DISTRIBUTION OF FLUORINATED SURFACTANTS IN BIVALVES ALONG COASTLINE OF JAPAN

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Abstract
Perfluorooctanesulfonate(PFOS) and five perfluorocarboxylates (PFCAs; C8~C12) levels in bivalve samples collected at 64 sampling sites were analyzed by the combination of strong alkaline digestion and LC/MS/MS in order to reveal their pollution status along coastline of Japan.
PFCAs have been used in the production of fluoropolymers and also for mold release agents. Fluoropolymers and other plastics/elastomers contaminated with PFCAs were eliminated from sample preparation process in order to attain good limits of detection (LOD) of 0.01~0.02 ng g^{-1} wet.
The concentration ranges and detection frequencies of PFOS and PFCAs were as follows; PFOA: <0.02 to 4.71 ng g^{-1}-wet (58/64), PFNA: <0.02 to 4.49 ng g^{-1}-wet (63/64), PFDA: <0.02 to 5.19 ng g^{-1}-wet (50/64), PFuDA: <0.01 to 7.19 ng g^{-1}-wet (64/64), PFdDA: <0.01 to 8.37 ng g^{-1}-wet (61/64)and PFOS: <0.01 to 3.80ng g^{-1}-wet (57/64),respectively. There are only a few sampling sites where more than 1ng g^{-1} of either PFOS or PFCAs were detected. In most sites, concentrations of PFCAs with odd number of carbons were higher than those with even number of carbons.
BCF in bivalves were estimated to be 700 for PFOS and 11 for PFOA, respectively. These data were very similar to those for rainbow trout.

Introduction
Fluorinated surfactants such as PFOS and PFCAs (Fig.1) have been actively investigated in various environmental samples due to their persistent, bioaccumulative and toxic properties.[1] Bivalve samples have been used as suitable species for biomonitoring of coastline pollution. Previously we developed and established analytical method of PFOS and PFCAs based on strong alkaline digestion with sufficiently low detection limit for their analysis even in remote area.[2] Using this method, we found, in bivalves, the presence of PFOS and PFOA derivatives which could liberate their free forms only after alkaline digestion.
The purpose of this study is to investigate PFOS and PFCAs levels in bivalves collected at 64 sampling sites based on strong alkaline digestion and to reveal their pollution status along coastline of Japan.

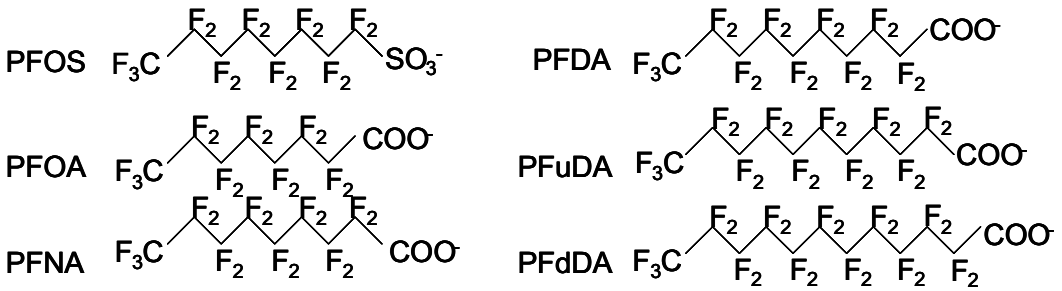


Fig. 1 The structure of PFOS and PFCAs

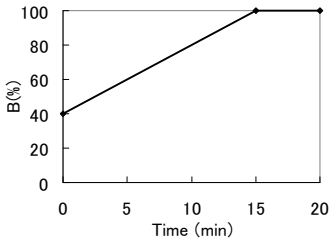
Materials and Methods
Mussel samples (*Mytilus galloprovincialis* and *Septifer virgatus)* and oyster samples (*Saccostrea mordax*) were collected from 64 locations along coastline of Japan. The samples were deshelled and frozen by liquid nitrogen on site or in the laboratory, transported and stored at frozen temperature, and cryo-homogenized as reported by Yoshikane et.al.(2006)[2]. Sample was treated with alkaline digestion (90 °C for 3hr in 2N NaOH) followed by ion-pair extraction (tetrabutyl ammonium as ion-pair reagent) and hexane acetonitorile partition (Chem Elut, Varian).
Analysis of PFOS and 5 PFCAs, i.e., perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA),

perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFuDA) and perfluorododecanoic acid (PFdDA), was performed by high performance liquid chromatography and tandem mass spectrometry (LC/MS/MS, API4000Qtrap, Applied Biosystems). (Table. 1) $^{13}C_4$ labeled PFOA, $^{13}C_4$ labeled PFOS, $^{13}C_2$ labeled PFDA and $^{13}C_2$ labeled PFdDA (Wellington. Lab. Inc.) were used as surrogate.

Table.1 Analytical method for PFOS and PFCAs using LC/MS/MS

HPLC		MS	
Instrument	Agilent 1100	Instrument	API4000Qtrap (Applied Biosystems)
Column	Zorbax XDB C-18 (2.1×150mm, 3.5μm)	Ionization	ESI
	Zorbax XDB C-8 (2.1×12.5mm, 5μm)	Polarity	Negative
Mobile Phase	A: Water(10mM CH_3COONH_4)	Ionspray voltage(V)	−4500
	B: CH_3CN	Nebulizer gas(psi)	40
Flow rate	200μl/min	Turbo gas(psi)	80
Column heater	40℃	Temperature(℃)	400
Injection Volume	10μl		

Gradient schedule



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

Results and Discussion

The concentrations of PFOS and PFCAs in bivalves were generally low, in the range of ng g^{-1} wet tissue or lower. Therefore, reduction of the blank level is a prerequisite for the PFOS and PFCAs analysis in bivalves. PFCAs have been used for processing aid in the production of fluoropolymers and also for mold release agents, thus fluoropolymers and other plastics/elastomers contaminated with PFCAs were carefully eliminated from sample preparation process. The blank levels, recoveries of surrogates for blank and LOD calculated from the fluctuation of the blank were shown in Table.2.

Table.2 Analytical performance of the method

	PFOS	PFOA	PFNA	PFDA	PFuDA	PFdDA
Blank level (ng g^{-1})	−	0.03	0.03	0.02	0.02	0.01
Recovery (%)	74	95	−	85	−	81
LOD (ng g^{-1})	0.01	0.02	0.02	0.02	0.01	0.01

*Blank levels were provided that 2 g of wet tissue was analyzed

Recoveries of target compounds during pretreatment procedure were assessed by using isotope labeled compounds and were found to be satisfactory, i.e., 58 to 133% for PFOS, 60 to 121% for PFOA, 73 to 127% for PFDA and 56 to109% for PFdDA, respectively, except for three samples in which PFdDA recoveries were low (10%, 41% and 15%, respectively). The concentration ranges and detection frequencies of PFOS and PFCAs were as follows; *PFOS: <0.01 to 3.80ng g^{-1}-wet (57/64), *PFOA: <0.02 to 4.71 ng g^{-1}-wet (58/64), PFNA: <0.02 to 4.49 ng g^{-1}-wet (63/64), *PFDA: <0.02 to 5.19 ng g^{-1}-wet (50/64), PFuDA: <0.01 to 7.19 ng g^{-1}-wet (64/64) and *PFdDA: <0.01 to 8.37 ng g^{-1}-wet (61/64), respectively. (Compounds with * were corrected by surrogate recoveries)

Geographical distribution of PFOS was shown in Fig.2.

There are only a few sampling sites where more than 1ng g^{-1} of either PFOS or PFCAs were detected. Those high concentration sites were located not only along enclosed coastal sea surrounded by densely populated or industrial areas, such as Tokyo Bay, Osaka Bay and Dokai Bay, but also in the areas faced to open sea, including

the area around Kanazawa, where water ventilation was good.
In most sites, concentrations of PFCAs with odd number of carbons were higher than those with even number of carbons (Fig.3). Concentration of PFNA and PFuDA were higher than 0.1ng g^{-1} in ca.75% of the sampling sites, while those of PFOA and PFDA were less than 0.1ng g^{-1} in ca.70% of the sites. PFdDA, however, tended to be higher among PFCAs with even number of carbons. As a result the number of sites above 0.1ng g^{-1} was nearly equal to those below 0.1 ng g^{-1}.

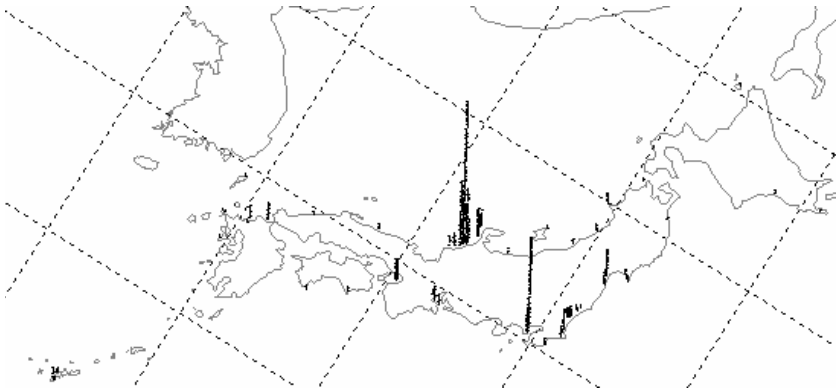


Fig.2 Distribution of PFOS in bivalves

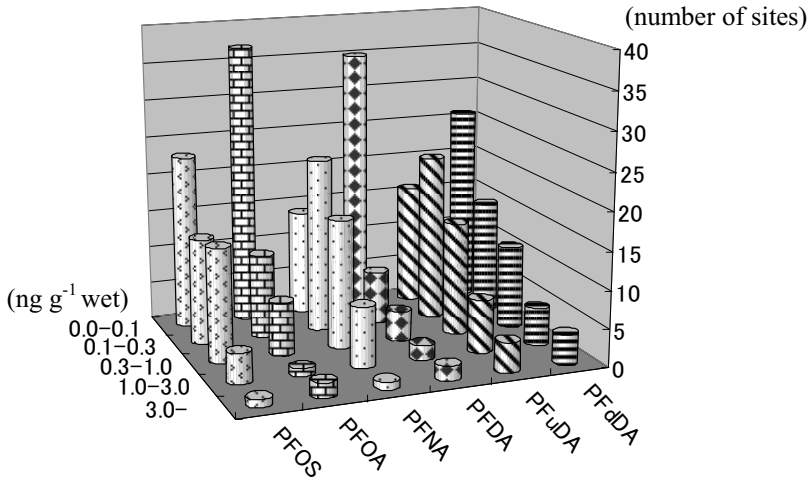


Fig.3 Histogram of PFOS and PFCAs in bivalves

Among high concentration areas, area around Kanazawa was specific, i.e., all the analytes were detected in more than 1ng g^{-1}levels. Their sources in Kanazawa area are not clear, but might be either fluorinated surfactants manufacturing factory or textile industries located in the area..
It was reported that bioconcentration factors (BCF) for PFCAs in rainbow trout increased by a factor of eight for each additional carbon in perfluoroalkyl chain between 8 and 12 carbons. In this study, BCF in bivalves were estimated to be 700 for PFOS and 11 for PFOA, respectively. These data were very similar to those for rainbow trout. Provided that BCF values of other compounds in bivalves were also similar to those in rainbow trout with similar increasing trend by the addition of carbon, it is interesting to note that the highest concentrations of each PFCAs in bivalves collected form all over Japan are in a same level in spite of their estimated large difference in BCFs. It is necessary to reveal their sources, fate and global dynamics of fluorinated surfactants for

understanding their pollution status and establishing their proper chemical management system.

References

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