COMPARISON OF PFCS POLLUTION CHARACTERISTICS BETWEEN KANTO AND KINKI, MOST DENSELY POPULATED AREAS IN JAPAN, USING DRAGONFLY AS BIOINDICATOR ORGANISM

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Introduction

In the past decade, perfluorinated compounds (PFCs), including perfluorooctanesulfonic acid (PFOS), have been investigated in many environmental fields, due to their persistent, bioaccumulative and toxic properties. The results of these studies have revealed ubiquitous occurrence of PFCs, and attracted much concern about their fate in the environment [1, 2]. Particular attention should be paid on highly populated / industrialized areas where their use and discharge are expected to be higher while the exposed population may also be higher than other areas. In previous study on the water concentrations of PFOS and perfluorooctanoic acid (PFOA)[3], Kanto and Kinki, most densely populated areas in Japan, were found to show higher levels than others, and detailed study on their pollution status and source identification are needed.

Biomonitoring is a useful tool to reveal the distribution of pollutants and to identify hot-spots / sources due to their bioaccumulation through the food web and representing magnified and averaged or integrated levels in bioindicator organisms. Previously, we found that dragonfly has a good potential as biomonitoring tool of PFCs in terrestrial environment[4]. The aim of this study was to compare PFCs pollution characteristics between Kanto and Kinki using the same species of dragonfly as bioindicator.

Materials and methods

Dragonfly samples (*Orthetrum albistylum speciosum*) were collected from 31 terrestrial sites of Japan with the help of people who showed interests to our activity of environmental monitoring by dragonfly, and were frozen at -80 C until analysis. Samples were prepared according to a procedure previously reported.[4] In brief, whole body samples (0.2~0.5g-wet) were treated with alkaline digestion (90 °C for 3hr in 4N NaOH) followed by ion-pair extraction (tetrabutyl ammonium as ion-pair reagent) and hexane acetonitrile partition (Chem Elut,Varian). The extracts were clean-upped with Oasis-HLB (Waters) and Oasis-MCX (Waters). PFCs analysis was performed by high performance liquid chromatography and tandem mass spectrometry (LC/MS/MS, 4000Qtrap, AB SCIEX). Original LC conditions and MSMS parameters used same conditions reported in 2009.[4] In accordance with the increase of PFCs in the standard, water-methanol LC gradient (Table 1) was employed for the LS separation in later stage of the study, and a delay column (Xterra C18 2.1x50mm, Waters) was added between the pump and autosampler in order to eliminate contamination from the LC system [5]. 6 PFCs, i.e., PFOS, PFOA, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnA) and perfluorododecanoic acid (PFDoA), were quantified using ^{13}C labeled standard mixture (MPFAC-MXA, Wellington. Lab. Inc.) as surrogate. The other PFCs included in the standard mixture (PFAC-MXB, MPFAC-MXA, Wellington Lab. Inc.) were also analyzed in later stage, but are not reported here. All statistical analysis was performed with PASW Statistics software. Principal Component Analysis (PCA) was applied on the summary data in which ND (not detected; less than MDL) were substituted with half of the method detection limit (MDL).

Table1. LCMSMS condition

LC	Bin pump: 1200 series (Agilent)				MS	4000QTrap (AB SCIEX)							
	Solvent A	Water (10mM ammonium acetate)				Scan Type	MRM		IS	-4500			
	Solvent B	MeOH				Polarity	Negative		TEM	400			
	Flow rate	0.2 ml/min				Ion Source	Turbo Spray		GS1	40			
	Gradient	Total time	A(%)	B(%)					GS2	80			
		0	50	50					CAD	4			
		3	40	60					EP	-10			
		25	0	100					CXP	-5			
		30	0	100									
		32	50	50									
		47	50	50									
	Column: 1200 series (Agilent)				MS/MS Parameter								
		ZorbaxXDB C-18 (2.1 × 150mm, 3.5 μm)			Native	Carbon No.	Q1	Q3	IS	Q1	Q3	DP	CE
	Guard column	ZorbaxXDB C-8 (2.1 × 12.5mm, 5 μm)			PFOS	C8	499	80	MPFOS	503	80	-105	-86
	Delay column	Xterra C18(2.1 × 50mm 3.5 μm)			PFOA	C8	413	369	MPFOA	417	372	-35	-16
	Oven (℃)	40			PFNA	C9	463	419	MPFNA	468	423	-35	-16
					PFDA	C10	513	469	MPFDA	515	470	-35	-16
	Autosampler: 1200 series (Agilent)				PFUnA	C11	563	519	MPFUnA	565	520	-45	-18
	Injection Volume(μl)	10			PFDoA	C12	613	569	MPFDoA	615	570	-45	-18

Results and discussion

(1) Sampling species and sites

The dragonfly, *O. albistylum speciosum*, is a common species in flat land area in Japan, and is abundant in both rural and urban areas by having territories in paddy fields, small tributaries, ponds, wetlands etc. As catching dragonfly is a popular play among children, especially boys, in Japan, we not only collected samples by ourselves but also asked people to collect samples and send them to our institute through a homepage of dragonfly project (http://www.nies.go.jp/timecaps1/dragonfly/dragonfly-top.htm). Sampling locations of *O. albistylum speciosum* is plotted in Figure 1. As we explained our intention to conduct environmental monitoring by dragonfly in the homepage, some people collected samples around 'suspected' contaminated sites, such as incinerators or waste dumping sites, while others collected them in town, remote area etc. Sampling sites fall into 3 groups, i.e. Kanto area, Kinki area and other area (Chubu, Hokuriku areas). In general, the sampling sites in Kanto and Kinki seem to be a good mixture of different categories, i.e. rural, urban and suspected contaminated sites.

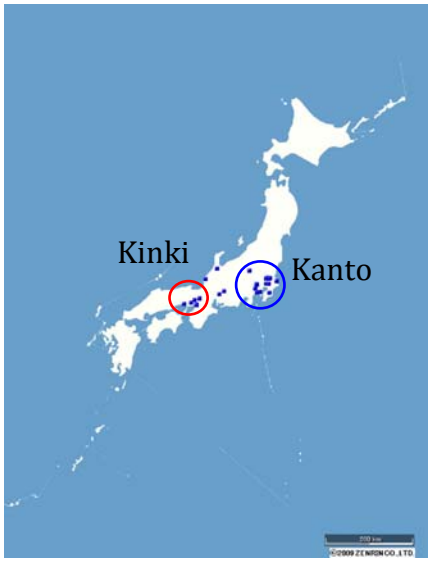

Figure 1. Sampling locations

(2) PFCs concentration and composition of 6 PFCs

PFCs were detected from all the samples collected from 31 sites, including highly contaminated sites located nearby PFOA and other fluorochemicals production factory (sites B-1, B-2). In 3 sites, dragonflies were caught and analyzed twice in different years. Concentration and compositions of PFCs in *O. albistylum speciosum* were shown in Figure 2.

In this study, we found that PFOS is a dominant component in most sites at Kanto area (>40% of total PFCs). Among perfluorinated carboxylic acid (PFCAs) in dragonfly collected at Kanto area, PFCAs with odd-numbers of carbon, i.e., PFNA and PFUnA, are higher than those with even numbers of carbon. On the other hand, PFOS composition is generally lower and PFCAs composition tend to vary much in Kinki area compared with Kanto area. It seems that longer chain PFCAs were relatively higher in composition in Kinki area than other areas.

Relatively high concentrations of PFCs were detected in several sites. Dragonflies at Site A, located near two waste incineration centers, contained highest level of PFOS (82.7 ng g^{-1}). Dragonflies at Site B located nearby fluorochemicals company, on the other hand, accumulated PFOA and PFDoA considerably.

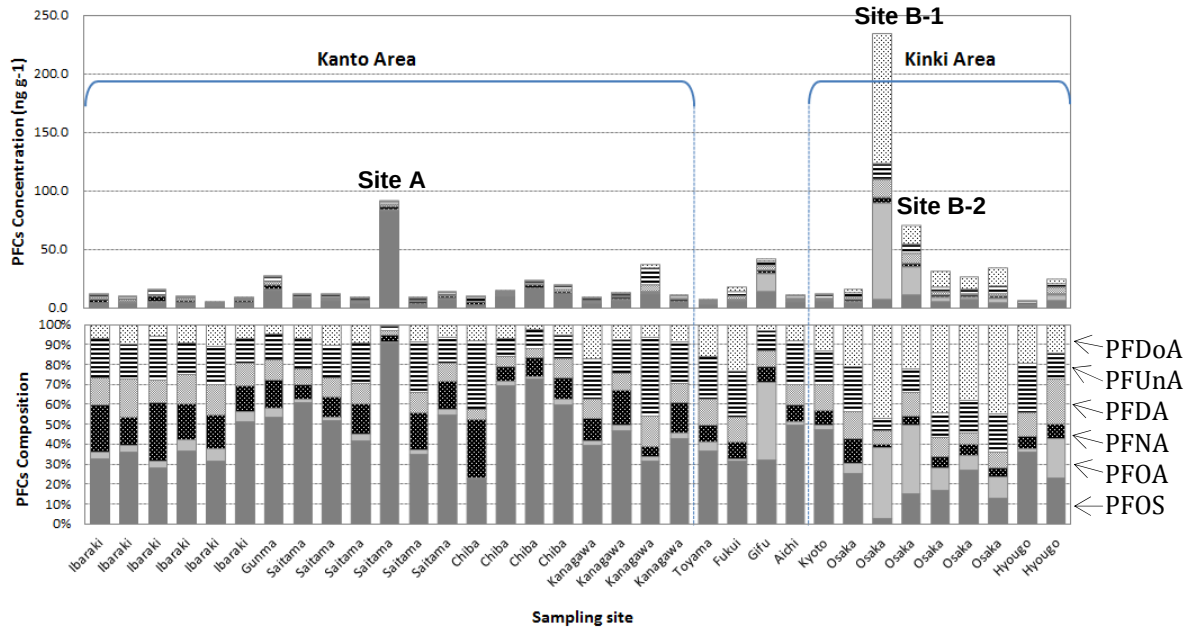

Figure 2. PFCs concentration and composition in dragonfly

(3) Comparison of PFCs compositions among sites

To reveal PFCs contamination characteristics between two densely populated areas, i.e., Kanto and Kinki, PCA was conducted using 6PFCs concentration data and5PFCAs composition data (Figure 3-6). In figures 3 and 4, circle, triangle and cross dots illustrate each sites located in Kanto, Kinki and other area.

Results of 6PFCs concentration data, which were changed in logalismic scale, were shown in Figures 3 and 5. From the result, 1st factor score seems to relate to total PFCs concentration while 2nd factor score to dominant component of PFCs, i.e., PFOS and PFNA in positive side and PFDoA in negative side. Kanto group and Kinki group separated along 2nd axis. Results of 5PFCAs composition data were shown in Figures 4 and 6. Both factor scores represent differences of carbon chain length; 1st score reflects either odd or even, while 2nd score reflects either short or long. Kanto group and Kinki group were separated along 1st axis. Dragonflies in Kanto area accumulate PFCAs with odd number of carbon, on the other hand, dragonflies in Kinki area accumulate those with even number.

According to the result of present study, it was suggested that there are differences in major source of PFCs between Kanto and Kinki. In Kanto, it is characterized by PFOS, however, the source in Kinki is dominated by PFCAs, particularly PFDoA. Among PFCAs, those having odd number of carbon dominate in Kanto, while those with even number of carbon in Kinki.

PFCAs other than PFOA were found to be accumulated in comparable or even higher levels than PFOS or PFOA in dragonfly. Generally, concentration of PFCAs with longer carbon chain are low in water, however tend to be accumulated in organisms considerably because of their high bioconcentration factors. [6,7] It is important to include PFCAs with longer carbon chain for risk assessment of wildlife in addition to PFOS and PFOA. The results of present study indicate the importance of biominitoring for appropriate risk assessment. Dragonfly monitoring is useful for this purpose.

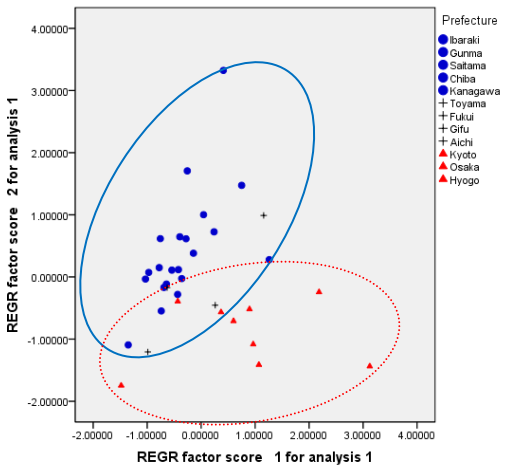

Figure 3. Results of PCA analysis (6PFCs)
Accumulative percentage (1^{st}+2^{nd}) 82.50%

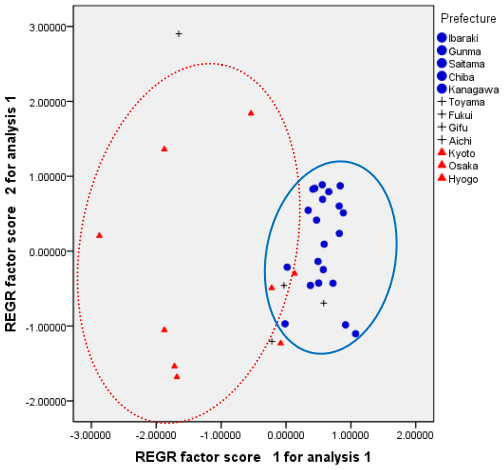

Figure 4. Results of PCA analysis (5PFCAs)
Accumulative percentage (1^{st}+2^{nd}) 74.42%

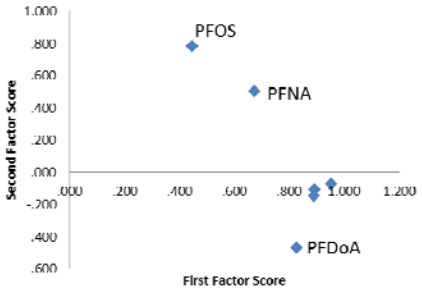

Figure 5. Plot of 1^{st} and 2^{nd} factor score (6PFCs)

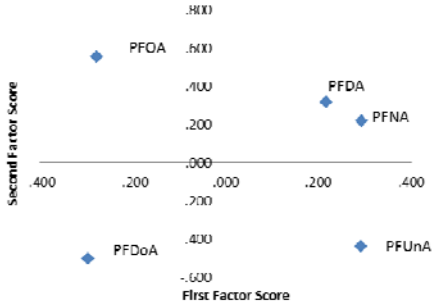

Figure 6. Plot of 1^{st} and 2^{nd} factor score (5PFCAs)

Acknowledgements:
Part of the study was supported by research funds from National Institute for the Environmental Studies and Ministry of the Environment (MOE), Japan.

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