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Odd-numbered perfluorocarboxylates predominate over perfluorooctanoic acid in serum samples from Japan, Korea and Vietnam

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ABSTRACT

Perfluorooctanoic acid (PFOA) has recently attracted attention as a potential health risk following environmental contamination. However, information detailing exposure to perfluorinated carboxylic acids (PFCAs) other than PFOA is limited. We measured the concentrations of PFCAs (from perfluorohexanoic acid to perfluorotetradecanoic acid) in serum samples obtained from patients in Japan (Sendai, Takayama, Kyoto and Osaka) between 2002 and 2009, Korea (Busan and Seoul) between 1994 and 2008 and Vietnam (Hanoi) in 2007/2008. Total PFCA levels (geometric mean) were increased from 8.9 ng mL^{-1} to 10.3 ng mL^{-1} in Japan; from 7.0 ng mL^{-1} to 9.2 ng mL^{-1} in Korea; and were estimated at 4.7 ng mL^{-1} in Vietnam. PFCAs of greater length than PFOA were significantly increased in Sendai, Takayama and Kyoto, Japan, and levels of long-chain PFCAs exceeded PFOA levels in serum. Among these PFCAs, perfluoroundecanoic acid (PFUnDA) was the predominant component (28.5%), followed by perfluorononanoic acid (PFNA 17.5%), perfluorodecanoic acid (PFDA 7.9%), perfluorotridecanoic acid (PFTrDA 6.1%) and perfluorododecanoic acid (PFDoDA 1.8%). Odd-numbered PFCAs (PFNA, PFUnDA and PFTrDA) were also observed in Korea and Vietnam and their presence increased significantly in Korea between 1994 and 2007/2008. The proportion of long-chain PFCAs in serum was relatively high compared to reports in Western countries. Further investigations into the sources and exposure routes are needed to predict the future trajectory of these serum PFCA levels.

1. Introduction

Perfluorinated compounds such as perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) have recently attracted attention owing to widespread contamination of the environment, wildlife and humans by these chemicals (Houde et al., 2006). In 2002, after 50 years of production, 3M Company phased out their manufacture of PFOS (Renner, 2001). PFOA is considered to be a major component of perfluorocarboxylate (PFCA) emission. However, in Japan, PFCA emissions consisted of not only PFOA but also perfluorononanoic acid (PFNA) and perfluoroundecanoic acid (PFUnDA) (of which 25 and 7 metric tons, respectively, were emitted in 2000) (Prevedouros et al., 2006). These odd-numbered PFCAs (PFNA, PFUnDA and perfluorotridecanoic acid (PFTrDA)) were detected at higher concentrations in samples from local wildlife than similar even-numbered PFCAs (PFOA, perfluorodecanoic acid (PFDA) and perfluorododecanoic acid (PFDoDA), respectively) (Furdui et al., 2008). Although studies using human samples from Western countries showed that PFOA was the most prevalent (followed by PFNA, PFDA and PFUnDA) (Haug et al., 2009; Joensen et al., 2009; Kato et al., 2009), our previous study of Japanese women in the Miyagi prefecture showed that PFNA and PFUnDA (average: 2.8 and 5.4 ng mL^{-1}, respectively) were found at broadly similar serum concentrations to PFOA (average: 4.9 ng mL^{-1}) (Kärrman et al., 2009).

PFCAs with longer chains than PFOA have higher bio-concentration factors suggesting persistency in the environment (Martin et al., 2003). Temporal trends in serum levels after 2002 showed no apparent

Abbreviations: PFCAs, perfluorinated carboxylic acids; PFOS, perfluorooctane sulfonate; PFOA, perfluorooctanoic acid; PFHxA, perfluorohexanoic acid; PFHpA, perfluoroheptanoic acid; PFNA, perfluorononanoic acid; PFDA, perfluorodecanoic acid; PFUnDA, perfluoroundecanoic acid; PFDoDA, perfluorododecanoic acid; PFTrDA, perfluorotridecanoic acid; PFTeDA, perfluorotetradecanoic acid; IDLs, instrumental detection limits; MDLs, method detection limits; RSD, relative standard deviation; SD, standard deviation; GM, geometric mean; GSD, geometric standard deviation.

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decline of PFNA, PFDA or PFUnDA in Norway (Haug et al., 2009), although serum levels of PFOA and PFOS both decreased in the United States, Norway and Japan (Harada et al., 2010; Harada and Koizumi, 2009; Haug et al., 2009; Olsen et al., 2008). These findings suggest a possibility that the origin and source of exposure to long-chain PFCAs could differ from those of PFOA and PFOS.

In the present study, we investigated current serum concentrations of PFCAs in three Asian countries (Japan, Korea and Vietnam). We selected the cities of Busan and Seoul in Korea because they are comparably urban and industrialized to Osaka, Japan. To confirm the temporal trends in Japan and Korea, we used archived historical serum samples stored in the human specimen bank (Koizumi et al., 2005; Koizumi et al., 2009). Hanoi in Vietnam was selected to evaluate the development of PFCA contamination following recent industrialization.

2. Material and methods

2.1. Experimental design and study population

To evaluate geographical differences and temporal trends in Asian countries, we compared 521 samples collected from Japan (Sendai, Takayama, Kyoto and Osaka) between 2002 and 2009; Korea (Busan and Seoul) between 1994 and 2008; and in Hanoi, Vietnam between 2007 and 2008. Samples from Sendai and Takayama in 2008, Osaka, Busan, Seoul and Hanoi are identical to a previous analysis of PFOS and PFOA (Harada et al., 2010; Kärrman et al., 2009). A total of 521 serum samples with information on donor age, sex and residential history (>5 years in each area) were selected from the archived samples in Kyoto Human Specimen Bank (Koizumi et al., 2005; Koizumi et al., 2009) (Table 1). Serum was separated from cellular components and stored at −30 °C until analysis.

The study population in Osaka and Kyoto consisted of residents that had been intensely exposed to PFOA from a local industrial source (the fluoropolymer manufacturer, Daikin Company) (Harada et al., 2004, 2007, 2010; Kärrman et al., 2009; Niisoe et al., 2010). In contrast, there is no known potential industrial source of PFCAs that would affect sample populations in the other cities studied.

For historical comparisons, samples were selected so that age and gender were matched among time points, except for Busan in 2000 and Osaka (Table 1).

The research protocol for the present study was reviewed and approved by the Ethics Committee of the Kyoto University Graduate School of Medicine on 14 November 2003 (E25).

2.2. Reagents

Ammonium acetate (purity: >99% by HPLC) was purchased from Aldrich (Steinheim, Germany). Acetonitrile (LC–MS grade) and water (distilled LC–MS grade) were obtained from Kanto Chemicals (Tokyo, Japan). Acetic acid and benzyl bromide were purchased from Wako pure chemicals (Osaka, Japan). Mixture of native PFCAs, $^{13}C_4$-labeled PFOA and $^{13}C_5$-labeled PFNA were obtained from Wellington Laboratories (Guelph, Ontario, Canada).

2.3. Determination of PFCAs in serum

Perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA and perfluorotetradecanoic acid (PFTeDA) were analyzed. Serum samples were subjected to a clean-up procedure using a dispersive carbon method described by Powley et al. (2005). Briefly, the serum samples (0.5 mL, except for Korean samples between 1994 and 2000, which were 0.25 mL) together with an internal standard ($^{13}C_4$-PFOA, 1 ng) were extracted with 5 mL of acetonitrile, followed by centrifugation at 1600×*g* for 15 min. The supernatants were transferred into new tubes with 25 mg of ENVI-Carb and 50 μL of acetic acid, and the solutions were mixed by vortexing for 30 s. After centrifugation at 1600×*g* for 15 min, the extracts were dried under a nitrogen stream. The residue was then re-dissolved in 100 μL of 100 mM benzyl bromide acetone containing the recovery performance standard $^{13}C_5$-PFNA (1 ng) for 1 h at 80 °C and transferred to an autosampler vial. Extracts were analyzed using gas chromatography–mass spectrometry (Agilent 6890GC/5973MSD, Agilent Technologies Japan, Ltd., Tokyo, Japan) in electron impact ionization mode using single ion monitoring. PFCA benzyl esters were separated on a DB-5MS column (30 m length, 0.25 mm i.d., 1 μm film thickness) with a helium carrier gas. Split-less injections (1 μL) were performed with the injector set at 220 °C, and the split was opened after 1.5 min. The initial oven temperature was 70 °C for 2 min, ramped at 20 °C min^{-1} to 100 °C, and then at 30 °C min^{-1} to 280 °C. Ion fragments ($[M]^+$) were monitored and used as quantification ions (Table 2).

Instrumental detection limits (IDL) were defined as the mass of analyte producing a peak with a signal-to-noise ratio of 3, and ranged from 1 pg (PFTeDA) to 0.25 pg (other PFCAs) (Table 2). Since blank samples (0.5 mL distilled water) contain no detectable concentrations, the method detection limit (MDL) value was considered to be equal to the IDL corresponding to 0.2 ng mL^{-1} for PFTeDA and 0.025 ng mL^{-1} for other PFCAs (Table 2).

Table 1
Study area and study population.

Sampling site	Population (×10^3)	Latitude and longitude	Year	n (%female)	Age[a]	(Range)
Japan						
Sendai	1031	38°17′04″ N 140°55′46″ E	2008	50 (100)	37.5 ± 9.44	(21–53)
	1023	–	2003	50 (100)	36.6 ± 10.1	(20–59)
Takayama	94 (65)[b]	36°08′13″ N 137°15′16″ E	2008	50 (100)	40.5 ± 4.78	(29–49)
	67	–	2003	50 (100)	39.9 ± 4.5	(31–45)
Kyoto	1466	35°01′18″ N 135°46′38″ E	2009	30 (50)	33.2 ± 14.7	(21–68)
	1469	–	2002	30 (50)	35.4 ± 11.3	(21–58)
Osaka	2652	34°45′31″ N 135°31′52″ E	2008	50 (100)	45.9 ± 8.92[A,*]	(30–63)
	2619	–	2004	10 (100)	60.9 ± 6.3[B]	(49–69)
Korea						
Busan	3711	35°14′39″ N 129°05′54″ E	2008	35 (100)	40.1 ± 6.44[A,*]	(18–49)
	3732	–	2000	30 (100)	35.4 ± 4.27[B]	(28–45)
	3961	–	1994	39 (100)	42.3 ± 4.65[A]	(34–52)
Seoul	10,421	37°27′52″ N 127°01′56″ E	2007	36 (100)	34.5 ± 8.24	(20–54)
	10,798	–	1994	24 (100)	38.0 ± 7.41	(24–51)
Vietnam						
Hanoi	6232	21°00′08″ N 105°49′50″ E	2007–2008	37 (100)	30.2 ± 5.76	(20–40)

* Means of age with different letters differed significantly ($p<0.05$ by Tukey's HSD test). For example, the letters A and B indicate that the corresponding values differ significantly at $p<0.05$.

[a] Data are presented as mean ± standard deviation.

[b] Takayama city merged with neighboring cities in 2005. Numbers in paretheses denote populations areas corresponding to those used in 2003.

Table 2
Recovery, detection limits and QA for PFCA analysis in human serum samples.

Compound	Quantification (confirmation)	Recovery and (reproducibility)% (RSD%)[a] (n=5)	Instrument detection limit[b] (pg)	Method detection limit[c] (ng mL^{-1})	SRM1957[d] (ng mL^{-1})
PFHxA	404 (385)	92.2 (8.41)	0.25	0.05	<0.05
PFHpA	454 (435)	94.5 (4.12)	0.25	0.05	0.27
PFOA	504 (485)	101.7 (6.99)	0.25	0.05	4.77
$^{13}C_4$ PFOA	508 (489)	102.8 (5.47)	–	–	–
PFNA	554 (535)	97.4 (7.61)	0.25	0.05	0.96
$^{13}C_5$ PFNA	559 (540)	–	–	–	–
PFDA	604 (585)	91.9 (8.63)	0.25	0.05	0.26
PFUnDA	654 (635)	94.1 (7.22)	0.25	0.05	0.16
PFDoDA	704 (685)	95.7 (4.87)	0.5	0.1	<0.1
PFTrDA	754 (735)	98.6 (9.41)	0.5	0.1	<0.1
PFTeDA	785 (786)	92.4 (8.18)	1	0.2	<0.2

[a] RSD: relative standard deviation.
[b] 1 μL injection.
[c] 0.5 mL serum sample.
[d] 0.5 mL serum sample of NIST SRM 1957 was analyzed.

2.4. Quality assurance

Quantification was performed using an internal standard method with the external standards dissolved in 10% methanol in water. $^{13}C_4$-PFOA was used as the internal standard for PFCAs. $^{13}C_5$-PFNA was used to calculate a recovery rate of $^{13}C_4$-PFOA. All samples were quantified using a seven-point calibration curve with a relative standard deviation (RSD) of the relative response factors <15% for all compounds. The recoveries were evaluated by five replicate fortifications (fortified by 10 times the original concentration of serum) of a human serum sample with low contamination (Table 2). The procedural blank levels were evaluated in duplicate for 11 samples each using 0.5 mL distilled water.

Using the above method, we reanalyzed 361 samples originally tested in a previous study by HPLC–MS/MS (Harada et al., 2010; Kärrman et al., 2009). The reanalyzed samples showed 5.14 ± 11.60 ng mL^{-1} for PFOA, which equates to 101.7% of the levels obtained in the previous study (5.05 ± 11.16 ng mL^{-1}, $p=0.478$ by paired t-test). Pearson's correlation coefficient, r and slope were 0.9882 and 1.128, respectively ($p<0.0001$). Levels (mean ± SD) of PFHpA, PFNA, PFDA and PFUnDA in Osaka in 2004 were also confirmed in this study (HPLC–MS/MS *vs* GC–MS: 0.26 ± 0.14 ng mL^{-1} *vs* 0.24 ± 0.09 ng mL^{-1}, 6.68 ± 1.78 ng mL^{-1} *vs* 6.16 ± 1.91 ng mL^{-1}, 2.55 ± 0.99 ng mL^{-1} *vs* 2.74 ± 1.32 ng mL^{-1}, 5.80 ± 2.13 ng mL^{-1} *vs* 5.12 ± 2.69 ng mL^{-1}, respectively; $p>0.05$ by paired t-test). RSDs of difference between methods were 33.1%, 9.8%, 13.6% and 11.5% for PFHpA, PFNA, PFDA and PFUnDA, respectively and average RSD was 17.0%.

To assess potential interlaboratory difference in analysis, NIST standard reference material (SRM) 1957 was analyzed (Table 2). The values from PFHpA to PFUnDA were comparable to those from interlaboratory comparison exercises (Keller et al., 2010; Lindstrom et al., 2009).

Mean recovery rate (RSD) of $^{13}C_4$-PFOA in 521 samples was 96.5% (8.8%). To evaluate possible matrix effect in serum sample, we further analyzed 100 samples extracts fortified with 1 ng of PFHpA, PFNA, PFDA, PFUnDA, PFDoDA and PFTrDA standards. Recoveries of fortified standards were 98.7%, 104.6%, 102.0%, 97.2%, 102.2% and 96.3% for PFHpA, PFNA, PFDA, PFUnDA, PFDoDA and PFTrDA, respectively. It is therefore considered that there was no substantial suppression or enhancement of target ions, if any.

2.5. Statistical analysis

All statistical analyses were carried out using the JMP software (Version 4; SAS Institute Inc., Cary, NC). Values of $p<0.05$ were considered to indicate statistical significance. Concentrations of less than the detection limit were all approximated to 'half of detection limit' for statistical analyses. Serum levels of PFCAs were assumed to distribute lognormally because the serum levels of PFCAs in the samples displayed right-skewed patterns and geometric means were comparable to medians. Statistical analyses were conducted after logarithmic transformation of the serum concentrations. Differences between mean values were tested by Tukey–Kramer's honestly significant difference (HSD) test after ANOVA. Correlation was tested by Spearman's rank correlation coefficient (ρ). Factor analysis was used to transform a number of contaminants into a smaller number of potential factors of sources. Factor analysis was conducted *via* correlation matrix. In essence, the factor analysis is a model which presumes the existence of a smaller set of factors that can reproduce exactly the correlation in the larger set of variable (Berenson et al., 1983). To achieve this goal, the linear combinations of factors (*i.e.*, principal component) will be generated in such a manner that each composite variate will account a smaller portion of the total variation *i.e.*, variance. Eigenvalues of a principal component is a measure how much this principal component can account for the variation and eigenvector indicates an associated set of coefficients with a principal component for each factor. Eigenvectors were employed through the analysis when eigenvalues were close to or greater than 1 which means its eigenvector can account the equivalent of one or more original variables. Normalized varimax rotation (an orthogonal rotation of the factor axes) was applied to these eigenvectors to simplify them into a few variables with high correlations.

3. Results

3.1. Temporal changes in PFCA concentrations in Japan

The descriptive statistics for PFCAs are presented in Table 3. Most samples contained PFOA, PFNA, PFDA, PFUnDA and PFTrDA at both time points. No samples contained PFHxA and PFTeDA at concentrations above MDL. PFHpA levels were significantly decreased in all sampling sites in Japan between 2002/2004 and 2008/2009 ($p<0.05$ by Student's t-test). PFOA was relatively high in Osaka and Kyoto although levels of this compound nevertheless significantly decreased in this period ($p<0.05$ by Student's t-test). In Sendai and Takayama, PFOA levels also decreased but this difference was not statistically significant. In contrast, PFCAs longer than PFOA showed significant increases in Sendai, Takayama and Kyoto with few exceptions. Among these PFCAs, PFUnDA was the predominant component, followed by PFNA, PFDA, PFTrDA and PFDoDA. These odd-numbered PFCAs (*i.e.* PFUnDA, PFNA and PFTrDA) were detected at higher concentrations than neighboring, even-numbered PFCAs (PFDA and PFDoDA).

In Osaka, levels of PFNA, PFDA and PFUnDA, as with PFOA, significantly decreased from 2004 to 2008. PFDoDA and PFTrDA levels did not change. Among four sampling sites in 2008/2009, Osaka and Kyoto had higher PFOA, PFNA and PFDA levels than Sendai and Takayama ($p<0.05$ by Tukey's HSD test) but PFUnDA, PFDoDA and PFTrDA showed no regional differences ($p>0.05$ by ANOVA).

As a consequence of the increase in long-chain PFCAs, the proportion of PFOA in the total PFCA content became less than 50% in all locations except Osaka.

Table 3
Serum concentrations of PFCAs in Japan.

Sampling site	Year	n		Concentration (ng mL^{-1})							
				PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	ΣPFCAs
Sendai	2008	50	GM(GSD)	0.06(2.17)*	2.44(1.56)	1.80(1.40)*	0.72(1.46)*	3.00(1.59)*	0.17(1.99)*	0.60(2.00)*	9.13(1.41)*
			Range	<0.05–0.37	0.85–6.05	0.90–3.58	0.31–1.58	1.15–8.08	<0.1–0.52	<0.1–1.43	3.81–17.52
			Detection%	58	100	100	100	100	82	96	
	2003	50	GM(GSD)	0.15(3.75)	2.65(1.61)	1.01(1.85)	0.52(1.71)	1.68(1.75)	0.10(1.85)	0.31(2.12)	6.92(1.51)
			Range	<0.05–1.25	0.87–7.59	0.21–4.94	0.09–1.57	0.32–5.70	<0.1–0.37	<0.1–1.13	2.74–17.94
			Detection%	72	100	100	100	100	58	100	
Takayama	2008	50	GM(GSD)	0.04(2.29)*	2.51(1.84)	1.78(1.42)*	0.85(1.51)*	3.12(1.51)	0.20(2.15)*	0.60(2.66)	9.87(1.39)
			Range	<0.05–0.49	0.82–11.25	1.01–4.50	0.26–2.68	1.28–7.13	<0.1–0.61	<0.1–2.46	5.44–22.09
			Detection%	38	100	100	100	100	82	94	
	2003	50	GM(GSD)	0.11(2.35)	3.19(1.62)	1.30(1.73)	0.65(1.63)	2.74(1.60)	0.14(1.88)	0.55(1.72)	9.18(1.49)
			Range	<0.05–1.72	1.36–20.28	0.64–9.88	0.18–2.26	0.77–7.81	<0.1–0.51	0.16–1.98	4.49–37.04
			Detection%	88	100	100	100	100	80	100	
Kyoto	2009	30	GM(GSD)	0.11(1.98)*	5.28(1.57)*	2.78(1.42)*	1.10(1.45)	3.20(1.64)*	0.24(1.87)*	0.45(1.57)*	13.67(1.42)
			Range	<0.05–0.31	2.60–16.52	1.34–4.40	0.60–2.25	1.20–11.26	<0.1–0.99	0.22–1.15	6.60–26.81
			Detection%	96.7	100	100	100	100	93.3	100	
	2002	30	GM(GSD)	0.23(1.89)	7.12(1.54)	2.09(1.67)	0.91(1.66)	1.89(1.65)	0.12(2.04)	0.31(1.83)	12.98(1.52)
			Range	0.08–1.25	2.69–19.64	0.81–5.37	0.35–2.54	0.72–5.44	<0.1–0.37	<0.1–1.00	5.38–33.75
			Detection%	100	100	100	100	100	66.7	96.7	
Osaka	2008	50	GM(GSD)	0.07(3.11)*	13.46(1.79)*	3.54(1.62)*	1.11(1.60)*	3.05(1.73)*	0.16(2.55)	0.52(2.62)	23.08(1.64)*
			Range	<0.05–1.11	5.59–201.68	0.85–14.57	0.36–2.80	1.01–8.79	<0.1–0.75	<0.1–1.95	10.77–220.07
			Detection%	48	100	100	100	100	68	94	
	2004	10	GM(GSD)	0.21(2.00)	29.54(1.29)	6.41(1.38)	2.38(1.48)	5.45(1.46)	0.25(2.28)	0.44(2.40)	45.42(1.27)
			Range	0.05–0.45	20.60–45.20	3.07–9.22	1.41–4.17	3.19–9.01	<0.1–0.51	<0.1–1.02	31.67–65.57
			Detection%	100	100	100	100	100	90	90	

GM: geometric mean; GSD: geometric standard deviation.
* GMs between time points are significantly different in each sampling site ($p<0.05$ by Student's t test after log transformation).

3.2. Temporal trends in the serum concentrations of PFCAs in Korea

The PFCA concentrations in the serum samples collected in Busan and Seoul between 1994 and 2008 are shown in Table 4. As is the case with Japan, PFOA, PFNA, PFDA, PFUnDA and PFTrDA were frequently detected in 2007/2008. PFHxA and PFTeDA were not detected in any samples at concentrations above MDL. In agreement with the previous report by Harada et al. (2010), PFOA levels were stable from 1994 to 2008 in Busan and Seoul ($p>0.05$ by ANOVA). In contrast, odd-numbered PFCAs (PFNA, PFUnDA and PFTrDA) were significantly increased during this period ($p<0.05$ by Tukey's HSD test or Student's t-test). The PFCA levels had the following order of prevalence in 1994: PFOA > PFNA ~ PFUnDA > PFDA > PFTrDA > PFHpA ~ PFDoDA. However, by 2007/2008 the order had changed to: PFOA > PFUnDA > PFNA > PFDA ~ PFTrDA > PFDoDA > PFHpA. Between 1994 and 2007/2008, total PFCA levels were significantly increased by 1.31- and 1.53-fold in Busan and Seoul, respectively ($p<0.05$ by Tukey's HSD test or Student's t-test). Samples from Busan contained higher concentrations of PFHpA, PFOA, PFNA, PFDA, PFUnDA and PFTrDA than did those from Seoul in both 1994 and 2007/2008 ($p<0.05$ by Student's t-test).

3.3. PFCA concentrations in Hanoi, Vietnam in 2008–2009

PFOA, PFNA, PFDA and PFUnDA were detected in all samples, and PFDoDA and PFTrDA were also detected, albeit less frequently (Table 5). PFHxA, PFHpA and PFTeDA were not detected in any samples from Hanoi. The concentration of PFUnDA was highest among the PFCAs studied, followed by PFNA, PFDA, PFOA, PFTrDA and PFDoDA. The proportion of PFOA relative to total PFCAs was only 12.9%.

3.4. Correlations among PFCA levels and factor analysis

Correlation coefficients among PFCAs in 521 samples are listed in Table 6. PFHpA was relatively less correlated with other PFCAs, except for PFOA ($\rho = 0.398$). PFOA also significantly correlated with PFNA and PFDA (ρ coefficient > 0.5) but was less well correlated with PFUnDA, PFDoDA and PFTrDA. In general, PFCA concentrations indicated a strong correlation between PFCAs of similar (*i.e.* adjacent) chain length.

To delineate potential patterns in the data, PFCA concentrations were examined using factor analysis. The contributions of factors 1 and 2 to the total variance were 49.72% and

Table 4
Serum concentrations of PFCAs in Korea.

Sampling site	Year	n		Concentration (ng mL^{-1})							
				PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	ΣPFCAs
Busan	2008	35	GM(GSD)	0.04(1.92)^{*A}	4.67(1.40)	1.91(1.45)^{*A}	0.91(1.38)	2.91(1.54)^{*A}	0.20(2.03)^{*AB}	0.94(1.92)^{*A}	11.87(1.38)^{*A}
			Range	<0.05–0.16	2.77–9.80	1.02–3.89	0.44–1.76	1.03–7.62	<0.1–0.81	<0.1–2.74	6.70–24.13
			Detection%	40	100	100	100	100	85.7	97.1	
	2000	30	GM(GSD)	0.10(1.59)^{B}	3.69(1.47)	1.77(1.41)^{A}	0.84(1.45)	2.06(1.66)^{AB}	0.14(1.61)^{A}	0.72(1.67)^{A}	9.58(1.39)^{B}
			Range	<0.1–0.28	1.19–7.33	0.89–3.61	0.32–1.47	0.58–3.95	<0.20–0.39	<0.20–1.73	4.31–16.00
			Detection%	30	100	100	100	100	33.3	100	
	1994	39	GM(GSD)	0.10(1.58)^{B}	4.11(1.43)	1.35(1.96)^{B}	0.89(1.65)	1.37(2.81)^{B}	0.11(1.61)^{B}	0.36(2.90)^{B}	9.05(1.46)^{B}
			Range	<0.1–0.32	1.72–9.63	<0.10–5.20	0.25–2.98	<0.20–13.16	<0.20–1.03	0.10–2.89	4.08–32.50
			Detection%	35.9	100	97.4	100	92.3	7.7	69.2	
Seoul	2007	36	GM(GSD)	0.03(1.48)	2.29(1.34)	1.13(1.32)*	0.58(1.38)	2.18(1.48)*	0.12(2.03)	0.59(2.10)*	7.10(1.35)*
			Range	<0.05–0.12	1.22–4.64	0.74–2.01	0.32–1.00	1.10–5.62	<0.10–0.38	<0.10–1.54	3.94–12.55
			Detection%	13.9	100	100	100	100	66.7	97.2	
	1994	24	GM(GSD)	0.08(1.00)	2.09(1.54)	0.65(2.01)	0.45(2.06)	0.54(3.89)	0.10(1.26)	0.16(2.40)	4.63(1.49)
			Range	<0.1	0.89–4.09	<0.1–1.73	<0.1–1.18	<0.20–3.59	<0.20–0.31	<0.20–1.08	2.56–10.69
			Detection%	0	100	95.8	95.8	70.8	4.2	25	

GM: geometric mean; GSD: geometric standard deviation.
* GMs among different time points are significantly different in each sampling sites ($p<0.05$ by Student's t test or Tukey's HSD test after log transformation). Alphabetic suffix was used for comparisons among three groups. For example, the letters A and B indicate that the corresponding values differ significantly at $p<0.05$., while A and AB or AB and B indicate that the corresponding values do not.

Table 5
Serum concentrations of PFCAs in Hanoi, Vietnam.

Sampling site	Year	n		Concentration (ng mL^{-1})							
				PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	ΣPFCAs
Hanoi	2007–2008	37	GM(GSD)	0.03(1.00)	0.61(1.55)	0.89(1.47)	0.82(1.67)	1.55(1.53)	0.09(1.85)	0.36(3.38)	4.73(1.38)
			Range	<0.05	0.20–1.43	0.35–1.65	0.19–2.03	0.57–3.95	<0.10–0.26	<0.10–1.99	2.58-9.43
			Detection%	0	100	100	100	100	51.4	86.5	

GM: geometric mean; GSD: geometric standard deviation.

19.40% (with an eigenvalue >1), respectively (Table 7). After varimax rotation, the first factor indicated a higher eigenvector for longer-chain PFCAs than PFNA. The second factor had a more positive eigenvector for shorter-chain PFCAs than PFDA. Since there is a point source of PFCAs in both Osaka and Kyoto, we evaluated whether this predominant source may perturb the results of the factor analysis. Eliminating Osaka and Kyoto samples, however, did not alter a correlation matrix among PFCAs with changes in eigenvalues being less than 5% (data not shown), indicating that the dominant point source had no substantial influence on the interpretation of factor 1 and factor 2.

Factor 1 is characterized by PFUnDA dominance (factor loading: 0.858) and another by PFOA dominance (0.819), respectively. This characteristic pattern indicates fingerprints of PFCAs sources in Asia. Temporal transition of factor scores is demonstrated by score plots shown in Supplemental Fig. 1. In sampling sites in Japan and Korea (except for Osaka), centers of score plot moved rightwards and downwards, indicating that the factor 1 score increased and factor 2 score decreased during these periods. Mean factor scores of each sampling site are also shown in Table 7. In Japan, factor 1 scores significantly increased from 2002/2003 to 2008/2009 ($p<0.05$ by Student's t-test), except for Osaka which already had a high factor 1 score (0.92) in 2004. This increase in factor 1 scores was also observed in Busan and Seoul from 1994 to 2007/2008 ($p<0.05$ by Tukey's HSD test or Student's t-test). Although the factor 1 score in Hanoi was lower than those in other sites in 2007–2009, it surpassed scores in Sendai and Kyoto in 2002/2003 and in Busan and Seoul in 1994. Contrary to factor 1, factor 2 scores in all sampling sites in Japan significantly declined between 2002/2004 and 2008/2009 ($p<0.05$ by Student's t-test) and also in Busan and Seoul from 1994 to 2007/2008 ($p<0.05$ by Tukey's HSD test or Student's t-test). Factor 2 in Hanoi was the lowest among all sampling sites.

4. Discussion

In the present study, we uncovered two major fingerprints (factor 1 and factor 2) by analyzing serum samples from three countries in East Asia. Characteristic PFCA composition was observed for odd-numbered PFCAs such as PFUnDA and PFTrDA with residual PFDoDA and PFDA, which can correspond to factor 1. Even in populations exposed to low levels of PFOA, notably Hanoi, PFUnDA showed substantial serum levels. Moreover, levels of those PFCAs with longer chain lengths than PFOA were significantly elevated in Japan and Korea in recent years. In the late-2000s, consequently, long-chain PFCA levels exceeded PFOA levels in most sampling sites. This finding suggests an emergence of specific sources of exposure in East Asia.

In several countries, serum PFOA has reportedly decreased (Harada et al., 2010; Olsen et al., 2008). In contrast, PFCAs of longer chain lengths than PFOA were frequently detected in serum samples in this study. Total levels of long-chain PFCAs were comparable to or greater than PFOA levels (except in Osaka) and showed trends towards increases in Japan and Korea. Correlation between PFOA and long-chain PFCAs was not strong which suggests that the sources of long-chain PFCA contamination have different exposure route than PFOA. Indeed, factor analysis demonstrated two major factors as sources of PFCAs. The first factor had loading on longer-chain PFCAs than PFOA and the second factor on PFHpA, PFOA and PFNA. Temporal trends of these factors were opposite and contamination derived from factor 1 might be expected to emerge in around a decade. This transition of factor scores was similar in Japan, Korea and Hanoi. Contamination derived from factor 1 may have been prevailing in East Asian countries.

Among long-chain PFCAs, odd-numbered PFCAs accounted for the major proportion. Serum or blood levels of PFCAs reported from populations in China, Sri Lanka, Australia, Norway, Sweden, Denmark,

Table 6
Correlation between different chain length PFCAs.

Combination		ρ	p value
PFOA	PFHpA	0.398	<0.001
PFNA	PFHpA	0.223	<0.001
PFNA	PFOA	0.734	<0.001
PFDA	PFHpA	0.165	<0.001
PFDA	PFOA	0.534	<0.001
PFDA	PFNA	0.727	<0.001
PFUnDA	PFHpA	0.019	0.660
PFUnDA	PFOA	0.323	<0.001
PFUnDA	PFNA	0.646	<0.001
PFUnDA	PFDA	0.689	<0.001
PFDoDA	PFHpA	0.055	0.208
PFDoDA	PFOA	0.235	<0.001
PFDoDA	PFNA	0.462	<0.001
PFDoDA	PFDA	0.563	<0.001
PFDoDA	PFUnDA	0.740	<0.001
PFTrDA	PFHpA	−0.117	0.008
PFTrDA	PFOA	0.063	0.151
PFTrDA	PFNA	0.264	<0.001
PFTrDA	PFDA	0.360	<0.001
PFTrDA	PFUnDA	0.552	<0.001
PFTrDA	PFDoDA	0.471	<0.001

ρ: Spearman's rank correlation coefficient.

Table 7
Factor analysis among PFCAs.

		Initial solution		Varimax rotated	
		F1	F2	F1	F2
Eigenvalue		3.48	1.36		
Contribution (%)		49.72	19.40		
Eigenvector					
PFHpA		0.092	0.618	−0.198	0.713
PFOA		0.365	0.480	0.327	0.819
PFNA		0.474	0.179	0.673	0.610
PFDA		0.469	0.036	0.745	0.459
PFUnDA		0.446	−0.230	0.858	0.168
PFDoDA		0.374	−0.266	0.760	0.066
PFTrDA		0.274	−0.481	0.719	−0.244
Factor score (mean ± standard deviation)					
Sendai	2008			0.31 ± 0.78*	−0.41 ± 0.67*
	2003			−0.84 ± 0.90	0.17 ± 0.92
Takayama	2008			0.50 ± 0.67*	−0.49 ± 0.85*
	2003			−0.10 ± 0.76	0.02 ± 0.81
Kyoto	2009			0.44 ± 0.68*	0.68 ± 0.52*
	2002			−0.46 ± 0.85	1.29 ± 0.50
Osaka	2008			0.91 ± 1.03	1.17 ± 0.78*
	2004			0.92 ± 0.71	2.42 ± 0.48
Busan	2008			0.68 ± 0.67†A	−0.28 ± 0.56†A
	2000			0.06 ± 0.61B	0.15 ± 0.46A
	1994			−0.48 ± 0.95C	0.42 ± 0.63B
Seoul	2007			0.02 ± 0.69†A	−0.92 ± 0.36†A
	1994			−1.49 ± 0.90B	−0.22 ± 0.48B
Hanoi	2007–2008			−0.27 ± 0.74	−1.48 ± 0.65

F1: 1st factor; F2: 2nd factor.
* Means between time points are significantly different ($p<0.05$ by Student's t test).
† Means among different time points are significantly different ($p<0.05$ by Student's t test or Tukey's HSD test). For example, the letters A and B indicate that the corresponding values differ significantly at $p<0.05$., while A and A or B and B indicated that the corresponding values do not differ significantly.

Poland, Belgium, Spain and USA are summarized in Table 8 (Ericson et al., 2007; Falandysz et al., 2006; Guruge et al., 2005; Haug et al., 2009; Joensen et al., 2009; Kärrman et al., 2006; Kuklenyik et al., 2004; Pan et al., 2010; Roosens et al., 2010; Toms et al., 2009). The PFCA composition in our current study, which was characterized by a large proportion of PFUnA, was apparently different from Western countries (Table 8). Although PFOA levels in these countries were comparable, long-chain PFCAs were not major components in Western countries, except for Antwerp, Belgium and Atlanta, USA. Therefore, this composition can be considered as a clear fingerprint for East Asian countries and is implicated in the origination of factor 1.

However, their source remains unclear due to insufficient monitoring data of PFCAs. Interestingly, a review by Prevedouros et al. (2006) indicated that PFNA has been manufactured in Japan *via* oxidation of fluorotelomer olefins together with PFUnDA and PFTrDA. Industrial application of these odd-numbered PFCAs, namely Surflon S-111, might contribute to the East Asian-specific pattern of serum body burdens. The temporal increase in long-chain PFCAs warrants further investigations of the sources and exposure routes to assist in predicting future changes in the serum levels of these contaminants.

In this study, there was a limitation in chemical analysis. $^{13}C_4$-PFOA was used for internal standard for PFCAs (C_7–C_{14}). Chemical properties of PFCAs may, however, be different even though they have similar structures. Matrix effects also might affect quantification of PFCAs other than PFOA. Thus it is logically possible that recovery rates of $^{13}C_4$-PFOA might be extensively deviated from those of other PFCAs. Nevertheless, such a possibility is unlikely because recovery rates of PFCAs were higher than 90% and RSD were within 10%, indicating that there was no substantial difference in recoveries among PFCAs in this method. Furthermore, a good agreement of results in SRM analysis by interlaboratory comparisons assured that our analytical method in this study is sound. Collectively, these findings consistently support that analytical method in this study was sufficiently qualified.

Recent epidemiological investigations have raised concern regarding developmental effects of PFOA on children (Steenland et al., 2010). In contrast, few studies have been conducted on the effects of PFCAs of different chain length. Even though PFCAs have similar structure, their chemical properties and biological activity are likely different. In several *in vitro* studies, long-chain PFCAs caused biological responses at lower doses than PFOA (Liao et al., 2009; Matsubara et al., 2006; Upham et al., 1998). The toxicokinetics of long-chain PFCAs are also unclear, especially in humans. These uncertainties necessitate more comprehensive toxicological studies on PFCAs.

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Appendix A. Supplemental data

Supplementary data to this article can be found online at doi:10.1016/j.envint.2011.04.011.

Table 8
Comparison of serum or whole blood concentrations of PFCAs with reported data.

Sampling site	Year	n	Sex		Sample	Concentration (ng mL^{-1})							
						PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	Reference
Japan													
Sendai	2008	50	F	Median	Serum	0.07	2.36	1.82	0.73	2.97	0.19	0.74	This study
	2003	50	F	Median	Serum	0.22	2.59	1.07	0.55	1.79	0.11	0.37	This study
Takayama	2008	50	F	Median	Serum	<0.05	2.08	1.72	0.80	3.11	0.24	0.77	This study
	2003	50	F	Median	Serum	0.13	3.21	1.29	0.69	2.69	0.15	0.56	This study
Kyoto	2009	15/15	M/F	Median	Serum	0.11	5.52	2.69	1.01	3.15	0.25	0.45	This study
	2002	15/15	M/F	Median	Serum	0.23	7.20	2.15	0.90	1.72	0.14	0.30	This study
Osaka	2008	50	F	Median	Serum	<0.05	12.80	3.32	1.10	2.98	0.19	0.72	This study
	2004	10	F	Median	Serum	0.23	28.90	6.87	2.53	5.83	0.35	0.51	This study
Korea													
Busan	2008	35	F	Median	Serum	<0.05	4.64	1.98	0.92	3.00	0.22	0.92	This study
	2000	30	F	Median	Serum	<0.1	3.98	1.92	0.87	2.27	<0.2	0.76	This study
	1994	39	F	Median	Serum	<0.1	3.98	1.28	0.94	1.82	<0.20	0.49	This study
Seoul	2007	36	F	Median	Serum	<0.05	2.21	1.11	0.57	2.37	0.14	0.74	This study
	1994	24	F	Median	Serum	<0.1	2.31	0.76	0.50	0.89	<0.20	<0.20	This study
Vietnam													
Hanoi	2007–2008	37	F	Median	Serum	<0.05	0.63	0.91	0.85	1.58	0.11	0.65	This study
Norway	2006	>20	M	Median	Serum	0.078	2.7	0.55	0.22	0.14	<0.05	0.071	Haug et al., 2009
Sri Lanka													
Colombo	2003	10	M	Median	Serum	0.146	9.32	0.299	0.18	0.186	0.015	–	Guruge et al., 2005
China													
Ningbo	2006–2008	8/12	M/F	Median	Pooled serum	<0.1	3.28	0.984	0.718	0.917	<0.18	–	Pan et al., 2010
Spain													
Catalonia	2002–2007	24/24	M/F	Median	Whole blood	<0.78	1.65	0.41	0.24	0.2	–	–	Ericson et al., 2007
Poland													
Gdańsk	2003	10/5	M/F	Median	Whole blood	0.086	2.8	0.49	0.17	0.078	0.012	–	Falandysz et al., 2006
Belgium													
Antwerp	2002–2005	182	F		Pooled serum	–	3.18	2.41	1.86	–	–	–	Roosens et al., 2010
Australia													
Queensland	2006–2007	42/42	M/F	Mean	Pooled serum	–	6.4	0.8	0.29	–	–	–	Toms et al., 2009
Denmark													
Copenhagen	2003	105	M	Median	Serum	0.2	4.9	0.8	0.9	0.1	0.08	<0.1	Joensen et al., 2009
Sweden													
Stockholm	1997–2000	40/26	M/F	Median	Whole blood	–	2.5	0.3	0.2	0.2	–	–	Kärrman et al., 2006
USA													
Atlanta	2003	10/10	M/F	Median	Serum	–	4.35	2.35	0.35	0.7	–	–	Kuklenyik et al., 2004

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ENVIRONMENTAL Science & Technology

ARTICLE

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Dietary Exposure to Short-Chain Chlorinated Paraffins Has Increased in Beijing, China

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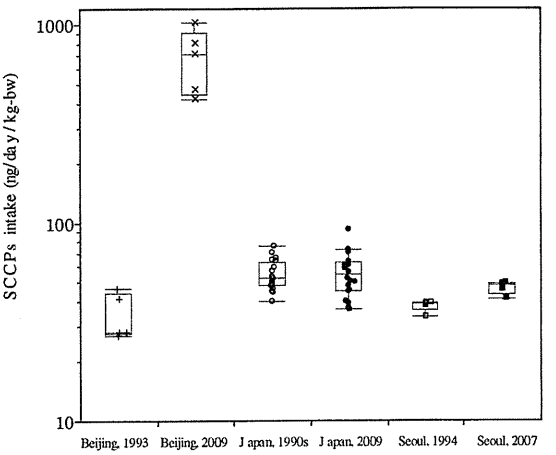
Supporting Information

ABSTRACT: Short-chain chlorinated paraffins (SCCPs) persist in the environment and bioaccumulate in biota and are under review by the Stockholm Convention on persistent organic pollutants. SCCP levels were measured semiquantitatively in pooled 24 h food composite samples from Chinese (n = 10), Korean (n = 10), and Japanese (n = 40) adults in the 1990s and 2007−2009. In Japan, SCCPs were detected in 14 of 20 pooled samples in the 1990s and 13 of 20 pooled samples in 2009. Between these two time points, the geometric mean (GM) of the dietary intake of total SCCPs per body weight was comparable in Japan (54 ng kg-bw^{-1} day^{-1} in the 1990s and 54 ng kg-bw^{-1} day^{-1} in the 2000s). In Beijing, SCCP levels were elevated by 2 orders of magnitude from 1993 to 2009 (GM: 620 ng kg-bw^{-1} day^{-1} in 2009). The 95th percentile estimate of the dietary intake was 1200 ng kg-bw^{-1} day^{-1} (>1% of tolerable daily intake). In Seoul, no samples in 1994 contained detectable SCCP levels and only one sample in 2007 showed trace levels of SCCPs. Preliminary evidence on the significant increase in SCCP exposure in Beijing in 2009 warrants urgent investigations to refine dietary intake estimates by targeting food types and source identification.



INTRODUCTION

Chlorinated paraffins (CPs), including short-chain chlorinated paraffins (SCCPs, C_{10-13}), are industrial products used as metal-working fluids and flame retardants for plastic materials.[1] SCCPs seem to persist in the environment and bioaccumulate in biota and are under review by the Stockholm Convention on persistent organic pollutants. At high exposure levels, SCCPs have been reported to cause liver toxicities in trout and rats.[2,3]

SCCPs have been produced in the USA, Europe, Japan, India, China, and other countries.[4] The production volumes of SCCPs were 1500−2500 t in the European Union (EU) in 2006, 8800 t in the USA in 2005, and 502 t in Japan in 2001.[4,5] In the EU, SCCPs have been regulated under EU Directive 76/769/EEC since 2004 owing to their potential environmental risk. In Japan, SCCPs have been listed as type-I monitored chemical substances under the Act of the Evaluation of Chemical Substances and Regulation of Their Manufacture since 2005. Metal-working industries voluntarily phased out the use of SCCPs by 2007. By contrast, the production of total CPs in China has continued to increase, reaching 600 000 t in 2007.[6] Although the huge production and use of CPs in China could imply potential contamination of various media, there is little information on exposure to SCCPs.[7]

A pioneering survey in Japan conducted in 2003 strongly suggested dietary intakes to be the major route of exposure,[8] but there has been limited information on the dietary intake of SCCPs. The production volume of SCCPs was shown to vary significantly over one decade, which may have affected the dietary intake of SCCPs.

In the present study, SCCP levels in dietary samples from Korea, China and Japan in the 1990s and 2007−2009 were investigated using archived food samples in the Kyoto University Human Specimen Bank.[9,10] The concentrations of SCCPs were determined semiquantitatively using a high-resolution gas chromatography and high-resolution mass spectrometry with electron-capture negative ionization (HRGC/ECNI/HRMS).

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MATERIALS AND METHODS

Ethical Approval of the Study Protocol. The Ethics Committee of Kyoto University (Kyoto, Japan) approved the study protocol. Written informed consent was obtained from all study participants.

Sampling and Preparation of Duplicate Food. Food samples from the Kyoto University Human Specimen Bank [9,10] were used for the evaluation. Those food samples were analyzed for several elements and were stored at −30 °C. Persistent organic pollutants were not analyzed upon sampling in the 1990s. Regarding with persistency of SCCPs, the estimated half-lives of polychlorinated decanes (65% Cl content) in freshwater and marine sediment were 1340 days and 335 days in the aerobic condition, respectively.[11] Those of polychlorinated tridecanes (65% Cl content) in freshwater and marine sediment were 1790 days and 680 days, respectively.[11] Under anaerobic conditions, significant mineralization was not observed over ~90 days.[11] It was assumed that the SCCPs in the frozen samples did not deteriorate due to their persistency.

Participants had been requested to donate duplicate portions of all food and drinks which they consumed during a 24 h period, which we called 24 h duplicate samples. Two hundred 24 h duplicate samples were collected from: Hokkaido (Japan) in 1992 and 1995; Okinawa (Japan) in 1992 and 1995; Kyoto (Japan) in 1996 and 1997; Beijing (China) in 1993 and 2009; and Seoul (Korea) in 1994 and 2007.[10,12] A 100 d supply of meals and water was purchased by volunteers from markets in Kyoto, Okinawa, and Hokkaido in 2009. The collected duplicate food samples were mixed and homogenized. From 300 homogenized duplicate food samples, five samples (30 g each) were pooled into 60 samples (Figure S1 of the Supporting Information). Therefore, the duplicate food samples from 5 subjects were treated as one pooled sample weighing 150 g. The duplicate food samples were stored in glass bottles at −30 °C until analyses.

Chemicals. Polychlorinated decanes (44.82%, 55.00%, and 65.02% Cl contents), undecanes (45.50%, 55.20%, and 65.25% Cl contents), dodecanes (45.32%, 55.00%, and 65.08% Cl contents), and tridecanes (44.90%, 55.03%, and 65.18% Cl contents) were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany) and used as reference solution for quantification. Thirteen isomer standards of polychlorinated decanes (i.e., 1,1,1,3-tetrachlorodecane, 2,5,6,9-tetrachlorodecane, 1,2,9,10-tetrachlorodecane, 1,2,5,6,9-pentachlorodecane, 1,1,1,3,9,10-hexachlorodecane, 1,2,4,5,9,10-hexachlorodecane, 1,2,5,6,9,10-hexachlorodecane, 1,2,5,6,9,10-hexachlorodecane, 1,2,4,5,6,9,10-heptachlorodecane, 1,2,5,5,6,9,10-heptachlorodecane, 3,4,5,6,7,8,9-octachlorodecane, 1,1,1,3,8,10,10,10-octachlorodecane, and 1,2,3,4,5,6,-7,8,9-nonachlorodecane) were obtained from Dr. Ehrenstorfer GmbH and Chiron AS (Trondheim, Norway) and used for analyses of the response factor. $^{13}C_{12}$-2,3,3′,5,5′-pentachlorobiphenyl (CB-111; Cambridge Isotope Laboratories, Andover, MA, USA) was used as the internal standard (syringe spike) for the SCCPs. Acetone, hexane, dichloromethane, and sodium sulfate were purchased as reagents and solvents for the residual pesticides test and polychlorinated biphenyl test (Kanto Chemical Company Incorporated, Tokyo, Japan).

Extraction, Cleanup Procedure, and Instrumental Analyses. Food composite samples were stirred and 20 g aliquots divided onto a weighing dish. A 20 g aliquot of food composite sample was extracted with 100 mL of 1:1 (v/v) acetone/hexane on a separatory funnel shaker (SFS) for 10 min (SR-2DS; Taitec Corporation, Limited, Saitama, Japan). Extracts were filtered and the residues extracted again with 100 mL of 1:1 acetone/hexane on a SFS for 10 min. Extracts were combined and washed with 500 mL of hexane-washed distilled water on a SFS for 10 min. The water layer was extracted twice with 50 mL of hexane on a SFS for 10 min. Organic layers were combined and washed again with 100 mL of hexane-washed distilled water on a SFS for 10 min. The organic fraction was dried with anhydrous sodium sulfate and evaporated to ~20 mL using a rotary evaporator. Crude extract was diluted to 20 mL using a volumetric flask. A 2 mL aliquot of the crude extract was dried on a balance and the weight of residue evaluated as fat content.

Another 2 mL aliquot of the crude extract was divided using a transfer pipet and loaded on an 8 g activated florisil column (Florisil PR; Wako Pure Chemicals, Osaka, Japan) that had been preconditioned with 90 mL of 1:4 (v/v) dichloromethane/hexane. SCCPs were eluted with 90 mL of 1:4 dichloromethane/hexane. The eluate was concentrated to 0.1 mL of decane and spiked with $^{13}C_{12}$-labeled CB-111 before HRGC/ECNI/HRMS analyses.

The HRGC/ECNI/HRMS system comprised a Hewlett-Packard 6890 Series Gas Chromatograph connected to a Thermo Fisher Scientific Finnigan MAT 95 XL (Thermo Fisher Scientific Incorporated, Yokohama, Japan). A short and thin capillary column (DB-5MS; 15 m × 0.25 mm i.d., 0.1-μm film thickness; Agilent Technologies, Palo Alto, CA, USA) was employed. The HRGC/ECNI/HRMS conditions are listed in Table S1 of the Supporting Information. All analytes were quantified by comparing the peak area of the particular compound in sample extracts with that of the internal standard ($^{13}C_{12}$-labeled CB-111). The highest peak in the $[M-Cl]^-$ ion group was employed as the quantification ion, as previously described.[13]

Quantification. Zencak et al. [14] showed that quantification using reference SCCP solutions with different chlorine content caused errors of >100% in ECNI/MS because the response factors of each congener were highly dependent upon chlorine numbers. In the present study, first, 13 isomer standards of polychlorinated decanes were analyzed and the relationship between chlorine number and response factor evaluated. Peak areas of each chlorinated decane isomers were compared between time-of-flight mass spectrometry with electron impact ionization (EI/MS) and ECNI/MS modes. A total ion chromatogram (TIC) was obtained in scan mode (m/z 50−550). $[M-2H3Cl]^+$ and $[M-Cl]^-$ were chosen as relatively specific ion fragments in EI/MS and ECNI/MS modes respectively and used for selected ion monitoring.

For each carbon chain length (C_{10-13}), 45%, 55%, 65% Cl and their 1:1:1 mixtures were prepared. The composition of congeners with different chlorine number was investigated in four reference solutions. Chlorine content was calculated based on peak areas of TIC peaks (m/z 50−550) in EI/MS and then compared with contents certified by the manufacturer.

The 1:1:1 mixtures of SCCPs with 45%, 55%, and 65% Cl contents were diluted and used for calibration curves of each congener based on the composition determined by EI/MS. Therefore, concentrations of SCCPs should be considered to be semiquantitative values.

Quality Control. Instrumental detection limits (IDLs) were defined as the mass of analyte producing a peak with a signal-to-noise ratio of 3. Procedural blank samples contained no detectable concentrations of SCCPs, so the method detection limit (MDL) value was considered to be equal to the IDL.

Extraction efficiencies and recoveries were evaluated by seven replicate fortifications (fortified by two reference solutions of different composition) of a 20 g food composite sample with low contamination. To evaluate the variation in analysis, including the preparation, extraction, purification, and instrumental conditions, intra- and interday variation was determined. The intraday variation was evaluated based on analyses of the five replicated samples prepared from a single fortified food composite sample. The interday variation was determined by comparing the five replicated samples prepared from a single fortified food composite sample on five different days.

Procedural blanks were processed in parallel with each batch of seven samples to check for interference or contamination by solvents and glassware.

Statistical Analyses. For calculation of the summary statistics and for undertaking statistical comparisons, data values below the MDLs were assumed to have concentrations equal to one-half of the highest MDL among congeners in each carbon chain length because there were large variations in the detection limits. To evaluate the effect of variations in the detection limits on estimation of dietary intake, calculations were carried out using zero for values below the MDLs. Statistical analyses were conducted using JMP (Version 4; SAS Institute Incorporated, Cary, NC, USA). The mean, range, and geometric mean (GM) were calculated for the dietary intake of SCCPs. There were large variations in the concentrations among groups, so log-transformed values were tested by the Tukey–Kramer honestly significant difference (HSD) test after ANOVA or the Student's *t*-test. Mean dietary intake of SCCPs was also compared using a nonparametric method (Steel–Dwass test). The 95th percentile estimate of the dietary intake was calculated by multiplying the GM by the geometric standard deviation (GSD) to the power of 1.64. Correlations were tested by Pearson's product moment correlation coefficient. Differences in proportions were tested by the Fisher's exact test. $P < 0.05$ was considered significant.

RESULTS

Quantification and Quality Assurance/Quality Control. In EI/MS, the difference in TIC peak areas of isomers with different chlorine number and position was less than 4-fold, whereas the ionization efficiency was significantly different in ECNI/MS (Figure S2 of the Supporting Information). TICs in EI/MS were considered to provide a relatively comparable response to different chlorine numbers of SCCPs. They were therefore employed to quantify SCCPs with the different chlorine contents in the reference solutions. Although selected isomers of SCCPs still showed different responses in EI/MS, this could be a surrogate for further validation of the method.

To validate the principle mentioned above, we planned to determine the compositions of congeners with different chlorine numbers. Good agreement between calculated chlorine contents and nominal chlorine contents was assumed to indicate accuracy in the analysis of compositions. In EI/MS, the congener composition of SCCPs was assumed to be proportional to the peak area percent of the TIC. This was despite the fact that SCCPs comprised various congeners with different molecular weight, positions of chlorine atoms, volatility, ionization efficiency, and other physicochemical characteristics. Reference solutions with a chlorine content of 45% containing di- and trichlorinated congeners showed deviations between calculated content and nominal content (56.04% vs 44.82% in polychlorinated decanes, respectively) (Table S2 of the Supporting Information), which indicated di- or trichlorinated SCCPs might have low responses in EI/MS mode. Conversely, reference solutions with 55% and 65% Cl provided good agreement between calculated content and nominal content (56.67% vs 55.00% for 55% solution; 65.62% vs 65.02% for 65% solution in polychlorinated decanes, respectively). Furthermore, 1:1:1 mixtures of SCCPs with 45%, 55%, and 65% Cl contents were analyzed in EI/MS (Table S2 and Figure S3 of the Supporting Information). Although the calculated chlorine contents in 1:1:1 mixtures of SCCPs indicated slightly higher than nominally estimated content (possibly due to a low response in di- and trichlorinated congeners), the deviation was <5% (60.03% vs 54.95% in polychlorinated decanes). Polychlorinated undecanes, dodecanes, and tridecanes also showed similar results to polychlorinated decanes. Therefore, the composition of 1:1:1 mixtures of SCCPs with 45%, 55%, and 65% Cl contents was considered to be optimal for calibration curves because it contained detectable amounts of 5–9 chlorinated congeners without losing agreement between calculated and nominal chlorine contents (2.2–28.9%, 3.5–22.9%, 7.8–22.3%, and 9.4–21.3% for polychlorinated decanes, undecanes, dodecanes, and tridecanes, respectively; Table S2 of the Supporting Information).

The calibration curves for quantification in ECNI/MS were made using peak areas of the $[M-Cl]^-$ ion chromatogram of each congener. It was assumed that the congener concentrations in the 1:1:1 mixtures of SCCPs were proportional to the percentage of peak areas determined in EI/MS. For example, 100 ng/mL concentration of 1:1:1 mixtures for decanes was assumed to contain $C_{10}H_{17}Cl_5$ at 28.93 ng/mL because its composition was calculated to be 28.93% based on the area of the TIC. Therefore, the concentrations shown in the present study should be considered to be semiquantitative values. The 1:1:1 mixtures of SCCPs with five dilutions were analyzed and values plotted using a linear fit (Table S3 of the Supporting Information). Linearity (r) for the congeners was >0.998.

The MDLs in ECNI/MS are shown in Table S4 of the Supporting Information. Nonachlorinated congeners were the most sensitive, whereas pentachlorinated congeners showed the highest MDLs for each carbon chain length.

Recoveries ranged from 81% to 134% in high-fortified samples and 97% to 119% in low-fortified samples (Table S4 of the Supporting Information). An isotope-labeled standard of SCCPs was not available, so a cleanup standard was not included in this analysis. Although the matrices of food composite samples were heterogeneous, it was assumed that recoveries in fortified samples were not different among analyzed samples. Intraday and interday variations (using relative standard deviations (RSDs)) were less than 10% in most of the congeners. The greatest variation was found for the interday $C_{11}H_{19}Cl_5$ (14.1% RSD). There were no detectable residues in any of the procedural blanks ($n = 9$). In addition, analyses of wash out with hexane from a control vacant container revealed SCCPs at lower-than-detectable limits.

For comparison with the work of other research teams, the composition of the technical mixture Chlorowax 500C was determined (Figure S4 of the Supporting Information). Its calculated chlorine content was 59.80%, which was comparable with the product specification (58%).

Characteristics of the Study Participants. Three-hundred samples were collected from the five study sites (Table 1; Figure S1 of the Supporting Information). A food duplicate

Table 1. Demographic Characteristics and Mean Food Intakes of the Study Participants in the 1990s and 2007−2009[a]

area	year	*n*	sex	age	HSD	height	weight	BMI	food consumption		HSD	fat	HSD
			male/female	(years)	test[b]	(cm)	(kg)		(g day^{-1})	(g kg-bw^{-1} day^{-1})	test[b]	content (%)	test[b]
Beijing	1993	25	0/25	35.5 ± 2.3	A	158.7 ± 2.7	55.0 ± 3.5	21.8 ± 0.9	2249 ± 408	41.0 ± 2.6	AB	3.0 ± 0.3	BC
	2009	25	0/25	26.5 ± 0.9	B	163.8 ± 2.3	69.8 ± 3.5	26.0 ± 1.9	3054 ± 365	43.8 ± 5.6	A	3.9 ± 0.4	A
Seoul	1994	25	0/25	37.8 ± 5.7	A	161.7 ± 0.6	56.3 ± 3.9	21.5 ± 1.6	1777 ± 457	31.7 ± 2.0	C	1.5 ± 0.6	E
	2007	25	0/25	35.8 ± 4.0	A	158.5 ± 3.1	53.4 ± 1.7	21.3 ± 0.8	2062 ± 152	38.7 ± 3.1	ABC	2.2 ± 0.3	CDE
Hokkaido	1992, 1995	35	0/35	51.7 ± 4.9	C	150.9 ± 1.6	54.5 ± 2.2	24.0 ± 1.2	2249 ± 274	41.3 ± 5.7	AB	1.8 ± 0.4	DE
	2009	35[c]	0/1	26−29		158	50.9	20.4	1901 ± 161	37.3 ± 3.2	ABC	2.8 ± 0.4	C
Kyoto	1996, 1997	30	0/30	21.5 ± 0.4	B	158.4 ± 1.3	50.7 ± 4.2	20.2 ± 1.9	1740 ± 335	34.4 ± 6.4	BC	2.2 ± 0.6	CDE
	2009	30[c]	0/1	26−29		158	50.9	20.4	1575 ± 73	30.9 ± 1.4	C	2.5 ± 0.6	CD
Okinawa	1992, 1995	35	15/20	49.4 ± 4.4	C	155.1 ± 6.8	61.8 ± 4.7	25.7 ± 1.8	2614 ± 433	42.4 ± 6.5	AB	2.2 ± 0.3	CDE
	2009	35[c]	0/1	26−29		158	50.9	20.4	1845 ± 137	36.3 ± 2.7	ABC	3.6 ± 0.5	AB

[a] BMI: body mass index; kg-bw: body weight in kilograms; HSD test: Tukey−Kramer honestly significant difference test. Data are mean ± SD. Samples were collected by the food duplicate method except sample taken inHokkaido, Kyoto and Okinawa in 2009. [b] Means of age, food consumption (g kg-bw^{-1} day^{-1}), and fat content with different letters differ significance ($p < 0.05$, Tukey−Kramer HSD test). For example, A and B indicate that the corresponding values differ significantly at $p < 0.05$, whereas A and AB or AB and B indicate that the corresponding values do not differ significantly. [c] Food samples were collected by five volunteers. Anthropometrics were assumed for females aged 26−29 years in 2007 in Japan (National Health and Nutrition Survey in Japan).

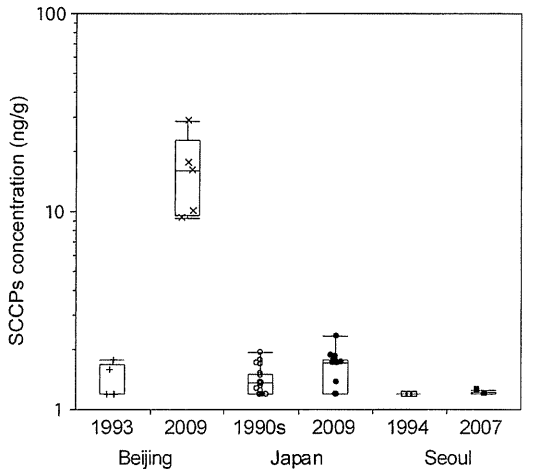

Figure 1. Box-and-whisker plot of the total SCCP amount in food duplicate samples. Box represents the first, second, and third quartiles. Lower whisker indicates lowest value within −1.5 interquartile range of the first quartile. Upper whisker indicates highest value within +1.5 interquartile range of the third quartile.

sampling technique was used to collect the samples (except for the 2009 samples from Japan). The Japanese samples in 2009 were purchased in markets by five volunteers, and we assumed that the mean body weight of a female aged 26−29 years in 2007 in Japan was 50.9 kg (National Health and Nutrition Survey in Japan). Apart from male subjects ($n = 15$) in Okinawa in the 1990s, all of the other participants were females. The mean (±SD) age of the participants was 36.5 ± 11.5 years. There was a significant difference in the mean ages between the time periods in Beijing ($p < 0.05$, Tukey−Kramer HSD test). Food consumption by individuals varied among the study sites ($p < 0.05$, Tukey−Kramer HSD test), which might be a reflection of the differences in age, weight, and food habits among the subjects. However, there was no significant difference between the 1990s and 2000s within each study site (in Beijing 41.0 g kg-bw^{-1} day^{-1} in 1993 and 43.8 g kg-bw^{-1} day^{-1} in 2009; bw denotes body weight). The fat contents of the food composites are shown in Table 1. Samples from China showed significantly higher fat contents than those from the other countries ($p < 0.05$, Tukey−Kramer HSD test), although the difference was, at most, less than 3-fold.

Changes of SCCPs in Daily Consumed Food Samples. Changes of the total SCCPs concentrations in daily consumed foods in three countries were shown in a box-whisker plot (Figure 1). Whereas total SCCPs concentrations were homogeneous as indicated small variations within each group, 1 SCCPs concentrations in samples from Beijing in 2009 were notably higher than those in other samples from Japan or Korea.

In Japan, SCCPs were detected in 14 of 20 pooled samples in the 1990s and 13 of 20 pooled samples in 2009 (Table 2). The total SCCP concentrations ranged from below the MDLs to 1100 pg g^{-1}. Among the SCCP congeners, polychlorinated dodecanes (especially hepta- to nonachlorinated congeners) were the most frequently detected, followed by polychlorinated undecanes.

In Beijing, SCCP levels were profoundly elevated by 2 orders of magnitude from 1993 to 2009 and were much higher than those in Japan (Table 3). The highest concentration of total SCCPs was 28 000 pg g^{-1} in 2009. In 1993, hepta- to nonachlorinated dodecanes were detected in a similar manner to that in Japan. In contrast, in 2009, various congeners were detected at comparable magnitudes from polychlorinated decanes to tridecanes. Even though the samples from Beijing in 2009 showed high total SCCP concentrations, highly chlorinated dodecanes (i.e., $C_{12}H_{18}Cl_8$ and $C_{12}H_{17}Cl_9$) showed comparable levels to those in Japanese samples.

In Seoul, no samples in 1994 contained detectable SCCP levels. Only one sample in 2007 showed trace levels of polychlorinated undecane.

The relationship between the total SCCP concentration and fat content was examined in the Japanese samples (Figure 2). The levels of SCCPs appeared to increase according to increases in fat content (Pearson's $r = 0.605$, $p < 0.001$).

The dietary intakes of SCCPs in East Asian countries are shown in Table 4. Between the two time-points, the GM

Table 2. Levels of SCCPs in the Composite Food Samples in Japan (pg g^{-1})[a]

	Hokkaido				Kyoto		Okinawa			
SCCP	1992/1995		2009		1996/1997	2009	1992/1995		2009	
congeners	range (n > MDL)	Q2	range (n > MDL)	Q2	range (n > MDL)	range (n > MDL)	range (n > MDL)	Q2	range (n > MDL)	Q2
$C_{10}H_{17}Cl_5$	<400 (0)		<400 (0)		<400 (0)	<400 (0)	<400 (0)		<400 (0)	
$C_{10}H_{16}Cl_6$	<200 (0)		<200–290 (1)		<200 (0)	<200 (0)	<200 (0)		<200 (0)	
$C_{10}H_{15}Cl_7$	<50 (0)		<50 (0)		<50 (0)	<50 (0)	<50 (0)		<50 (0)	
$C_{10}H_{14}Cl_8$	<20 (0)		<20 (0)		<20 (0)	<20 (0)	<20 (0)		<20 (0)	
$C_{10}H_{13}Cl_9$	<10 (0)		<10 (0)		<10 (0)	<10 (0)	<10 (0)		<10 (0)	
total $C_{10}Cl_x$	<400		<400		<400	<400	<400		<400	
$C_{11}H_{19}Cl_5$	<500 (0)		<500 (0)		<500 (0)	<500 (0)	<500 (0)		<500 (0)	
$C_{11}H_{18}Cl_6$	<300 (0)		<300 (0)		<300 (0)	<300 (0)	<300 (0)		<300 (0)	
$C_{11}H_{17}Cl_7$	<100 (0)		<100 (0)		<100–110 (1)	<100 (0)	<100–120 (2)		<100–120 (2)	
$C_{11}H_{16}Cl_8$	<50 (0)		<50 (0)		<50–67 (2)	<50 (0)	<50–79 (1)		<50 (0)	
$C_{11}H_{15}Cl_9$	<20 (0)		<20 (0)		<20 (0)	<20 (0)	<20 (0)		<20 (0)	
total $C_{11}Cl_x$	<500		<500		<500	<500	<500		<500	
$C_{12}H_{21}Cl_5$	<600 (0)		<600 (0)		<600 (0)	<600 (0)	<600 (0)		<600 (0)	
$C_{12}H_{20}Cl_6$	<400 (0)		<400 (0)		<400 (0)	<400 (0)	<400 (0)		<400 (0)	
$C_{12}H_{19}Cl_7$	<200–410 (4)		<200 (0)	240	<200–410 (2)	<200–380 (3)	<200–350 (2)		<200–490 (5)	370
$C_{12}H_{18}Cl_8$	<100–120 (4)		<100–110 (3)	100	<100–130 (2)	<100–120 (3)	<100–130 (6)	120	<100–150 (5)	120
$C_{12}H_{17}Cl_9$	<50–64 (4)	61	<50–63 (4)	63	<50–64 (3)	<50–65 (3)	<50–63 (4)	62	<50–64 (4)	64
total $C_{12}Cl_x$	<600		<600		<600	<600	<600		<600	
$C_{13}H_{23}Cl_5$	<900 (0)		<900 (0)		<900 (0)	<900 (0)	<900 (0)		<900 (0)	
$C_{13}H_{22}Cl_6$	<700 (0)		<700 (0)		<700 (0)	<700 (0)	<700 (0)		<700 (0)	
$C_{13}H_{21}Cl_7$	<300 (0)		<300 (0)		<300 (0)	<300 (0)	<300 (0)		<300–300 (1)	
$C_{13}H_{20}Cl_8$	<200 (0)		<200 (0)		<200 (0)	<200 (0)	<200 (0)		<200 (0)	
$C_{13}H_{19}Cl_9$	<50 (0)		<50 (0)		<50 (0)	<50 (0)	<50 (0)		<50 (0)	
total $C_{13}Cl_x$	<900		<900		<900	<900	<900		<900	
total $C_{10-13}Cl_x$	<200–590 (5)	61	<50–170 (4)	530	<50–760 (3)	<200–570 (3)	79–540 (7)	302	<200–1100 (5)	600

[a] SCCP: short-chain chlorinated paraffins; MDL: method detection limit; Q2: median.

of the dietary intake of SCCPs decreased slightly from 3000 to 2800 ng day^{-1} in Japan, and the difference was not statistically significant (p = 0.2, Student's t-test). Among the three study sites in Japan, SCCP intake was significantly higher in Okinawa than in Hokkaido in the 1990s (p < 0.05, Tukey–Kramer HSD test) and there was no difference in 2009 (p = 0.4, ANOVA). Participants in Okinawa in the 1990s consisted of males and females, so dietary intakes between males and females were compared. However, there was no significant difference in intakes of SCCPs (66.3 ng kg-bw^{-1} day^{-1} for males and 60.0 ng kg-bw^{-1} day^{-1} for females; p = 0.45 by Student's t test). In Beijing, the dietary intake of SCCPs in 1993 was within the same range as that seen in Japan. However, in 2009, SCCP intake in Beijing had increased up to tens of micrograms per day (GM: 43 μg day^{-1}) and was the highest among the three countries (p < 0.05, Tukey–Kramer HSD test). In Seoul, accurate exposure levels could not be estimated owing to the low detection frequency. Dietary intake of SCCPs was also estimated using zero for values below the MDLs (Table S5 of the Supporting Information). Mean intake in Japan in 2009 was 14 ng kg-bw^{-1} day^{-1}, which was 24.4% of the estimated intake using one-half of MDLs for values below the MDLs. Even though estimates differed among the different assumptions, statistical comparisons among the sampling sites produced the same results (Table S5 of the Supporting Information).

Exposure to SCCPs in China in 2009 was compared with the tolerable daily intake (TDI) for the non-neoplastic effects of SCCPs (100 μg kg-bw^{-1} day^{-1}).[15] The 95th percentile estimate of the dietary intake was 1.2 μg kg-bw^{-1} day^{-1}, which was >1% of the TDI (Table 4).

Correlations Among SCCPs in Food. The correlations among SCCP congeners were examined for the samples from Beijing in 2009 (n = 5) and Japan (n = 15) with detectable SCCP concentrations (Table 5). In Japan, there were significant correlations between $C_{12}H_{19}Cl_7$ and $C_{12}H_{18}Cl_8$ and between $C_{12}H_{19}Cl_7$ and $C_{12}H_{17}Cl_9$ (Pearson's r = 0.61 and 0.52, respectively, p < 0.05). In China, the congeners within polychlorinated dodecanes and tridecanes were significantly correlated (p < 0.05). In contrast, the congeners of polychlorinated decanes and undecanes showed significant correlations only between $C_{10}H_{16}Cl_6$ and $C_{10}H_{15}Cl_7$ and between $C_{11}H_{18}Cl_6$ and $C_{11}H_{17}Cl_7$ (Pearson's r = 0.97 and 0.95, respectively, p < 0.05). The congeners between polychlorinated dodecanes and tridecanes showed significant correlations in 5/16 combinations (p < 0.05), whereas the congeners between polychlorinated decanes and undecanes were significantly correlated in 4/12 combinations (p < 0.05). In other combinations, significant correlations were found only in 2/56 combinations (p < 0.05). The proportions of significant correlations were significantly higher in the C_{10}–C_{11} and C_{12}–C_{13} combinations than in the other combinations (p < 0.001, Fisher's exact test).

Table 3. Levels of SCCPs in Composite Food Samples from Beijing and Seoul (pg g^{-1})[a]

	Beijing			Seoul	
SCCP	1993	2009		1994	2007
congeners	range (n > MDL)	range (n > MDL)	Q2	range (n > MDL)	range (n > MDL)
$C_{10}H_{17}Cl_5$	<400 (0)	1000–3800 (5)	3000	<400 (0)	<400 (0)
$C_{10}H_{16}Cl_6$	<200 (0)	670–1800 (5)	1200	<200 (0)	<200 (0)
$C_{10}H_{15}Cl_7$	<50 (0)	180–520 (5)	330	<50 (0)	<50 (0)
$C_{10}H_{14}Cl_8$	<20 (0)	<20–68 (2)		<20 (0)	<20 (0)
$C_{10}H_{13}Cl_9$	<10 (0)	<10–24 (1)		<10 (0)	<10 (0)
total $C_{10}Cl_x$	<400	1900–6200	4400	<400	<400
$C_{11}H_{19}Cl_5$	<500 (0)	1400–1900 (5)	1600	<500 (0)	<500 (0)
$C_{11}H_{18}Cl_6$	<300 (0)	990–2000 (5)	1700	<300 (0)	<300 (0)
$C_{11}H_{17}Cl_7$	<100 (0)	440–1000 (5)	780	<100 (0)	<100 (0)
$C_{11}H_{16}Cl_8$	<50 (0)	83–240 (5)	140	<50 (0)	<50–56 (1)
$C_{11}H_{15}Cl_9$	<20 (0)	<20 (0)		<20 (0)	<20 (0)
total $C_{11}Cl_x$	<500	2900–4800	4400	<500	<500
$C_{12}H_{21}Cl_5$	<600 (0)	<600–1300 (1)		<600 (0)	<600 (0)
$C_{12}H_{20}Cl_6$	<400 (0)	<400–1300 (4)	940	<400 (0)	<400 (0)
$C_{12}H_{19}Cl_7$	<200–430 (2)	670–1400 (5)	1100	<200 (0)	<200 (0)
$C_{12}H_{18}Cl_8$	<100–110 (1)	240–630 (5)	420	<100 (0)	<100 (0)
$C_{12}H_{17}Cl_9$	<50–56 (2)	<50–170 (4)	120	<50 (0)	<50 (0)
total $C_{12}Cl_x$	<600	910–3900	2700	<600	<600
$C_{13}H_{23}Cl_5$	<900 (0)	<900–3600 (1)		<900 (0)	<900 (0)
$C_{13}H_{22}Cl_6$	<700 (0)	<700–4100 (4)	1400	<700 (0)	<700 (0)
$C_{13}H_{21}Cl_7$	<300 (0)	770–4100 (5)	1200	<300 (0)	<300 (0)
$C_{13}H_{20}Cl_8$	<200 (0)	390–1700 (5)	620	<200 (0)	<200 (0)
$C_{13}H_{19}Cl_9$	<50 (0)	91–340 (5)	120	<50 (0)	<50 (0)
total $C_{13}Cl_x$	<900	1500–14000	3300	<900	<900
total $C_{10-13}Cl_x$	<200–600 (2)	8500–28 000 (5)	15 000		<50–56 (1)

[a] SCCP: short-chain chlorinated paraffins; MDL: method detection limit; Q2: median.

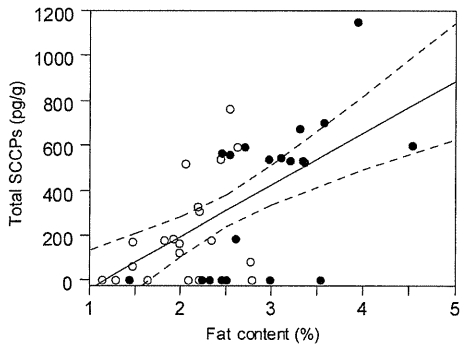

Figure 2. Correlation between the total SCCP amount in food duplicate samples and the fat content in Japan (Pearson's r = 0.605, p < 0.001). Open circles indicate samples from the 1990s and closed circles indicate samples from 2009. Solid line indicates a linear regression line. Dotted lines indicate 95% confidence interval of the linear regression line.

DISCUSSION

The daily intakes of SCCPs in Beijing showed a rather startling increase of more than 2 orders of magnitude between 1993 and 2009, whereas those in Japan did not change within a decade and were far lower than those in Beijing. By contrast, food samples from Seoul contained no detectable amounts of SCCPs. The food duplicate samples in Beijing in 2009 contained various SCCP congeners and, in contrast to Japan, high-chlorinated dodecanes were the major congeners, indicating that the sources of the contamination may differ between Japan and China.

There is limited information regarding the environmental distribution of SCCPs because of the difficulties associated with their analysis. Yuan et al. [7] examined SCCP levels in soil samples from Chinese e-waste dismantling sites, including control areas (10–20 km apart from e-waste dismantling sites), and found that they were higher than those in industrial areas in the United Kingdom.[16] CPs were mainly used for plasticizers for polyvinyl chloride in China (Table S6 of the Supporting Information). Further investigation was needed to clarify where the Chinese food samples were contaminated with SCCPs (e.g., cultivation, food processing, or distribution).

The median dietary intake of SCCPs per body weight in Japan in recent years was ~50 ng kg-bw^{-1} day^{-1}. A survey based on a market basket study produced a 50th percentile estimated intake of SCCPs of ~100 ng kg-bw^{-1} day^{-1} in adults.[8] That study was conducted in one site in Japan and analyzed one sample for each food category, which might have been influenced by the chance of incorporating high SCCP-containing products. Indeed, fat and fish products occupied 49% of the total SCCP intake. There is a possibility that cooking processes may eliminate SCCPs, similar

Table 4. Dietary Intakes of SCCPs in East Asian Countries[a]

	year (number of samples detected/total)		ng day^{-1}	ng kg-bw^{-1} day^{-1}	HSD test[a]	
			total	total	1990s	2000s
Beijing	1993 (2/5)	range	ND−2400	ND−36	A	
	2009 (5/5)	range (Q2)	26 000−69000 (53 000)	390−1000(700)		A
		mean ± SD	46 000 ± 18 000	660 ± 260		
		GM (GSD)	43 000 (1.5)	620 (1.5)		
		P95	85 000	1200		
Seoul	1994 (0/5)	range	ND	ND	A	
	2007 (1/5)	range	ND−2700	ND−50		B
Japan	1990s	range (Q2)	ND−5100 (2800)	ND−76 (53)	B	
	(14/20)	mean ± SD	3100 ± 760	55 ± 9.6		
		GM (GSD)	3000 (1.3)	54 (1.2)		
		P95	4400	72		
	2009	range (Q2)	ND−4700 (2800)	ND−93 (55)		C
	(13/20)	mean ± SD	2800 ± 710	56 ± 14		
		GM (GSD)	2800 (1.3)	54 (1.3)		
		P95	4100	81		
Hokkaido	1992/1995	range (Q2)	ND−3500 (2800)	ND−64 (51)	A	
	(4/7)	mean ± SD	2900 ± 340	53 ± 7.0		
		GM (GSD)	2900 (1.1)	52 (1.1)		
		P95	3400	65		
	2009 (5/7)	range (Q2)	ND−3600 (3000)	ND−71 (60)		n.s.
		mean ± SD	2900 ± 470	57 ± 9.2		
		GM (GSD)	2900 (1.2)	57 (1.2)		
		P95	3800	74		
Kyoto	1996/1997	range (Q2)	ND−2700 (2400)	ND−54 (48)	AB	
	(3/6)	mean ± SD	2400 ± 220	48 ± 4.9		
		GM (GSD)	2400 (1.1)	48 (1.1)		
		P95	2800	57		
	2009 (3/6)	range (Q2)	ND−2900 (2300)	ND−57 (45)		n.s.
		mean ± SD	2300 ± 450	46 ± 8.8		
		GM (GSD)	2300 (1.2)	45 (1.2)		
		P95	3100	62		
Okinawa	1992/1995	range (Q2)	3000−5100(3700)	48−76 (66)	B	
	(7/7)	mean ± SD	3900 ± 640	63 ± 9.6		
		GM (GSD)	3900 (1.2)	63 (1.2)		
		P95	5000	81		
	2009 (5/7)	range (Q2)	ND−4700 (3200)	ND−93 (63)		n.s.
		mean ± SD	3200 ± 890	63 ± 18		
		GM (GSD)	2300 (1.2)	61 (1.3)		
		P95	4900	96		

[a] SCCPs: short-chain chlorinated paraffins; ND: not detected; Q2: median; GM: geometric mean; GSD: geometric standard deviation; kg-bw: body weight in kilograms; HSD test: Tukey−Kramer honestly significant difference test; n.s.: not significant. 95th percentile (P95) estimates were calculated by multiplying the GM by the GSD to the power of 1.64. An HSD test was conducted to compare the GMs among the sampling sites in each time period.

to the case for various organic pollutants.[17] The estimated intake differed between different assumptions regarding values less than MDLs. Low-chlorinated SCCPs were less sensitive in ECNI/MS, and their MDLs were high compared with high-chlorinated SCCPs. The composition of SCCPs in food-stuffs should be investigated to evaluate the contribution of low-chlorinated SCCPs for precise estimation of dietary intake. In addition, there was uncertainty in comparisons with the results of other research teams due to differences in the methodology of collection of foods sample and chemical analysis.

In Japan, samples from Okinawa showed higher levels of SCCPs than those from other areas in the 1990s. Fifteen of 35 participants were male in Okinawa in the 1990s, although no difference in dietary intake between male and female participants was noted. The fat content of samples in Okinawa was comparable with that in Kyoto. This finding might be related to food habits because food habits in Okinawa have been reported to be unique in Japan.[18] The difference in intake of SCCPs was not significant in 2009, whereas samples from Hokkaido and Okinawa showed high levels compared with those in Kyoto. The fat content

Table 5. Pearson's Correlation Coefficients between the Concentrations of SCCP Congeners[a]

Beijing in 2009		C_{10}			C_{11}				C_{12}				C_{13}			
		Cl_5	Cl_6	Cl_7	Cl_5	Cl_6	Cl_7	Cl_8	Cl_6	Cl_7	Cl_8	Cl_9	Cl_6	Cl_7	Cl_8	Cl_9
$n = 5$	$C_{10}H_{17}Cl_5$															
	$C_{10}H_{16}Cl_6$	0.80														
	$C_{10}H_{15}Cl_7$	0.70	0.97[b]													
	$C_{11}H_{19}Cl_5$	0.72	0.35	0.21												
	$C_{11}H_{18}Cl_6$	0.94[b]	0.73	0.70	0.76											
	$C_{11}H_{17}Cl_7$	0.91[b]	0.90[b]	0.89[b]	0.59	0.95[b]										
	$C_{11}H_{16}Cl_8$	0.20	0.70	0.80	−0.08	0.29	0.56									
	$C_{12}H_{20}Cl_6$	0.77	0.39	0.38	0.42	0.75	0.63	−0.18								
	$C_{12}H_{19}Cl_7$	0.86	0.59	0.59	0.51	0.90[b]	0.82	0.09	0.96[b]							
	$C_{12}H_{18}Cl_8$	0.79	0.66	0.71	0.27	0.80	0.81	0.23	0.91[b]	0.96[b]						
	$C_{12}H_{17}Cl_9$	0.75	0.39	0.37	0.36	0.70	0.59	−0.21	0.99[b]	0.93[b]	0.90[b]					
	$C_{13}H_{22}Cl_6$	0.73	0.69	0.76	0.15	0.74	0.80	0.35	0.83	0.90[b]	0.99[b]	0.83				
	$C_{13}H_{21}Cl_7$	0.62	0.78	0.89[b]	0.04	0.67	0.82	0.64	0.61	0.75	0.88[b]	0.59	0.94[b]			
	$C_{13}H_{20}Cl_8$	0.66	0.75	0.85	0.06	0.69	0.81	0.53	0.70	0.82	0.94[b]	0.69	0.98[b]	0.99[b]		
	$C_{13}H_{19}Cl_9$	0.64	0.74	0.85	0.06	0.69	0.82	0.57	0.68	0.80	0.92[b]	0.66	0.97[b]	0.99[b]	0.99[b]	
Japan		Cl_7	Cl_8	Cl_9												
$n = 15$	$C_{12}H_{19}Cl_7$															
	$C_{12}H_{18}Cl_8$	0.614[b]														
	$C_{12}H_{17}Cl_9$	0.520[b]	0.434													

[a] SCCPs: short-chain chlorinated paraffins. [b] $p < 0.05$, significant correlation between two congeners.

was increased in samples from Hokkaido and Okinawa in 2009, which was associated with changes in the dietary intake of SCCPs. In Seoul, SCCPs were not detected in 1994. The fat content of those samples was the lowest of those tested, which might result in a lack of detection. Information on SCCPs levels in individual foodstuffs is needed for further analyses.

SCCP concentrations in food samples showed no substantial changes in Japan between the 1990s and 2009, even though SCCPs were withdrawn from metal-working fluids by 2007 (Table S6 of the Supporting Information). In Japan, SCCPs have been used as flame retardants, from which emission may be one of the sources of exposure. However, reports on the production volume for flame retardants are limited, and current production should be surveyed.

The congener patterns of SCCPs were investigated in one study.[19] This revealed comparable concentrations among different chain lengths, and the highest congeners had six or seven chlorine atoms. In the present study, the major components of SCCP congeners were polychlorinated dodecanes in Japan, and this was also seen in Beijing in 1993. However, the samples from Beijing in 2009 showed an entirely different composition, suggesting that the sources responsible for the temporal increase in SCCPs in Beijing have their own wide range of congeners. The associations among the congeners also showed characteristic patterns. The congeners seemed to share similar physicochemical properties and to be concomitantly transferred in the environment. The patterns of these congeners and their associations, such as chlorine content and chain length, may provide insights to identify exposure sources of SCCPs in East Asian countries. Further monitoring activities are needed for comparisons of congener patterns among technical CP mixtures, environmental media, and biota.

SCCPs were consistently present at elevated levels in food samples from Beijing in 2009. In the present study, only SCCPs currently evaluated by the Reviewing Committee for Persistent Organic Pollutants were analyzed. Medium-chain CPs and long-chain CPs were not analyzed because they are not listed or suggested to be persistent organic pollutants in the Stockholm Convention. The results of the present study indicate that SCCPs in particular may be of concern in China. China is considered to be the largest manufacturer of SCCPs in the world (Table S6 of the Supporting Information). The high production and use of SCCPs results in the high levels of SCCPs detected in dietary samples from Beijing (and possibly the whole of China).

The present study had several limitations. We chose five sampling sites from Japan, Korea, and China. This does not necessarily represent exposure to SCCPs over all of these countries. Sampling time periods were also limited to 2 years in each country. Further confirmation is required if the increase in dietary intake of SCCPs in China corresponded to a large increase in the production of CPs. In addition, samples archived in the specimen bank for >10 years may deteriorate during storage. The homogeneity of SCCPs in frozen conditions should be confirmed in future studies. There was no available isotope-labeled reference solution, which might assess a difference in recoveries due to the heterogeneity of sample matrix. Finally, the analysis of SCCPs is associated with difficulties due to their enormous number of isomers and congeners. In this study, compositions of SCCPs were estimated using EI/MS, so concentrations were considered to be semiquantitative values. There has been no agreed method for the analysis, so interlaboratory calibration and certification in standard reference material will circumvent this problem.

SCCPs have been reported to cause liver toxicities.[20] Induction of peroxisomes was considered to be a mode of action of SCCP toxicity.[21] However, peroxisome proliferation in the human liver is controversial.[22] Toxicological investigations for SCCPs are required.

We found changes in dietary exposure to SCCPs in Beijing, Seoul, Kyoto, Okinawa, and Hokkaido. With the exception of Seoul, consumers in all study sites were exposed to SCCPs. Although median dietary exposure was 100-fold less than the TDI, the significant change in SCCP exposure observed in Beijing was quite alarming. It is therefore essential to refine the estimates of dietary intake by targeting food types and source identification to ensure that food provided for consumers is safe. In addition, temporal comparisons of pollutants provide important information regarding the contributions of historical production and/or the effects of regulatory actions.

■ ASSOCIATED CONTENT

Supporting Information. Information regarding GC and ionization conditions for the determination of SCCPs as well as schematic presentation of the food sampling process. This material is available free of charge via the Internet at http://pubs.acs.org.

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ARTICLE

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Marine Sponge: A Potential Source for Methoxylated Polybrominated Diphenyl Ethers in the Asia-Pacific Food Web

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Supporting Information

ABSTRACT: Marine sponges collected in Palau, Micronesia, were investigated for hydroxylated or methoxylated analogues of brominated diphenyl ethers (BDEs), brominated dibenzo-*p*-dioxin (BDD), and brominated biphenyls. The neutral fractions of *Haliclona* sp. and *Callyspongia* sp. contained 2′-methoxy-2,3′,4,5′-tetraBDE, 6-methoxy-2,2′,4,4′-tetraBDE, 2′,6-dimethoxy-2,3′,4,5-tetraBDE 2,2′-dimethoxy-3,3′,5,5′-tetrabromobiphenyl, several methoxy-triBDEs, and dimethoxy-penta-/hexaBDEs. The methoxylated BDEs in sponges were strikingly similar to those of local fish living in the western Pacific Ocean. The total concentrations of these compounds (ΣMeO-PBDE) in both sponges were 63.5 μg/g extractable organic matter (EOM) for *Haliclona* sp. and 36.5 μg/g EOM for *Callyspongia* sp., which were about 2 orders of magnitude higher than the levels seen in tropical coral reef fish (unicornfish or surgeonfish) (280−290 ng/g lipid) and groupers (550 ng/g lipid) from Okinawan coastal waters. The phenolic fractions of both sponges contained hydroxy-methoxy tetra-/pentaBDEs as well as hydroxy-tetraBDD, in addition to the corresponding phenolic tetraBDE analogues. Although the total concentrations of phenolic products (27−80 μg/g EOM) in both sponges fell within a range comparable to the methoxylated products, ΣOH-PBDE in local fish were trace level (less than 10 ng/g lipid of) or undetectable. This survey indicates that marine sponges are a possible source of the MeO-PBDE analogues that biomagnify via the food chain to the higher trophic organisms in the western Pacific, whereas the distribution of the corresponding hydroxylated analogues is limited.

KEYWORDS: OH-PBDE, MeO-PBDE, marine sponge, fish, natural production, Palau

INTRODUCTION

The marine environment is an abundant source of halogenated compounds produced by marine plants, animals, and bacteria.[1] Chemical investigations of marine sponges of the family *Dysideidae* have resulted in the isolation of various bioactive brominated aromatic metabolites, including bromophenol, polybrominated diphenyl ethers (PBDEs), and brominated dibenzo-*p*-dioxins (BDD).[2−5]

Methoxylated tetrabromodiphenyl ethers (MeO-tetraBDEs) have been isolated not only in marine sponges[5,6] but also in marine algae,[7,8] mussels,[7] fish,[9−12] and marine mammals.[13−17] In some cases, the levels of MeO-tetraBDEs in marine mammals reach the parts per million range, making them comparable to or higher than levels of anthropogenic persistent organic pollutants such as polybrominated diphenyl ethers (PBDEs). Furthermore, dimethoxylated tetrabromobiphenyl (diMeO-BB) and dimethoxylated tetraBDE (diMeO-tetraBDE) have been detected in the blubber of whales and in shark livers from Japan[12,16] as well as in Australian marine mammals.[13,17] These compounds are thought to originate from biogenic sources (e.g., cyanobacteria)[18] and likely biomagnify in higher trophic organisms via the food chain.

In addition to methoxylated analogues, hydroxylated PBDEs (OH-PBDEs) have been isolated in algae, mussels,[19] and marine sponges.[5,6] It has been suggested that OH-PBDEs from *Dysidea* sp. are produced by sponge-associated cyanobacteria[20] or a *Vibrio* sp. bacterium.[21] Most OH-PBDEs exhibit a variety of bioactivities, including antibacterial and antifungal properties,[22] cytotoxicity, and enzyme inhibition.[2] Some of them have been found in the marine food web, such as in fish plasma,[9,23] and even in human blood.[24] However, it is still unclear whether the OH-PBDEs distributed in higher trophic organisms are secondary metabolites of natural MeO-PBDEs or metabolites of anthropogenic PBDEs.

In an attempt to identify the origin of the PBDE analogues distributed in higher trophic organisms, we screened several marine sponges collected from Palau in 2005 and found that *Haliclona* sp. and *Callyspongia* sp. contained abundant MeO- or OH-analogues of tri-, tetra-, penta-, and hexaBDEs. The aim of this study was to investigate whether brominated secondary metabolites in sponges are identical to the brominated compounds distributed in fish samples from the western Pacific. The present study describes the gas chromatography/mass spectrometry (GC/MS) profile and concentrations of sponge brominated products compared to those in coral reef fish from coastal waters of Palau, Guam, and Japan.

EXPERIMENTAL PROCEDURES

Sponge Collection. *Haliclona* sp. (class Demospongia, order *Haplosclerida*, family *Haliclonidae*) and *Callyspongia* sp. (class Demospongia,

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Table 1. Mass Spectral Characteristics of Brominated Compounds Isolated from *Haliclona* sp. (Figure 1) in Palau

product	RRT[a]	M^+ (m/z)	major fragment ion (ion abundance %)[b]	target ion/confirmed ion
		Neutral Fraction		
peak a	0.703	434	M-CH_3Br (80), M-Br_2(40)	438/342
peak b	0.720	434	M-CH_3Br (50), M-Br_2(50)	438/342
peak c	0.750	434	M-CH_3Br (55), M-Br_2(40)	438/342
peak d	0.799	434	M-CH_3 (40), M-Br_2(10)	438/421
2′-MeO-BDE28	0.758	434	M-CH_3Br (50), M-Br_2(80)	438/340
3′-MeO-BDE28	0.788	434	M-Br_2 (100)	438/282
peak e	0.871	512	M-CH_3Br (80), M-Br_2(75)	516/422
peak f	0.881	526	M-CH_3Br (30), M-CH_3Br-CH_3(30)	530/436
peak g	0.901	512	M-CH_3Br (70), M-Br_2(60)	516/422
peak h	0.955	542	M-CH_3OCH_3 (20), M-CH_3Br (50)	546/452
6′-MeO-BDE49	0.842	512	M-CH_3Br (80), M-Br_2(75)	516/422
2′-MeO-BDE68	0.871	512	M-CH_3Br (80), M-Br_2(75)	516/422
2,2′-diMeO-BB80	0.881	526	M-CH_3Br (40), M-CH_3Br-CH_3(30)	530/436
6-MeO-BDE47	0.901	512	M-CH_3Br (80), M-Br_2(75)	516/422
3-MeO-BDE47	0.930	512	M-Br_2 (100), M-Br_2CH_3(90)	516/360
5-MeO-BDE47	0.940	512	M-Br_2 (90), M-Br_2CH_3 (75)	516/360
2′,6-diMeO-BDE68	0.955	542	M-CH_3OCH_3 (20), M-CH_3Br (50)	546/452
4′-MeO-BDE121 (IS)	1.000	590	M-CH_3 (85) M-Br_2(20) M-CH_3CO-Br_2 (30)	594/579
6′-MeO-BDE90	1.048	590	M-CH_3Br (65), M-Br_2(60)	594/579
		Phenolic Fraction (Methylated)		
peak i	1.108	620	M-$COCH_3$ (25), M-CH_3Br (40)	626/583
peak j	1.207	620	M-$COCH_3$ (25), M-CH_3Br (50)	626/583
peak k	1.435	698	M-$COCH_3$ (20), M-CH_3Br (60)	704/661
6-MeO-[^{13}C]BDE47	0.901	524	M-$COCH_3$ (25), M-CH_3Br (50)	528/485

[a] Relative retention time to IS. [b] Relative intensity of the isotopic ion.

order *Haplosclerida*; family *Callyspongiidae*) were collected by scuba diving at Nikko Bay, Koror, Palau in August 2005. Freshly collected sponge material was immediately frozen and stored at −20 °C until molecular and chemical analyses were performed. The voucher specimens were deposited at the Department of Analytical Chemistry, Daiichi College of Pharmaceutical Sciences, Japan (Cord No. SP05-301 and SP05-319).

Fish Sampling. Twenty-five coral fish (unicornfish, *Naso lituratus*, n = 7, body size 20−30 cm) were collected by fishing from the same geographic regions of Palau (Nikko Bay) in which the sponge habitats were located. Surgeonfish (*Acanthurus xanthopterus*, n = 7, body size 20−30 cm) from Guam Island, Micronesia, and groupers (*Epinephelinae* sp., n = 11, body size 20−35 cm) from Okinawa Island, Japan, were collected for analysis of brominated compounds. The other samples investigated in this study were yellowfin tuna (*Thunnus albacores*), horse mackerel (*Trachurus murphyi*), parrotfish (*Chlorurus microrhinos*), kawakawa (*Euthynnus affinis*), and cornetfish (*Fistularia corneta*), which were purchased from local markets in the three islands in 2005.

Chemical Standards. Standards of five MeO-PBDE analogues, 2′-methoxy-2,3′,4,5′-tetrabromdiphenyl ether (2′-MeO-BDE68), 6-methoxy-2,2′,4,4′-tetrabromodipheyl ether (6-MeO-BDE47), 2′,6-dimethoxy-2,3′,4,5′-tetrabromodiphenyl ether (2′,6-diMeO-BDE68), 2,2′-dimethoxy-3,3′,5,5′-tetrabromobiphenyl (2,2′-diMeO-BB80), and 4′-methoxy- 2,3′,4,5′,6-pentabromodiphenyl ether (4′-MeO- BDE121), were kindly provided to us by Dr. G. Marsh (Stockholm University). 4′-MeO-BDE121 and 6-OH-[^{13}C]BDE47 (Cambridge Isotope Laboratories, Inc.) were used as internal standards for the determination of neutral and phenolic compounds, respectively. 2′-OH-6-MeO-BDE68 was synthesized by demethylation of 2′,6-diMeO-BDE68 in the presence of boron tribromide/dichloromethane. Standards of five phenolic products, 2′-OH-BDE68, 3-OH-BDE47, 5-OH-BDE47, 6-OH-BDE47, 2′-OH-BDE28, and 3′-OH-BDE28 were purchased from Cambridge Isotope Laboratories, Inc.

Sample Cleanup. Each wet sponge sample (2 g) was cut into pieces, and the homogenate was extracted with MeOH (50 mL) for a week. The extract was concentrated, and the residue was partitioned between 0.1 M HCl and ethylacetate (EtOAc). The EtOAc extractable organic matter (EOM) in the sponge sample was determined gravimetrically. Fish materials (5−20 g) were cut into pieces, and lipids were extracted with n-hexane and acetone (2:1 v/v). Lipid weights were then determined. A portion of each material was spiked with an internal standard solution of 4′-MeO-BDE121 and 6-OH-[^{13}C]BDE47. The extracts were subjected to gel permeation chromatography (GPC, Bio-Beads S-X3, Bio-Rad Laboratories Inc.) and eluted with dichloromethane (DCM)/n-hexane (1:1). After GPC, brominated products were partitioned by 1 M KOH/EtOH (7:3, v/v) and n-hexane. The organic phase was concentrated and purified by silica gel column chromatography (0.2 g, Wako gel S-1, Wako Pure Chemicals Inc.) with elution with 12% DCM in n-hexane (15 mL) (neutral fraction). The aqueous layer was acidified by HCl and back-extracted with n-hexane/diethylether (8:2, v/v) (phenolic fraction), which was then concentrated and reacted with diazomethane (methylated phenolic fraction). These fractions (500 μL) were subjected to GC/MS.

Identification and Quantification. Analyses of natural organohalogens were performed using a gas chromatograph (GC, Agilent 6980N) equipped with a mass-selective detector (5973i) in both electron-ionization (EI) and selected ion monitoring (SIM) modes. The GC was equipped with an HP-5MS column (30 m × 0.25 mm, 0.25-μm film thickness, J&W Scientific Inc.), and all ions in the range of

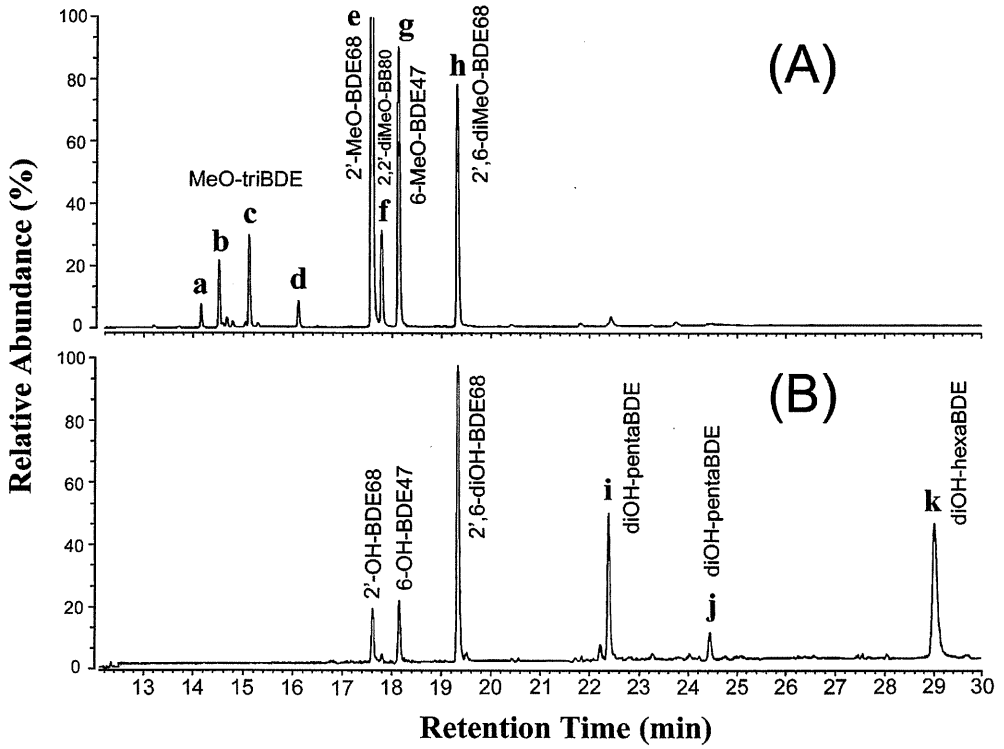

Figure 1. GC-MS (EI-TIC) chromatograms of the brominated compounds present in the neutral fraction (A) and in the methylated phenolic fraction (B) of *Haliclona* sp.

m/z 300 to 800 were recorded in EI mode to select the target ions for monitoring. Helium was used as a carrier gas at a constant flow rate of 1.0 mL/min. The injector and transfer line temperatures were 250 and 280 °C, respectively. The GC oven program was as follows: after injection at 70 °C (1.5 min), the temperature was increased at a rate of 20 °C/min to 230 °C (2 min) and then at a rate of 4 °C/min to 280 °C (20 min). The total run time was 45 min. In SIM mode, the selected ion channels (target and confirmed ions, *m/z* values) were used for quantification as shown in Table 1.

Quality Control. Standard materials, 2′-OH-BDE68 and 2′-OH-6-MeO-BDE68 were used for the recovery test of internal standard (IS). Cod liver oil was spiked with approximately 10−100 ng of each isomer. Recoveries of all analytes ranged from 85% to 102%. The limit of quantification (LOQ) in EI-GC/MS, which was determined using a signal-to-noise of 10, ranged from 1 to 20 pg on the column (0.2−1.5 ng/g lipid) for all analytes. Levels of MeO-PBDEs in laboratory blanks were all below the detection limit (0.2− 0.8 ng/g for all analytes). Solvent blanks did not contain any of the analytes under investigation, indicating no carryover effect between GC/MS runs. Total concentrations of MeO-tri/tetra/pentaBDE analogues and diMeO-tetra/penta/hexaBDEs were calculated by comparing their peak areas relative to the internal standard (4′-MeO-BDE121), assuming the same molar response factors. Quantification of OH-tetra/penta/hexaBDEs, diOH-tetra/-penta/-hexaBDEs, and OH-tetraBDDs was performed assuming the same response as 6-OH-[^{13}C]BDE47 after derivatization by diazomethane. The levels of diOH-PBDEs were calculated as diMeO-BDEs by subtracting the levels of the corresponding hydroxyl-methoxy BDEs.

RESULTS

Brominated Products in *Haliclona* sp. Selected ion chromatograms of brominated compounds in the neutral and phenolic fractions from a marine sponge (*Haliclona* sp.) are shown in Figure 1. The mass spectral characteristics and relative retention times (RRTs) of each peak and authenticated references to IS are listed in Table 1. In the neutral fraction, peaks **a**−**d** (monitored at *m/z* 438) were identified as methoxy-triBDEs (MeO-triBDEs) by full scan EI mass spectra (Figure S1, Supporting Information). Among them, the mass spectra of peaks **a**−**c** showed abundant fragment ions $[M\text{-}CH_3Br]^+$, indicating the *ortho* substitution of the methoxy group to the diphenyl ether bond, whereas the mass spectrum of peak **d** showed a characteristic fragment ion $[M\text{-}CH_3]^+$ for the *para* substitution of the methoxy group.[25] The RRTs of peaks **a**−**d** were not identical to those for the two authentic reference standards available for this study. Peaks **e** and **g** (*m/z* 516) were identified as 2′-MeO-BDE68 and 6-MeO-BDE47, respectively, whereas peaks **f** (*m/z* 530) and **h** (*m/z* 546) were identified as 2,2′-diMeO-BB80 and 2′,6-diMeO-BDE68, respectively, by RRTs and EI-MS comparison with the reference standard. For minor brominated compounds, a signal monitored at *m/z* 420 (t_R = 15.6 min) was tentatively identified as tribromodibenzo-*p*-dioxin (triBDD, M^+, *m/z* = 418 and $[M\text{-}BrCO]^+$, *m/z* = 311). As shown in Figure S2 (Supporting Information), the compound was interfered with by fragment ions derived from the coeluting compound (M^+, *m/z* = 448; $[M\text{-}CH_3Br]^+$, *m/z* = 354), which was identified as MeO-triBDD.

In the phenolic fraction, we isolated the corresponding four hydroxylated PBDEs (2′-OH-BDE68 and 6-OH-BDE47, 2,2′-diOH-BB80, and 2′,6-diOH-BDE68) as the methoxylated analogues (Figure 1). Furthermore, both peaks **i** and **j** were identified as diMeO-pentaBDEs (M^+, *m/z* = 620), whereas peak **k** was identified as diMeO-hexaBDE (M^+, *m/z* 698) (Figure 1 and Table 1). In the phenolic fraction without derivatization, we

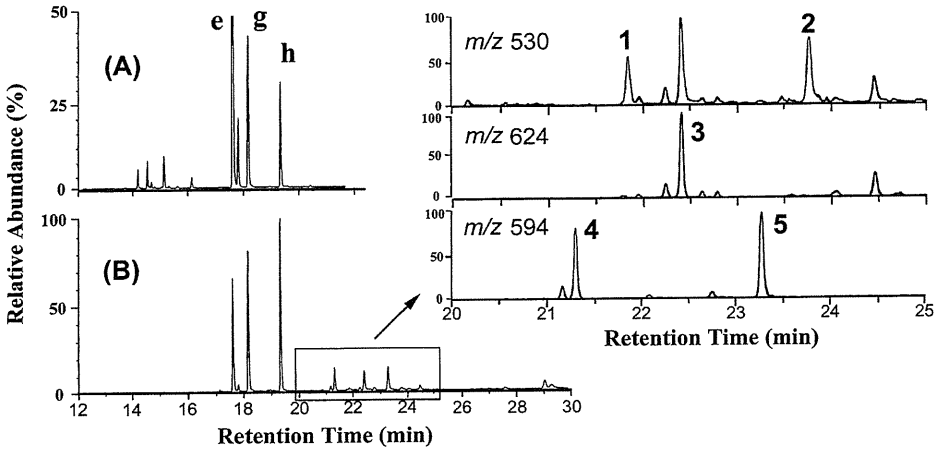

Figure 2. GC/MS (EI-TIC) chromatograms of the brominated compounds present in the neutral fraction (A) and in the methylated phenolic fraction (B) of *Callyspongia* sp. The selected ion chromatograms of hydroxylated tetraBDDs (*m/z* 530), dihydroxylated pentaBDEs (*m/z* 624), and hydroxylated pentaBDEs (*m/z* 594) between 20 and 25 min are also illustrated as their methoxylated derivatives.

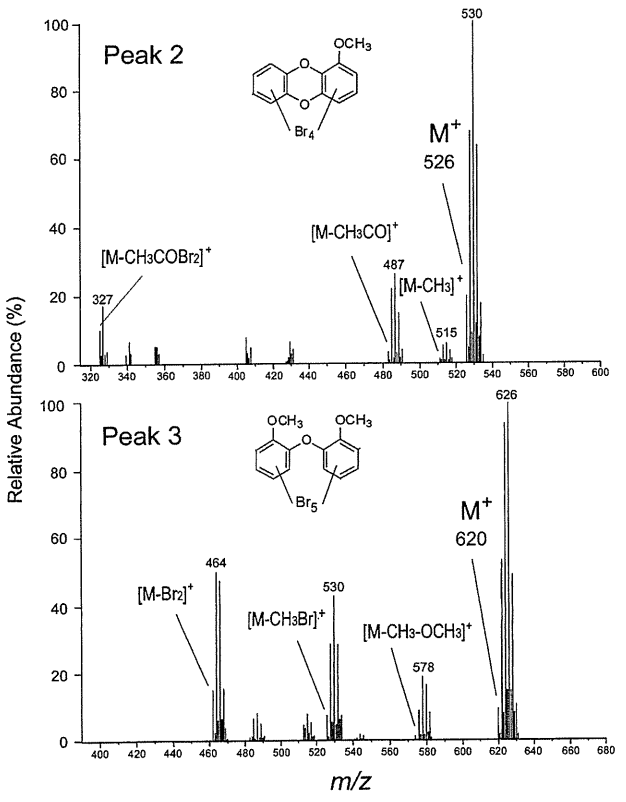

Figure 3. EI mass spectra of the methoxylated tetraBDD (peak 2) and dimethoxylated pentaBDE (peak 3) in Figure 2 isolated from the *Callyspongia* sp. Peak 3 was at the same GC retention time as peak i in Figure 1. EI-MS of peaks 1, 4, and 5 are presented in Figure S4 (Supporting Information).

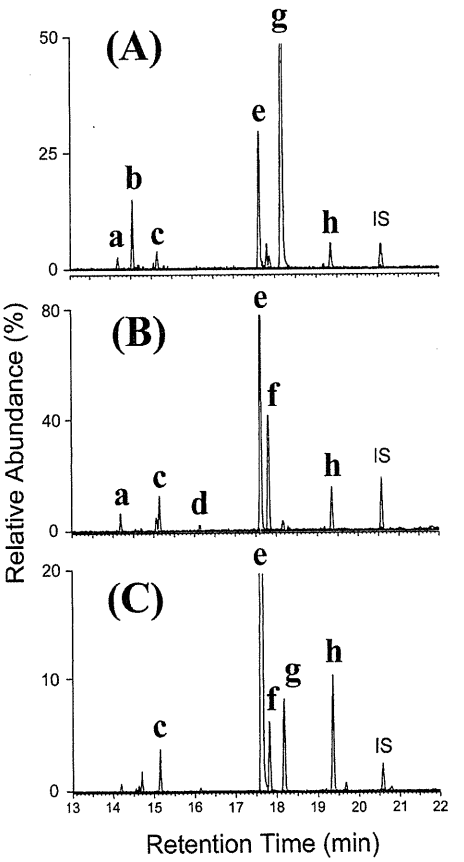

Figure 4. GC/MS chromatograms of brominated compounds in neutral fractions of unicornfish (*N. lituratus*) from Palau Island (A), surgeonfish (*A. xanthopterus*) from Guam Island (B), and grouper (*Epinephelinae* sp.) from Okinawa Island (C). Peak labeling is according to Table 1, corresponding to MeO-triBDEs, MeO-tetraBDEs, diMeO-tetraBB, and di-MeO-tetraBDEs in sponges (Figure 1). IS represents 4′-MeO-BDE121.

detected hydroxy-methoxy analogues of tetra- and pentaBDEs, one of which was tentatively identified as 2′-OH-6-MeO-BDE68 (t_R = 19.2 min, Figure S3, Supporting Information).

Brominated Products in *Callyspongia* sp. The GC profile of brominated compounds in the neutral fraction of *Callyspongia* sp. was similar to that of *Haliclona* sp. However, the phenolic fraction contained additional brominated components (peaks 1–5)