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Original Article

Bioconcentration of perfluorinated compounds in wild medaka is related to octanol/water partition coefficient

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ABSTRACT — Perfluorinated compounds (PFCs) have been used widely, detected worldwide in the environment, and have accumulated highly in animals. As far as we know, there have been no reports which relate the PFC concentration in wild animals to the physicochemical properties. Therefore, we measured the concentrations of 15 currently available PFCs (perfluorocarboxylic acids with x carbons: Cx, perfluorosulfonic acids with x carbons: CxS) in medaka and the environmental water where medaka live. Samples were obtained from 7 points in Japan (Iwate, Ibaraki, Niigata, Hyogo, Yamaguchi, Ehime, and Nagasaki) from July to September in 2013. Twenty to forty medaka were collected from each point, as well as 2 L of water in a clean PET bottle. PFCs were extracted and concentrated using a solid-phase cartridge, and were measured by LC/MS/MS. The medaka samples were treated individually. C5-C9 and C8S were detected mainly in the water, C11-C13 and C8S were detected mainly in medaka. C8S was always detected in high concentrations in the water and medaka. The bioconcentration factors (BCFs) of PFCs were calculated from PFC concentrations of the water and the medaka. The BCFs of C8-C11 were increased exponentially with the length of carbon chain. The BCF of C8S (approx. 5,500) was far greater than C8 (approx. 330) or C9 (approx. 480). However, the BCFs of C8-C11 and C8S tended to increase in proportion with octanol/water partition coefficient (log K_{ow}).

Key words: Perfluorinated compound (PFC), Medaka, Bioconcentration factor (BCF), log K_{ow}

INTRODUCTION

Perfluorinated compounds (PFCs) are chemically stable and very useful surfactants, and have been used widely. Among them, perfluorooctan sulfonate (PFOS, C8S) is a typical compound of perfluorosulfonic acids (PFSAs), and perfluorooctanoic acid (PFOA, C8) is a typical compound of perfluorocarboxylic acids (PFCAs). C8S has been used in hydraulic oil for planes, fire extinguishers, and etching agents for metal working, while C8 has been used as an additive for polytetrafluoroethylene composition.

C8S was first detected in the serum of 3M employees in 1999 (Olsen *et al.*, 1999), and has also been detected in mammals, birds, fish, and surface waters (Giesy and Kannan, 2001; Stahl *et al.*, 2014; Nakayama *et al.*, 2005; Kannan *et al.*, 2001).

PFCs have a certain characteristic known as persistent organic pollutants (POPs), and C8S and its salts became the subject of worldwide regulations. In May 2009, C8S and its salts were additionally registered in Annex B of the Stockholm Convention on Persistent Organic Pollutants. In Japan, they were additionally registered as Class I Specified Chemical Substances in "The Chemical Substances Control Law" in 2010, and their production or importation have been regulated. As for C8, it was registered with C8S by this Japanese law in 2002. Now it is registered as a general chemical substance. Although regulation of C8 by law has not begun yet, through the 2010/2015 PFOA Stewardship Program in 2006, in which the makers committed to eliminate emissions to the environment and product content by 2015, USEPA and eight major makers of PFOA have started regulating C8 to reduce its use.

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While such these regulations are in effect, PFCs with different carbon numbers from C8S or C8 began to be used. In Japan, Perfluorohexanoic acid (PFHxA, C6) with six carbons has been produced in the Kinki district and detected in the environment. Not much is known about the fate of alternative substances, which includes C6 in the environment. Although there have been several reports which compared the concentration of PFCs in wild animals with their environment (Shirasaka and Kadokami, 2014; Ahrens *et al.*, 2015; Zhou *et al.*, 2013), as far as we know, there have been no reports relating the bioconcentration factor (BCF) of PFCs to their physicochemical characteristics.

In this study, we measured PFC concentrations in wild medaka and its environmental water. There were 15 congeners of PFCs we measured, including C8S, C8, and non-regulated PFCs. In this study, we found correlations in PFC concentrations between the water and the medaka, and we first related the BCFs with their physicochemical properties.

MATERIALS AND METHODS

Target PFCs for analysis

We measured 15 congeners of PFCs: PFCAs of C5-C14 and PFSAs of C4S, C6S, C7S, C8S, and C10S (Table 1).

Samples

We selected medaka (killifish) as a biological sample, a widely used sample for various studies because they can be found all over Japan and can be obtained easily. We collected the environmental water in which medaka live. Samples were obtained from July to September in 2013. Seven sampling points in Japan, which were intended to be geographically equal, were selected (Iwate, Ibaraki, Niigata, Hyogo, Yamaguchi, Ehime, and Nagasaki) (Fig. 1). The water samples were collected from each point in a 2 L PET bottle which has been cleaned 3 times with methanol, and approximately 20-40 samples of medaka were collected.

Table 1. Target PFCs and monitor ions (m/z) for analysis.

compound	abbreviation	m/z
Perfluoropentanoic acid	PFPeA(C5)	263 → 219
Perfluorohexanoic acid	PFHxA(C6)	313 → 269
Perfluoroheptanoic acid	PFHpA(C7)	363 → 319
Perfluorooctanoic acid	PFOA(C8)	413 → 369
Perfluorononanoic acid	PFNA(C9)	463 → 419
Perfluorodecanoic acid	PFDA(C10)	513 → 469
Perfluoroundecanoic acid	PFUdA(C11)	563 → 519
Perfluorododecanoic acid	PFDoA(C12)	613 → 569
Perfluorotridecanoic acid	PFTrDA(C13)	663 → 619
Perfluorotetradecanoic acid	PFTeDA(C14)	713 → 669
Perfluorobutanesufonate	PFBS(C4S)	299 → 80
Perfluorohexanesufonate	PFHxS(C6S)	399 → 80
Perfluoroheptanesufonate	PFHpS(C7S)	449 → 80
Perfluorooctanesufonate	PFOS(C8S)	499 → 80
Perfluorodecanesufonate	PFDS(C10S)	599 → 80

Chemicals

Commercially available reagents were used. Sodium carbonate (special grade), tert-butyl methyl ether (MTBE; for LC/MS), 25% ammonia solution (super special grade), ammonium acetate (special grade), acetic acid (for LC/MS), sodium acetate (special grade), methanol (for LC/MS), acetonitrile (for LC/MS) were obtained from Wako Pure Chemical Industries (Osaka, Japan), and tetrabutylammonium hydrogen sulfate (99%, for HPLC) was provided by ACROS ORGANICS (Geel, Belgium).

Standard chemicals of PFCs were used for calibration. PFC-MXA (mixture of native perfluoroalkylcarboxylic acids), PFS-MXA (mixture of native perfluoroalkylsulfonates) were obtained from Wellington Laboratories (Ontario, Canada).

For surrogate compounds, MPFAC-MXA (mixture of mass-labelled perfluoroalkylcarboxylic acids and mass-labelled perfluoroalkylsulfonates) from Wellington Laboratories was used.

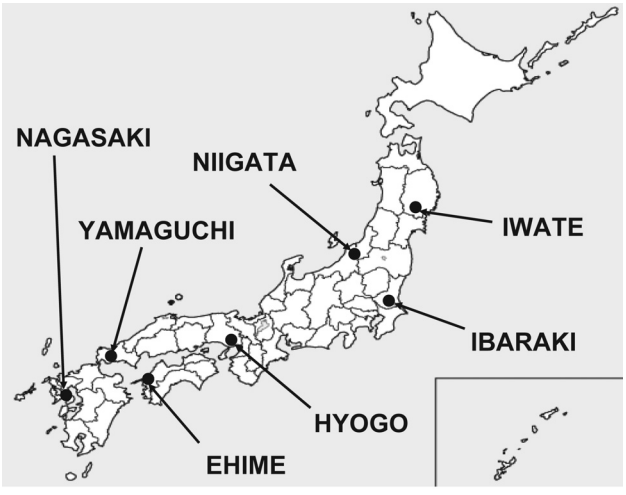

Fig. 1. Sampling points of environmental samples.

Pretreatment of the water samples

The concentration of PFCs in the water samples was very low, so we concentrated them with a concentrator. The concentrator (Sep-Pak Concentrator Plus; Waters Corp., Milford, MA, USA) and a solid phase cartridge (Oasis WAX Plus type 225 mg; Waters Corp.) were used to concentrate the water samples from 2 L to 1 mL. This procedure is shown in Fig. 2. Surrogate compounds were added to the 2 L PET bottle water sample first. The water sample was controlled to approximately pH 4 with a few drops of formic acid. The solid phase cartridge was conditioned with 5 mL of 0.1% ammonia solution-methanol, methanol and acetate buffer successively, where it was then set on the concentrator. The water sample was loaded into the cartridge on the concentrator at a pace of 10 mL/min. The empty PET bottle was washed with 20 mL of ultrapure water, acetate buffer, and methanol successively. The washing was also loaded into the cartridges. PFCs were eluted from the cartridge with 5 mL of 0.1% ammonia solution-methanol and concentrated using N_2 gas to approximately 0.5 mL, where they were then reconstituted to 1mL by methanol. The prepared samples were measured by LC/MS/MS.

Pretreatment of the medaka samples

PFCs in the medaka samples were extracted with organic solvent, and concentrated by using a solid phase cartridge. The medaka were individually weighed and treated. The aliquot of 2.5 mL of sodium carbonate solution (0.25 M) was added to the medaka samples. The samples were stirred or shaken, then left to stand overnight. Surrogate compounds were added to the samples, together with 1 mL of tetrabutylammonium hydrogen sulfate (0.5 M). After stirring, 5 mL of tert-butyl methyl ether (MTBE) was added. The samples were shaken for 2 min and centrifuged (3,000 rpm) for 10 min. The MTBE layers were moved to another sample tube, and the remaining water layers were re-treated similarly. The resulting MTBE layers were again moved, and combined with the former MTBE layers. The MTBE was purged with N_2 gas, and 2 mL of 2% formic acid-methanol solution (1 mL twice) was added to the dried samples. The samples were dissolved and mounted (1 mL twice) to solid phase cartridges conditioned with 5 mL of 0.1% ammonia water-methanol solution, 5 mL of methanol and 5 mL of 2% formic acid. The cartridges were also successively washed with 2 mL of methanol and

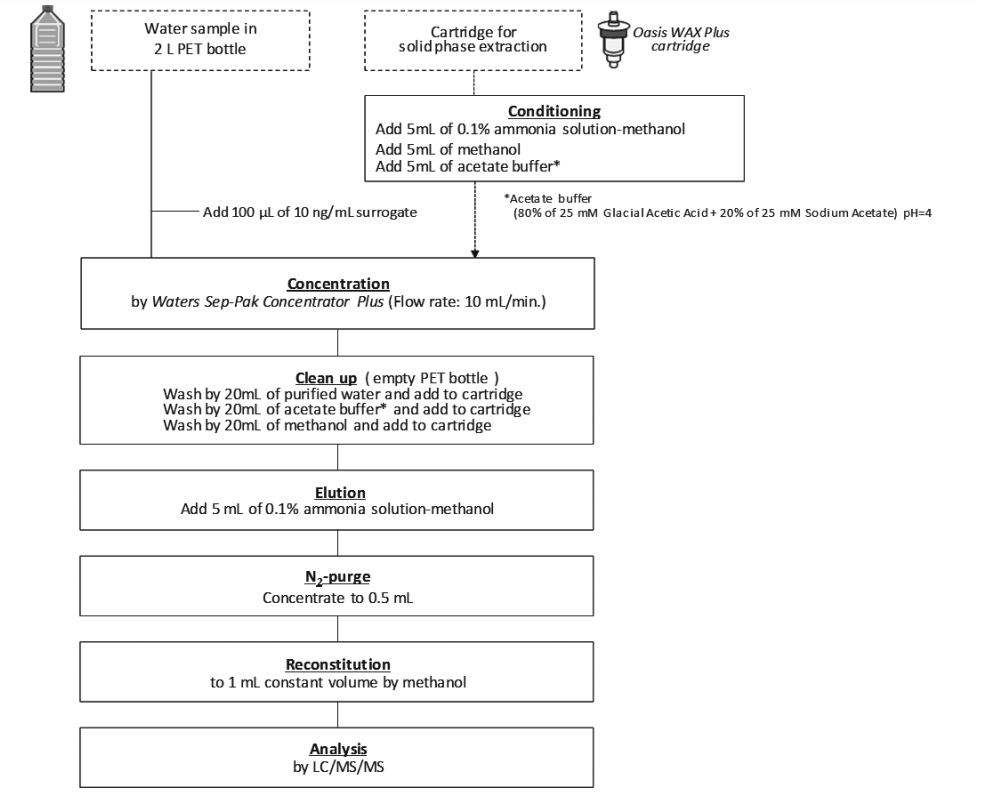

Fig. 2. Flow chart of the pretreatment procedure for water samples.

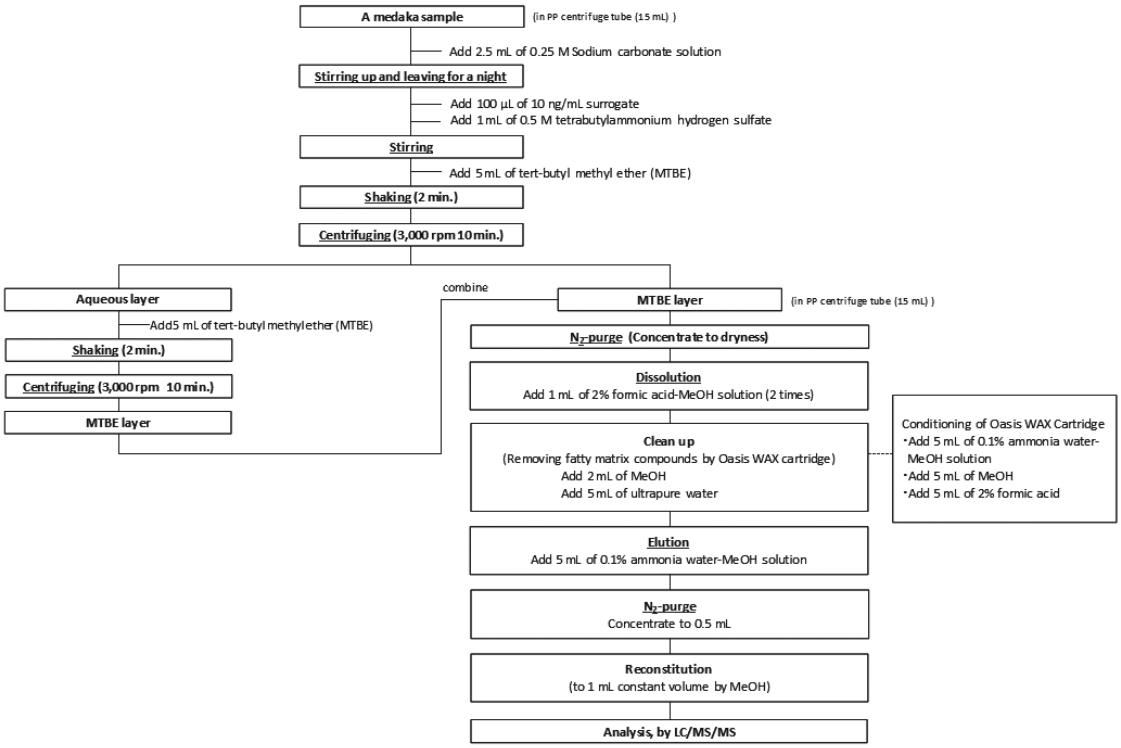

Fig. 3. Flow chart of the pretreatment procedure for medaka samples.

5 mL of ultrapure water. The target PFCs were eluted from the cartridge with 5 mL of 0.1% ammonia water-methanol solution, concentrated to approximately 0.5 mL using N_2 gas, and reconstituted to 1 mL by methanol. The prepared samples were measured by LC/MS/MS. This procedure is shown in Fig. 3.

Instruments

Due to repeated measurement of PFCs, experimental instruments may have been contaminated. To eliminate the PFCs contaminants, all experimental instruments that were used to extract and concentrate PFCs were washed thoroughly 3 times with methanol. The concentrator was washed with methanol for 2 hr or more. It was then rinsed with ultrapure water for no less than 2 hr with its respective sole circulating operation.

Analyzing devices

Prepared samples were measured by the LC/MS/MS system, which consists of HPLC (1200 series; Agilent Technologies, Santa Clara, CA, USA) and mass spectrometer (6410; Agilent Technologies). The conditions for analyzing devices were as follows;

HPLC : Agilent Technologies 1200 series

guard column :	ZORBAX Eclipse Plus C18 (2.1∗30 mm 1.8 µm) (Agilent Technologies)
analysis column :	ZORBAX Eclipse Plus C18 (2.1∗100 mm 1.8 µm) (Agilent Technologies)
column temperature :	40°C
mobile phase :	(A) 10 mM Ammonium acetate (B) Acetonitrile

gradient (linear)

time (min)	0	4	20	28
(B) (%)	25	25	75	75

post time 17 min (Acetonitrile 95% to 25%)

flow :	0.2 mL/min
injection volume :	10 µL

MS : Agilent Technologies 6410

ionization :	ESI (Negative)
gas temperature :	350°C
gas flow :	5 L/min
nebulizer :	60 psi
capillary voltage :	2000 V
monitor ion (m/z) :	see Table 1

Calibration, LOD and LOQ

A standard sample for each of the 15 PFCs was prepared for calibration on a range of 0.05-80 ng/mL. For accurate measurement, two calibration curves were prepared for each PFC: a low concentration curve of 0.05-5.0 ng/mL and a high concentration curve of 5.0-80 ng/mL. They showed high linearity, and their coefficients of correlations were above 0.995 for all 15 PFCs. The S/N ratio of the lowest concentration of each sample was close to or above 3, therefore the limit of detection (LOD) was set to 0.05 ng/mL for each PFC. The S/N ratio of 0.1 ng/mL concentration was close to or above 10 for each PFC, thus the limit of quantitation (LOQ) was set to 0.1 ng/mL.

RESULTS

PFCs concentration of the water samples

As shown in Table 2, PFCAs of C5-C10 were detected in all of the water samples, with 73.0 pg/mL for C7 as the highest total concentration. PFCAs in C11-C14 were hardly detected or under the limit of detection (ND). As for PFSAs, C8S was detected in all of the water samples, with 14.0 pg/mL as the highest total concentration. The others were detected only in some areas at low concentrations. The highest concentration among PFCs was detected in Hyogo with 34 pg/mL for C6. The total amount of PFCs was the highest in Hyogo, followed by Ehime.

PFCs concentration of the medaka samples

The mean concentrations (arithmetical mean ± S.E.) of PFCs in medaka samples are shown in Table 3. Long-chain-PFCAs (C11-C13) and C8S were detected at high concentrations in almost all of the medaka samples. Among PFCAs, C11 showed the highest total concentration with 66 ng/g, while C8S was the highest with 26 ng/g among PFSAs.

Short-chain-PFCAs such as C5-C8 were detected in some samples at low concentrations, while they showed high concentrations in all of the water samples. In the Hyogo sample, C8S was detected with the highest concentration at 13 ng/g, and was approximately 2-14 times higher than the other samples. C8S was detected in both the water and medaka samples. The total amount of PFCs was the highest in Nagasaki, followed by Hyogo.

Relation between the water and the medaka

C8-C10 and C8S were detected in both the water and medaka samples, so the relation in the concentrations between water and medaka of each of those PFCs was examined by scatter graph (Fig. 4). The concentrations of C8S, C9 and C10 in the medaka increased with those of the water, with the coefficients of correlation between them being 0.99, 0.96 and 0.86, respectively. The coefficients of correlation for C9 and C10 were 0.99 and 1.00 respectively, after eliminating the unexpectedly high Nagasaki sample data. However, C8 showed no clear relation between water and medaka concentrations, with the coefficient of correlation of 0.43.

Bioconcentration factor of PFCs and carbon number

We calculated the BCFs of C8, C9, C10, C11, and C8S, which were detected at high rates in both the water and medaka samples. Each BCF was calculated from the PFC concentration ratio of the mean medaka concentration and the mean water concentration. BCFs were approximately 330 (C8), 480 (C9), 8,700 (C10), 110,000 (C11), and 5,500 (C8S). As shown in Fig. 5, the larger the carbon numbers of the PFC, the greater the BCFs are among the PFCAs. The BCF of C8S was far greater than the BCF of C8 with the same carbon number.

BCF and log K_{ow}

The log K_{ow} values of PFCs have been reported as 4.30 (C8), 4.84 (C9), 5.30 (C10), 5.76 (C11), and 5.25 (C8S), respectively (Arp *et al*., 2006). As shown in Fig. 6, BCF of the PFC was related to the log K_{ow} with the coefficient of determination of 0.90.

DISCUSSION

The typical PFCs of C8 and C8S, of which production had been stopped, were still detected in the water. Measurement of the concentrations in the sediment samples of these PFCs is necessary in order to understand the environmental fate of these PFCs more thoroughly.

New PFCs which are not regulated were also detected. C6 was detected at a high concentration in Hyogo's water sample, which indicates the dominant source of the new PFC may be located in or near Hyogo.

The reason for the unexpectedly high concentration in the Nagasaki medaka samples compared to those in the water samples may be due to the fact that the medaka had been exposed to water with higher concentrations before the sampling. The reason for why C8 showed no clear relation between water and medaka concentrations is still unknown and should be clarified.

The BCF of the PFC was related to the log K_{ow} for wild medaka. The BCF and log K_{ow} of PFCA were both related to the carbon numbers. Therefore, C5-7 may not concentrate in the biological body because of a short carbon

Table 2. PFCs concentration of environmental water samples (pg/mL).

Sampling points	Perfluorocarboxylic acids										Perfluorosulfonates					total
	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUdA	PFDoA	PFTrDA	PFTeDA	PFBS	PFHxS	PFHpS	PFOS	PFDS	
	(C5)	(C6)	(C7)	(C8)	(C9)	(C10)	(C11)	(C12)	(C13)	(C14)	(C4S)	(C6S)	(C7S)	(C8S)	(C10S)	
Iwate	1.8	0.2	0.5	0.4	0.1	0.1	ND	ND	ND	ND	ND	ND	ND	0.1	ND	3.1
Ibaraki	10.5	6.0	5.7	4.9	1.4	0.2	0.1	ND	ND	ND	0.2	0.2	ND	0.1	ND	29.4
Niigata	0.6	2.5	4.4	2.1	0.9	0.2	0.1	ND	ND	ND	0.8	0.1	ND	0.4	ND	12.2
Hyogo	24.7	34.2	26.0	33.3	13.9	3.8	3.1	0.3	0.1	ND	2.0	2.1	0.2	7.7	ND	151.3
Yamaguchi	0.3	0.9	2.1	1.5	0.2	0.1	ND	ND	ND	ND	0.1	ND	ND	0.3	ND	5.4
Ehime	13.0	8.2	25.3	16.9	2.0	0.4	0.1	ND	ND	ND	2.4	0.6	0.1	4.6	ND	73.7
Nagasaki	4.8	3.3	8.8	6.5	1.0	0.1	0.1	ND	ND	ND	0.6	0.5	ND	0.9	ND	26.5

ND : below LOD

Table 3. PFCs concentration of medaka samples (ng/g).

Sampling Points		Perfluorocarboxilates										Perfluorosulfonates					total
		PFPeA (C5)	PFHxA (C6)	PFHpA (C7)	PFOA (C8)	PFNA (C9)	PFDA (C10)	PFUdA (C11)	PFDoA (C12)	PFTrDA (C13)	PFTeDA (C14)	PFBuS (C4S)	PFHxS (C6S)	PFHpS (C7S)	PFOS (C8S)	PFDS (C10S)	
Iwate	(n=30)	ND	ND	ND	0.2 ± 0.06	0.2 ± 0.06	0.2 ± 0.05	0.7 ± 0.08	0.2 ± 0.05	0.4 ± 0.06	ND	ND	ND	ND	1.4 ± 0.18	ND	3.3
Ibaraki	(n=22)	ND	ND	ND	0.5 ± 0.17	0.2 ± 0.05	0.8 ± 0.17	4.8 ± 0.84	6.5 ± 0.98	6.1 ± 0.83	0.8 ± 0.12	ND	ND	0.0 ± 0.02	1.1 ± 0.22	ND	20.9
Niigata	(n=29)	0.4 ± 0.16	ND	0.2 ± 0.11	1.3 ± 0.34	0.0 ± 0.02	0.8 ± 0.18	9.3 ± 1.62	7.9 ± 1.39	6.7 ± 0.88	0.8 ± 0.13	ND	ND	0.2 ± 0.17	1.0 ± 0.23	ND	28.7
Hyogo	(n=21)	ND	ND	ND	2.0 ± 0.33	2.4 ± 0.26	7.6 ± 0.53	16.2 ± 1.09	13.7 ± 1.44	17.9 ± 1.73	3.0 ± 0.28	ND	ND	ND	12.5 ± 1.36	ND	75.4
Yamaguchi	(n=42)	0.3 ± 0.09	ND	ND	1.5 ± 0.26	0.0 ± 0.02	0.6 ± 0.09	4.6 ± 0.48	2.6 ± 0.27	3.3 ± 0.28	0.2 ± 0.05	ND	ND	ND	0.9 ± 0.13	ND	14.0
Ehime	(n=35)	0.2 ± 0.05	ND	ND	0.2 ± 0.06	0.2 ± 0.03	0.9 ± 0.12	3.9 ± 0.47	3.2 ± 0.47	2.1 ± 0.31	0.3 ± 0.07	ND	ND	0.0 ± 0.01	6.5 ± 1.06	0.0 ± 0.01	17.6
Nagasaki	(n=19)	ND	ND	ND	0.3 ± 0.09	0.7 ± 0.09	4.4 ± 0.72	26.6 ± 5.36	21.6 ± 5.06	25.5 ± 5.35	3.1 ± 0.56	ND	ND	0.1 ± 0.1	2.3 ± 0.50	0.1 ± 0.06	84.7

Average concentrations of medaka are shown as mean ± S.E.

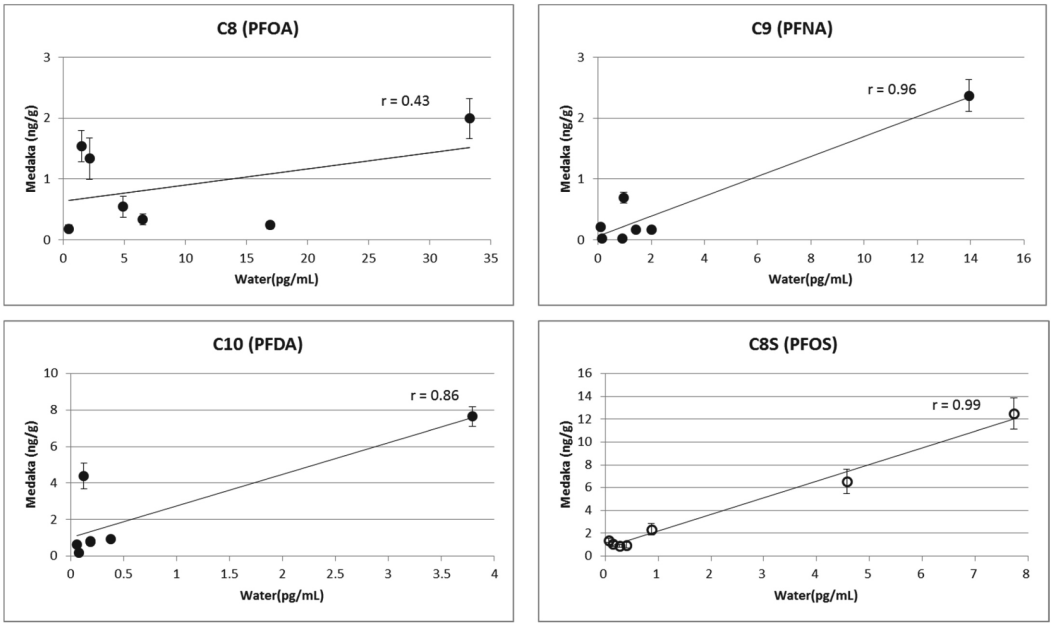

Fig. 4. Correlation between PFCs(C8-C10, C8S) concentrations of water and medaka. Each point ± vertical line = mean ± S.E. ●: PFCA ○: PFSA (PFOS).

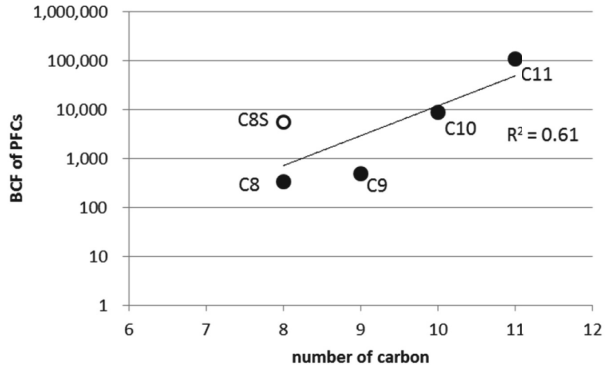

Fig. 5. Correlation between number of carbon (C8-C11, C8S) and BCF.

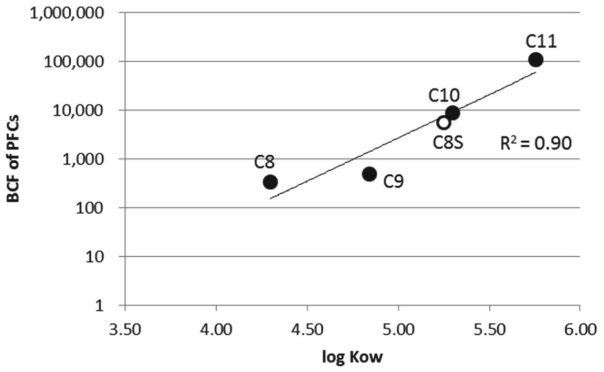

Fig. 6. Correlation between log K_{ow} (C8-C11, C8S) and BCF.

chain and a small log K_{ow} value. If the lipid solubility is a main determinant of log K_{ow} and BCF, this accumulation of PFCs in wild medaka may be applied to other aquatic life.

Inoue *et al.* (2012) reported the BCFs in carp (*Cyprinus carpio* L.) which was examined according to OECD test guideline (TG) 305. Their results were considerably lower than our results, as shown in Table 4. In their study, the BCF was not related to the log K_{ow}. The reasons behind these results are unclear, but may be due to:

(1) The samples of OECD TG 305 were exposed to a much higher concentration than our environmental samples.
(2) A different species was used (carp vs medaka).
(3) According to OECD TG 305 the exposure phase was 28-60 days, but environmental samples had been exposed for about 1 year, considering the life-cycle of medaka.

Table 4. Comparison of concentration of exposure and BCFs between wild medaka (environmental samples) and carp (OECD TG 305 samples).

PFCs	wild medaka (environmental samples) (a)		carp (OECD TG 305 samples) [1] (b)		ratio (b)/(a)	
	concentration of exposure[2] [3]	BCF	concentration of exposure[2]	BCF	concentration of exposure	BCF
C8	9.4	330	4,710-47,600	3.1-9.4	501-5,063	0.0094-0.028
C9	2.8	480	-	-	-	
C10	0.7	8,700	-	-	-	
C11	0.6	110,0000	91.1-946	2,300-3,700	152-1,577	0.020-0.034
C8S	2.0	5,500	1,880-16,000	720-1,300	940-8,000	0.13-0.24

1) Results of Inoue *et al.*
2) Concentrations are in pg/mL
3) Mean concentration of 7 samples of environmental water

The BCF data derived by the OECD test guidelines may be verified by the environmental data.

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Conflict of interest---- The authors declare that there is no conflict of interest.

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