INVESTIGATION OF PFOS POLLUTION IN THE TERRESTRIAL ENVIRONMENT OF JAPAN USING DRAGONFLY AS BIOINDICATOR ORGANISM

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Abstract

Concentrations of perfluooctane sulfonic acid (PFOS) in dragonfly samples collected from 40 sites in Japan were analyzed in order to get general idea of the PFOS pollution in terrestrial environment in Japan. Elevated levels of PFOS were found in densely populated / industrialized areas, i.e., Kanto and Kinki, as well as Hokuriku where known or suspected sources of perfluorinated compounds (PFCs) are present while their levels were much lower in less populated, remote areas. Higher levels, however, were detected in dragonflies caught at rural areas distant from known or suspected sources, i.e., factories of fluorochemical industries or semiconductor factories, suggesting the presence of new, unidentified sources in terrestrial environment. These results support our view that dragonfly is a useful tool for biomonitoring terrestrial environment.

Introduction

Perfluorinated compounds (PFCs), including perfluooctane sulfonic acid (PFOS), which was listed in ANNEX B of the Stockholm Convention at COP-4 in May 2009, have attracted much attention because of their persistent, bioaccumulative and toxic properties. However, the information on the sources and pollution status of PFCs are still in insufficient stage. It is known that biomonitoring is one of the effective methods for understanding pollution status by chemicals. In the Environmental Specimen Bank (Environmental Time Capsule program) at our institute, bivalves and fishes were selected and stored as bioindicator organism for monitoring marine environment.[1, 2]On the other hand, no particular bioindicator organism has been identified in terrestrial environment.

During the extensive survey of PFCs pollution in Japanese environment, we found relatively higher concentrations of PFCs accumulated in carnivorous species of insects or other organisms feeding on insects (such as dragonfly, mantis, spider and lizard).[3] Dragonflies are particularly interesting because of their relatively high trophic levels, wide distribution in many countries, and representation of wide spatial area due to their flying capability. Besides, according to our previous study, PFCs composition and levels in individual dragonfly

apparently reflected differences of pollution status in each habitat. [4, 5]This results support the view that dragonfly will be an appropriate bioindicator organism for monitoring PFCs in terrestrial environment.

In this study, we analyzed PFCs concentrations in dragonfly collected from 40 sites in Japan for the purpose of obtaining general view of PFCs pollution in terrestrial environment.

Materials and Methods

9 species of dragonflies were used for this study; *Sympetrum infuscatum* (Noshime tonbo, NT), *S. baccha matutinum* (Konoshime tonbo), *S. darwinianum* (Natsuakane) *S. frequens* (Akiakane, AT), *S. eroticum eroticum* (Mayutateakane), *Orthetrum albistylum speciosum* (Shiokara Tonbo, ST), *Deielia phaon* (Kofuki tonbo), *Crocothemis servilia mariannae* (Shojo tonbo, JT), *Pseudothemis zonata* (Koshiaki tonbo, KT). These species belong to the same family Libellulidae in sub order Anisoptera. Samples were collected and sent to the institute and stored in -80C freezer until the analysis.

Table.1 Details of LC condition and MSMS parameters

HPLC

Instrument	Agilent 1200
Column	Zorbax XDB C-18 (2.1×150mm, 3.5μm)
	Zorbax XDB C-8 (2.1×12.5mm, 5μm)
Mobile Phase	A: Water(10mM CH_3COONH_4)
	B: CH_3CN
Flow rate	200μl/min
Column heater	40 C
Injection Volume	10μl

Gradient schedule

Total time (min)	A (%)	B (%)
0	60	40
15	0	100
20	0	100
25	60	40
40	60	40

MS

Instrument	4000QTRAP (Applied Biosystems)
Ionization	ESI
Polarity	Negative
Ionspray voltage(V)	−4500
Nebulizer gas(psi)	40
Turbo gas(psi)	80
Temperature(C)	400

Peak Name	Analyte mass Ranges Precursor ion	Product ion	DP(V)	CE(V)
PFOS	499	80	−105	−86
$^{13}C_4$ PFOS	503	80	−105	−86
PFOA	413	369	−35	−16
$^{13}C_4$ PFOA	417	372	−35	−20
PFNA	463	419	−30	−16
$^{13}C_5$ PFNA	468	423	−30	−16
PFDA	513	469	−30	−18
$^{13}C_2$ PFDA	515	470	−30	−18
PFuDA	563	519	−45	−18
$^{13}C_2$ PFuDA	565	520	−45	−18
PFdDA	613	569	−50	−18
$^{13}C_2$ PFdDA	615	570	−50	−18

Sample (0.2~0.5g-wet) was treated with alkaline digestion (90C for 3hr in 4N-NaOH) followed by ion-pair extraction (tetrabutyl ammonium as ion-pair reagent) and hexane acetonitorile partition (Chem Elut, Varian). [6] Part of the samples with low apparent recoveries was clean-upped further with Oasis-HLB (Waters) and

Oasis-MCX (Waters). Analysis of PFOS was performed together with other perfluorocarboxylic acids (PFCAs) by high performance liquid chromatography and tandem mass spectrometry (LC/MS/MS, 4000QTRAP, Applied Biosystems) Quantification was conducted based on the peak area of straight-chain PFOS, i.e., perfluoro-*n*-octane sulfonic acid, alone by using PFAC-MXB and $^{13}C_4$-PFOS (Wellington Lab, Inc) as standard and surrogate, respectively.

Results and Discussion

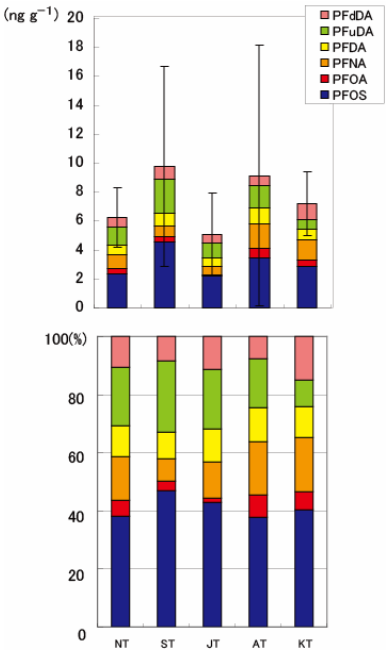

Fig. 1 PFCs levels and compositions in dragonfly

PFCs levels and compositions in different dragonfly species collected in the same location

5 species of dragonfly, i.e., *S. infuscatum* (NT; n=46), *S. frequens* (AT; n=7), *O. albistylum speciosum* (ST; n=8), *C. servilia mariannae* (JT; n=8), *P. zonata* (KT; n=3), were collected at our institute, and the results of the analysis were summarized in Fig.1.

Bar graph in upper side of the figure shows geometric mean of PFCs concentration in each species. Several to ten ng g^{-1} levels of total PFCs were detected in all the dragonflies analyzed in this study with PFOS as the major component. (40%; see lower side) These dragonflies are rather similar in size and show similar lifestyle in general, i.e., living around still water in lowlands, such as pond and paddy fields, except for *S. frequens*, which is well known of its long-range migration between lowlands, its birth / breeding place, and uplands, where they spend a couple of months for foraging but postponing maturation.[7] *O. albistylum speciosum*, on the other hand, may be in a higher trophic level than the other species because *O. albistylum speciosum* sometimes catch and eat other dragonflies. Although higher average levels and larger variations observed in *O. albistylum speciosum* and *S. frequens* may reflect these characteristics, the differences are not significant and PFCs compositions were similar among the 5 species collected in our institute, suggesting that bioaccumulation properties of PFCs may not vary much among different species. Therefore we proceeded the comparison of data obtained from several different species collected in various places in Japan in order to reveal general pollution status of PFCs in Japan.

Geographical distribution of PFOS in dragonfly

7 dragonfly species, i.e., 5 species in genus *Sympetrum* and 1 each in *Orthetrum* and *Deielia* respectively, were collected from 40 sites in Japan and were analyzed their PFOS concentrations. Three individual samples were analyzed and averaged in elevated sites while one in others.

Distribution of PFOS levels in dragonfly were shown in Fig. 2. PFOS levels were much higher in densely populated / industrialized areas, such as Kanto and Kinki, than others. Highest levels of PFOS were detected in Hokuriku. Geographical distribution of PFOS in dragonfly looked similar to the distribution in bivalves. i.e., elevated levels of PFOS had been detected in bivalves collected at Tokyo Bay in Kanto, Osaka Bay in Kinki, and Ishikawa in Hokuriku, suggesting the presence of large PFCs sources in these areas. In fact, there are some known or suspected sources in the areas, such as fluorochemical industries and semiconductor factories. By the closer look of the sampling sites, however, it was found that elevated levels of PFOS were detected in dragonflies living in rural sites distant from the above factory sources rather than those living nearby the sources, suggesting the presence of new, unidentified sources in terrestrial environment.

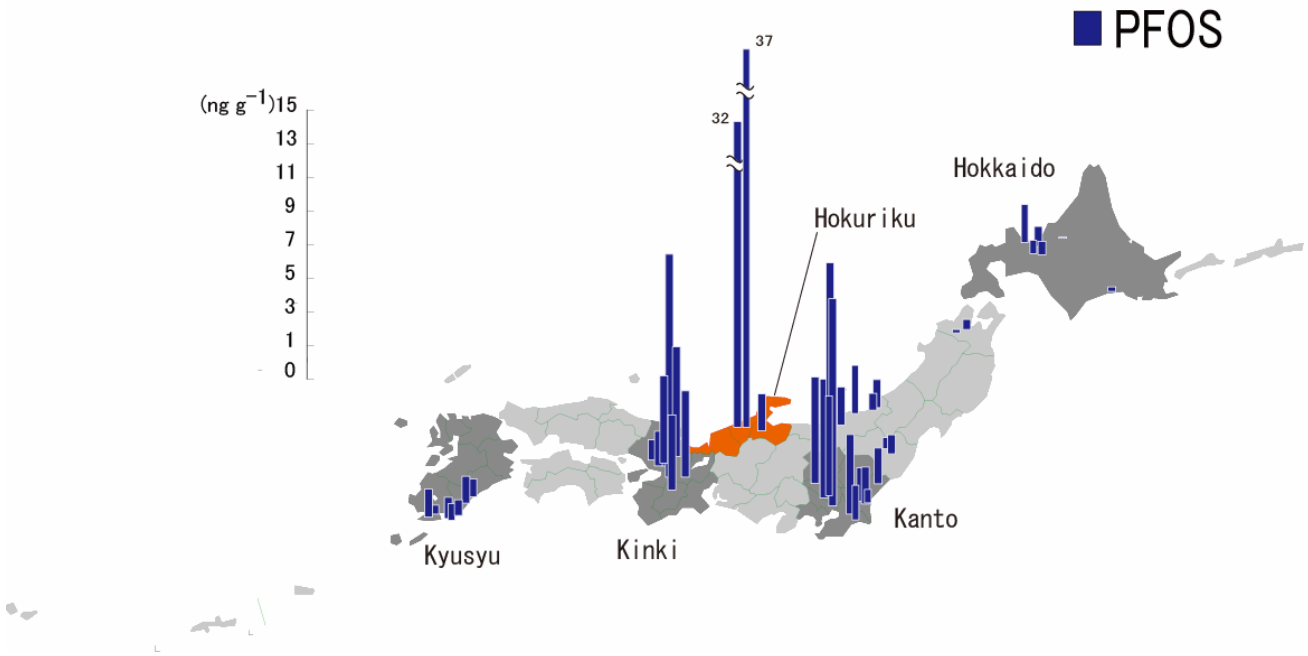

Fig.2 Geographical distribution of PFOS in dragonfly

Comparison of PFOS levels between Dragonfly（S. infuscatum）and surface water

Detailed mechanisms of PFCs accumulation in dragonfly have not been clarified yet, though it is suspected that PFCs in surface water will be accumulated in dragonfly through food web. Concentration ratio of surface water and dragonfly, *S. infuscatum*, were analyzed at 11 sites around our institute, where no particular sources of PFCs were identified, in order to get idea of apparent bioaccumulation factor (BAF) of PFOS in dragonfly. Concentrations of PFOS in dragonfly were between 0.49~2.9 ng g^{-1}, while those of water were between 0.42～

3.1 pg ml^{-1}. Geometric mean of PFOS ratios between *S. infuscatum* and water was 1300, similar to BCF in other organisms, such as bivalves[1] and rainbow trout.[8]

Conclusion

Dragonfly species analyzed in this study belong to the same family, are widely distributed in Japan and show relatively similar lifestyle. Furthermore, the result of this study showed that dragonflies accumulate PFCs with BAF values comparable to BCF in marine bioindicator organisms, such as bivalves and fishes, and that bioaccumulation properties of PFCs were similar among species. Therefore, we took a combination approach of using several similar species together in order to reveal the status of PFCs pollution in terrestrial environment of Japan.

PFCs levels in dragonfly were considerably higher in densely populated / industrialized areas where known or suspected PFCs sources located. However, distances between the sampling sites and the suspected sources were longer than their expected flying distance, suggesting the presence of new, unidentified sources in terrestrial environment.

These results support our view that dragonfly is a useful tool for biomonitoring. Several species, such as in genus *Sympetrum*, *Orthetrum* or *Crocothemis*, are widely distributed in Asia and other countries, and may be useful for regional / global monitoring. Further research will be necessary to establish biomonitoring method of terrestrial environment by using dragonfly as bioindicator organism.

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