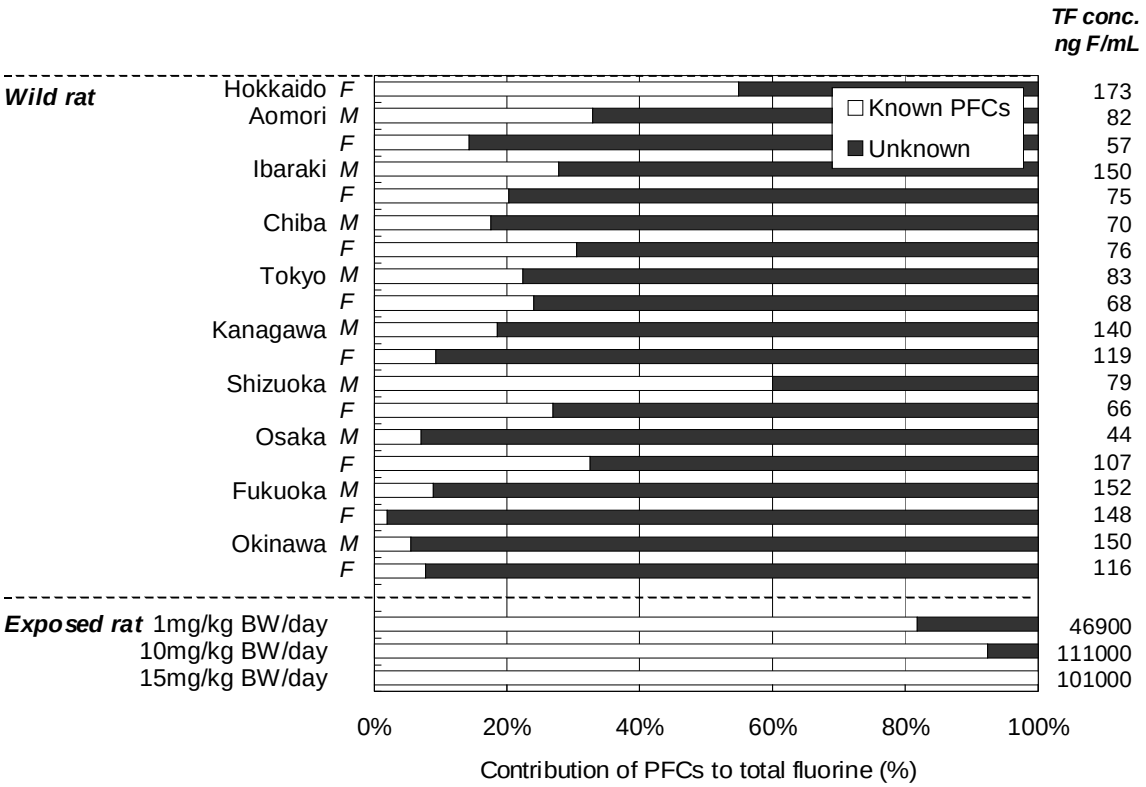

Figure 1. Contribution of PFCs to total fluorine (TF) in wild and laboratory rat blood.

References

1. Taniyasu, S., Kannan, K., So, M.K., Gulkowska, A., Sinclair, E., Okazawa, T., and Yamashita, N. *J. Chromatog. A* 2005; 1093: 89-97.
2. Miyake, Y., Yamashita, N., So, M.K., Rostkowski, P., Taniyasu, S., Lam, P.K., Kannan, K. *J. Chromatogr. A* 2007; 1143: 98-104.
3. Miyake, Y., Yamashita, N., So, M.K., Rostkowski, P., Taniyasu, S., Lam, P.K., Kannan, K. *J. Chromatogr. A* (In press).
4. Kannan, K., Corsolini, S., Falandysz, J., Oehme, G., Focardi, S., Giesy, J.P. *Environ. Sci. Technol.* 2002; 36:

3210-3216.
5. Taniyasu, S., Kannan, K., Horii, Y., Hanari, N. and Yamashita, N. *Environ. Sci. Technol.* 2003; 37: 2634-2639.
6. Vanden Heuvel, J., Kuslikis, B. and Van Rafelghem, M. *Biochem. Toxicol.* 1991; 6: 83-92.
7. 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).*
8. Yeung LWY, Miyake Y, Zhan JB, Wang Y, Hagino Y, Taniyasu S, Yamashita N, Lam PKS Extractable organic fluorines, perfluoroocatane sulphonates and other fluorochemicals in Indo-Pacific humpback dolphin (*Sousa chinensis*) and finless porpoise (*Neophocaena phocaenoides*) in Hong Kong, China. *Organohalogen compounds* 2007 *(Submitted).*