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).

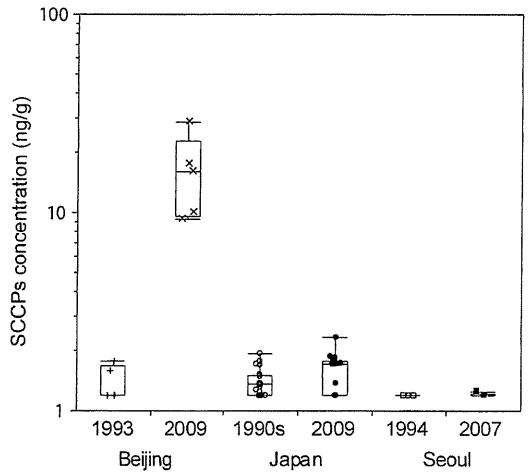

Figure 1. Box-and-whisker plot of the total SCCP amount in food duplicate samples. Box represents the first, second, and third quartiles. Lower whisker indicates lowest value within −1.5 interquartile range of the first quartile. Upper whisker indicates highest value within +1.5 interquartile range of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

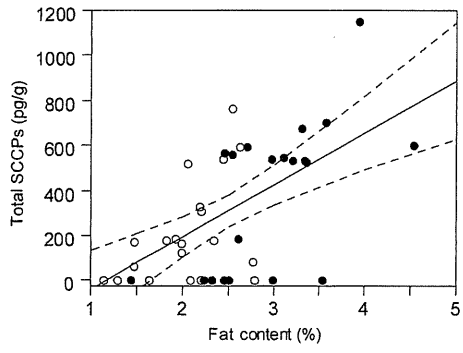

Figure 2. Correlation between the total SCCP amount in food duplicate samples and the fat content in Japan (Pearson's r = 0.605, p < 0.001). Open circles indicate samples from the 1990s and closed circles indicate samples from 2009. Solid line indicates a linear regression line. Dotted lines indicate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

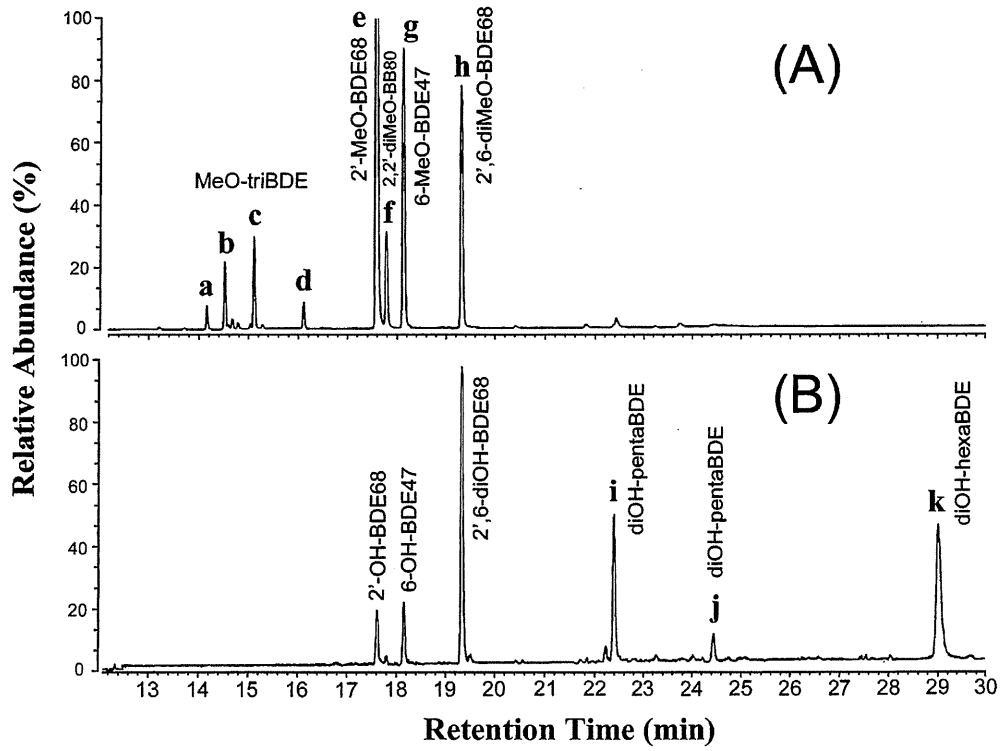

Figure 1. GC-MS (EI-TIC) chromatograms of the brominated compounds present in the neutral fraction (A) and in the methylated phenolic fraction (B) of *Haliclona* sp.

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

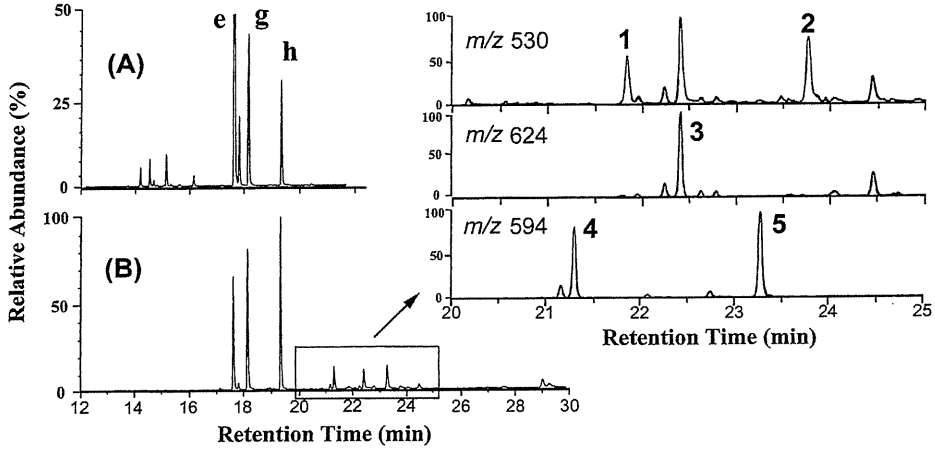

Figure 2. GC/MS (EI-TIC) chromatograms of the brominated compounds present in the neutral fraction (A) and in the methylated phenolic fraction (B) of *Callyspongia* sp. The selected ion chromatograms of hydroxylated tetraBDDs (m/z 530), dihydroxylated pentaBDEs (m/z 624), and hydroxylated pentaBDEs (m/z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

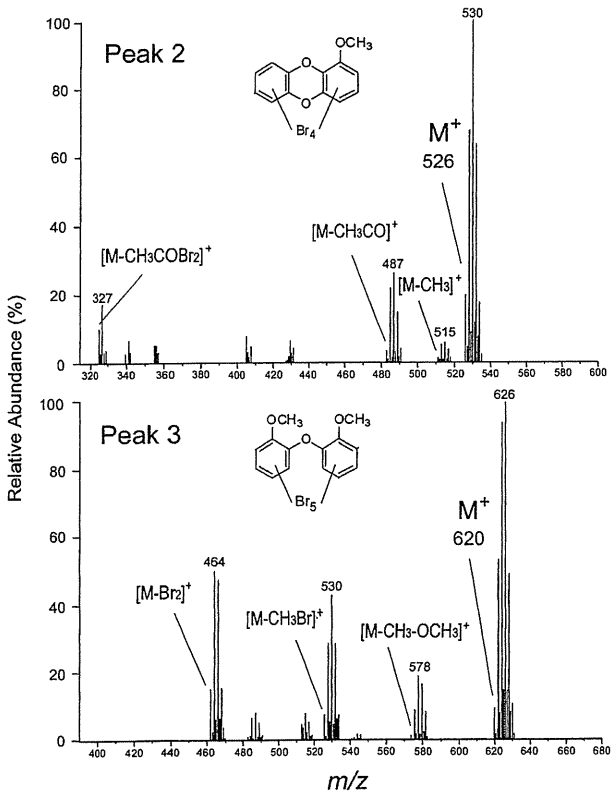

Figure 3. EI mass spectra of the methoxylated tetraBDD (peak 2) and dimethoxylated pentaBDE (peak 3) in Figure 2 isolated from the *Callyspongia* sp. Peak 3 was at the same GC retention time as peak i in Figure 1. EI-MS of peaks 1, 4, and 5 are presented in Figure S4 (Supporting Information).

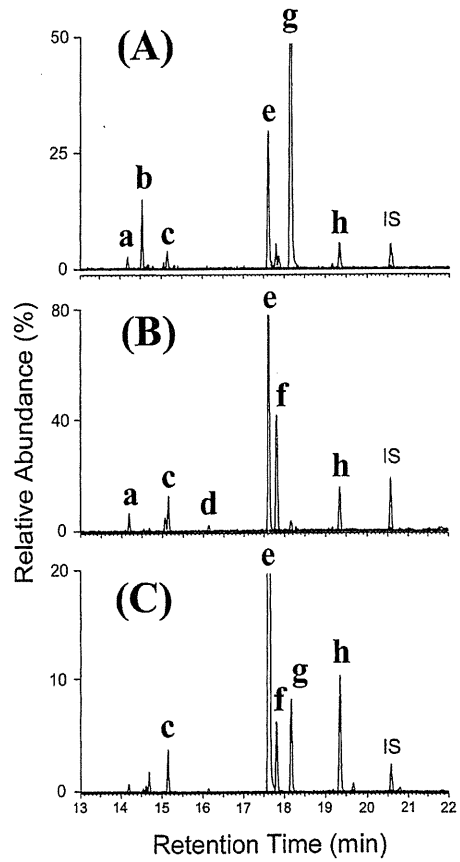

Figure 4. GC/MS chromatograms of brominated compounds in neutral fractions of unicornfish (*N. lituratus*) from Palau Island (A), surgeonfish (*A. xanthopterus*) from Guam Island (B), and grouper (*Epinephelinae* sp.) from Okinawa Island (C). Peak labeling is according to Table 1, corresponding to MeO-triBDEs,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

Table 2. Concentrations of Brominated Products in Marine Sponges and Fish Samples Collected between 2005 and 2009 from Palau and the Western Pacific

	marine sponge (Palau)		unicorn fish (n = 7, Palau)	surgeon fish (n = 7, Guam)	grouper (n = 11, Japan)
	Haliclona sp.	*Callyspongia* sp.	*N. lituratus*	*A. xenthopterus*	*Epinephelinae* sp.
extracts (%)	14.5[a]	21.6[a]	2.5 ± 1.2[b]	3.3 ± 1.1[b]	8.5 ± 4.2[b]
	concentration (μg/g EOM)			concentration (ng/g lipid)	
			Neutral Fraction		
2′-MeO-BDE68	43.2	19.1	130 (20–280)	110 (18–220)	290 (65–840)
6-MeO-BDE47	8.22	5.90	80 (20–190)	120 (14–240)	330 (40–950)
2,2′-diMeO-BB80	2.84	2.92	25 (10–120)	28 (15–60)	120 (75–600)
2′,6-diMeO-BDE68	6.72	4.51	41 (15–110)	43 (10–80)	82 (30–250)
sum of four congeners	**61.0**	**32.4**	**510 (70–1320)**	**301 (57–500)**	**1072 (210–3060)**
Σ_4MeO-triBDE	4.29	4.17	31 (10–87)	20 (5–38)	46 (15–160)
Σ_2MeO-tetraBDE	51.5	25.0	210 (40–470)	230 (32–460)	620 (105–1790)
ΣMeO-pentaBDE	0.31	1.56	nd	nd	nd
ΣdiMeO-tetraBDE	7.24	5.56	41 (15–110)	43 (10–80)	82 (30–250)
ΣdiMeO-pentaBDE	0.16	0.17	nd	nd	nd
ΣdiMeO-hexBDE	nd	nd	nd	nd	nd
total	**63.5**	**36.5**	**280 (65–670)**	**290 (47–580)**	**550 (150–2200)**
			Phenolic Fraction		
2′-OH-BDE68	4.71	20.4	nd	nd	nd
6-OH-BDE47	3.41	20.5	nd	nd	10 (<0.2–20)
2,2′-diOH-BB80	0.39	1.25	nd	nd	nd
2′,6-diOH-BDE68	18.7	37.9	nd	nd	nd
sum of four congeners	**27.2**	**80.1**			**10 (<0.2–20)**
ΣOH-triBDE[c]	nd	nd	nd	nd	nd
ΣOH-tetraBDE[c]	8.12	54.8	nd	nd	nd
ΣOH-pentaBDE[c]	2.59	12.9	nd	nd	nd
ΣdiOH-tetraBDE[c,d]	18.7	37.9	nd	nd	nd
ΣdiOH-pentaBDE[c,d]	9.67	9.03	nd	nd	10 (<0.2–24)
ΣdiOH-hexaBDE[c,d]	10.7	3.65	nd	nd	nd
ΣOH-tetraBDD[c]	0.21	0.45	nd	nd	0.2 (<0.2–3)
total	**50.0**	**119**			**10 (<0.2–24)**
			Ratio		
6-OH-BDE47/6-MeO-BDE47	0.41	3.47			0.03

[a] Ethylacetate extractable organic matter (%). [b] Arithmetic mean of hexane extractable lipid (%) ± standard deviation. [c] Calculated by selected ion abundance on the assumption of the same response of target analytes relative to 6-OH-[^{13}C]BDE47 (IS). [d] diOH-PBDEs include OH-MeO-PBDEs. Concentrations are medians, along with 10th–90th percentiles in parentheses. nd = not detected (less than LOQ = 0.2 ng/g lipid for 6-OH-[^{13}C]BDE47 and 0.5 ng/g lipid for 2′,6-diMeO-BDE68).

(Figure 2). The SIMs at m/z 530, 624, and 594 were indicative of OH-tetraBDDs (peaks **1** and **2**), diOH-pentaBDE (peak **3**), and OH-pentaBDEs (peaks **4** and **5**), respectively. The EI mass spectra of peaks **2** and **3** are shown in Figure 3, and those of peaks **1**, **4**, and **5** are available in Figure S4 (Supporting Information). The spectrum of peak **2** exhibited M^+ (m/z 526), $[M\text{-}CH_3]^+$ (m/z 511), and $[M\text{-}CH_3CO]^+$ (m/z 483), which undergoes further fragmentation of a $[M\text{-}CH_3COBr_2]^+$ ion, characteristic of MeO-tetraBDD. The EI mass spectrum of peak **3** exhibited M^+ (m/z 620), $[M\text{-}CH_3\text{-}OCH_3]^+$ (m/z 576), $[M\text{-}CH_3Br]^+$ (m/z 526), and $[M\text{-}Br_2]^+$ (m/z 462), characteristic of diMeO-PBDE.[16]

Profiles from Coral Fishes. Figure 4 shows the SIM profiles of tri- and tetraBDE products in the neutral fraction of unicornfish (*Naso lituratus*) from Palau Island, surgeonfish (*Acanthurus xanthopterus*) from Guam Island, and groupers (*Epinephelus* sp.) from Okinawa Island, Japan. Major brominated components in fish samples (muscles) were basically the same as brominated products from sponges (MeO-triBDEs, 2′-MeO-BDE68, 6-MeO-BDE47, 2′,6-diMeO-BDE68, and 2,2′-diMeO-BB80). Several fish samples (i.e., kawakawa, (*Euthynnus affinis*) from Palau and yellowfin tuna (*Thunnus albacores*) from Okinawa) also contained these methoxylated analogues with similar profiles to sponge brominated compounds (Figure S5, Supporting Information).

Levels of Brominated Compounds in Sponge and Fish Samples. The concentrations of major brominated compounds in sponges (EOM weight basis) and fish (lipid weight basis) are listed in Table 2. The total concentrations of MeO-tetraBDE analogues (sum of four compounds) were 61.0 μg/g EOM in *Haliclona* sp. and 32.4 μg/g EOM in *Callyspongia* sp., whereas the

corresponding OH-tetraBDE analogues were present at the levels of 27.2 and 80.1 μg/g EOM, respectively. The concentrations of MeO-PBDE analogues in nearby fish ranged from 65 to 670 ng/g lipids (median, 280 ng/g lipid), which accounted for 0.4% and 0.8% of ΣMeO-tetraBDE in *Haliclona* sp. and *Callyspongia* sp., respectively. OH-PBDE and diOH-pentaBDE in fish were present in concentrations of up to 40 ng/g lipid. The concentration ratios of ΣOH-PBDE/ΣMeO-PBDE were about 0.4 in *Haliclona* sp. and 3.5 in *Callyspongia* sp., whereas the ratios were smaller than 0.03 in all fish samples.

DISCUSSION

Sponge Brominated Products. Previous chemical studies on the isolation of brominated products from marine sponges have been extensively performed on the genus *Dysidea*,[3,6,21,26] where OH-/MeO-PBDEs may represent as much as 12% of the dry weight.[20] This genus contains a large population of cyanophytes within its tissues,[3,27] and the OH-PBDE analogues are likely produced by the symbiotic filamentous cyanobacterium *Oscillatoria spongeliae*.[20] In the present study, we screened brominated analogues in sponge families other than *Dysidea* sp., of which two genera, *Haliclona* and *Callyspongia*, that are common marine sponges in Palau, had an abundance of OH- and MeO-PBDEs.

In the neutral fractions of both sponge extracts, we isolated four MeO-triBDE congeners (unidentified structures), two MeO-tetraBDEs (2′-MeO-BDE68 and 6-MeO-BDE47), and three dimethoxylated analogues (2,2′-diMeO-BB80, 2′,6-diMeO-BDE68, and diMeO-pentaBDEs). Although the profiles and amounts of these products in two species seems to be different from those from *Dysidea* sp. in the Indo-Pacific,[6] 2′-MeO-BDE68 and 6-MeO-BDE47 were commonly found in the present study (*Haliclona* and *Callyspongia* sp.) as well as in *Dysidea* sp.[2,4] These products were also isolated from an aquatic sponge (*Ephydatia fluviatilis*) and marine red algae (*Ceramium tenuicorne*) from the Baltic sea[7,27] and marine algae (e.g., *Sargassum* sp.) from the Philippines,[8] indicating that these products are widespread in the marine food web.[28] For other products, 2,2′-diMeO-BB80 isolated in this study has not hitherto been reported in sponges or algae. This compound is likely derived from 2,2′-diOH-BB80 that has been isolated in the bacteria *Pseudoalteromonas phenolica* sp. from the Pacific.[29] Furthermore, MeO-triBDEs (four products) and diMeO-tetraBDE (as 2′,6-diMeO-BDE68) detected in both sponges are also produced by *Dysidea* sp.[27] Most of these products have been seen in whale blubber from Japanese coastal waters[15,16] and in Australia.[13,17]

In the phenolic fraction of marine sponges, 2′-OH-BDE68 and 6-OH-BDE47 were the predominant congeners in both species. Although diOH-penta- and diOH-hexaBDE were determined as methoxylated derivatives, these products included OH-MeO-analogues of tetra-, penta-, and hexaBDEs, some of which were confirmed by direct GC/MS measurement in EI mode (Figure S3, Supporting Information). One of the structures was tentatively identified as 2′-MeO-6-OH-BDE68 due to characteristic $[M\text{-}CH_3Br]^+$ for an ortho-substituted MeO group and *ortho*-bromine in the other ring.[25] Since it seems difficult to directly determine the diOH-PBDEs, the ratios of OH-MeO- and diOH-analogues present in the sponges remain unclear.

Interestingly, tetraBDE products were isolated as a mixture of hydroxy and methoxy analogues (about 1:2 for *Haliclona* sp. and 2:1 for *Callyspongia* sp.). However, the triBDE products were present only as methoxylated analogues, whereas the penta- and hexaBDE products were present primarily as dihydroxylated or hydroxy-methoxy PBDE analogues in both species. These findings suggest that bacterial O-methylation may occur in sponges, depending on the degree of bromination.[30] Alternatively, the biosynthesis process of diOH-PBDEs may be different from that of MeO-PBDEs.

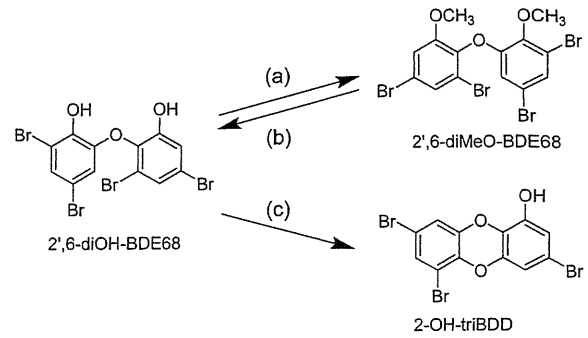

Figure 5. Proposed pathway for the biotransformation of dihydroxylated PBDEs. (a) bacterial *O*-methylation (ref 30); (b) microsomal demethylation (ref 37); and (c) bromoperoxidase-mediated condensation (ref 31) in marine biota.

In red algae and cyanobacteria from the Baltic Sea, Malmvarm et al.[18] demonstrated the presence of tri-, tetra-, and pentaBDDs. Although we could not confirm the occurrence of such PBDDs in sponges investigated, we studied the occurrence of at least two hydroxy-tetraBDDs (OH-tetraBDDs) in the phenolic fraction. The OH-tetraBDDs isolated in this study may be the same as products from an Australian marine sponge, *Dysidea dendyi*,[4,5] where diOH- or OH-MeO-pentaBDEs have been present as well. As proposed in Figure 5, we hypothesize that sponge-associated organisms produce OH-PBDDs by dehydrobromination of diOH-PBDEs (e.g., 2′,6-diOH-BDE68) present in *Callyspongia* sp. due to the loss of phenolic protons and bromine in the ortho position of the other ring. The levels of OH-tetraBDDs are estimated to be 0.2−0.45 μg/g EOM, which account for about 2−5% of diOH-PBDEs in the both sponges (Table 2). Bromoperoxidase may contribute to the internal cyclization from diOH-PBDEs[31] as it is suggested that chloroperoxidase can be involved in the condensation of chlorophenols to tetrachloro-dibenzo-*p*-dioxin.[32] Alternatively, OH-tetraBDDs could potentially be formed by photolysis of the diOH-PBDEs present in the sponges.[33]

Brominated Compounds in Fish. Because fish frequently feed on marine sponges in the coral reef environment, sponge-produced brominated compounds could have been transferred to fish such as unicorn fish included in this study living in the same regions of Palau. Our study shows that sponge-produced MeO-PBDEs are observed not only in Palauan fish, but also in the other fishes (surgeonfish and groupers) from the coastal waters of Guam (Micronesia) and Okinawa (Japan) (Figure 4S, Supporting Information). Similar profiles in fish indicate the wide distribution of MeO-PBDEs produced by marine sponges, likely explaining the higher concentrations observed in whales and dolphins in the Pacific and Oceania.[15,16] The present levels of MeO-tetraBDEs in fish from the western Pacific are comparable to the results from shark liver in Ishigaki Island, in the southern coast of Japan,[12] and blubber of killer whales from northern Japan.[15] In particular, the levels of MeO-tetraBDEs in groupers

from Okinawa seem to be 1 order of magnitude higher than those in bluefin tuna (up to 80 ng/g lipid) from Okinawa Island,[34] farmed tuna (up to 63 ng/g lipid) from the Mediterranean Sea,[11] Baltic salmon (up to 7 ng/g lipid), and Arctic cod liver (up to 17 ng/g lipid).[10] In addition, MeO-triBDEs, 2,2′-diMeO-BB80, and 2′,6-diMeO-BDE68 distributed in marine biota are also major products in the marine sponges investigated.[13,16] Considering that their levels in sponges on an EOM weight basis are about 2 orders of magnitude higher than those observed in fish on a lipid-weight basis (Table 2), it is evident that marine sponges are a potential source of MeO-triBDEs and diMeO-BB/BDEs, which could biomagnify via the food chain to higher trophic organisms.[35]

Total concentrations of 2′-OH-BDE68 and 6-OH-BDE47 are similar to those of the methoxylated analogues in both sponges. Nevertheless, these phenolic products were trace (less than 10 ng/g lipid) or undetectable in the local fish of Micronesian and Japanese coastal waters. A few fish samples (three of eleven groupers) from Okinawa Island contained diOH-pentaBDEs and OH-tetraBDDs, but the levels observed were low (up to 24 ng/g lipid) in this study. These results indicate that sponge-produced OH-PBDE analogues are less transferable to higher trophic animals or accumulate less in the fatty tissues of mammals. In fact, OH-tetraBDEs were present at 2% of the corresponding MeO-tetraBDE levels found in shark livers from Okinawa.[36] These phenolic PBDEs may be more concentrated in fish blood[9] and water-soluble body tissues.[24] In some cases, it is possible that the OH-PBDEs identified in the blood may originate from metabolites of anthropogenic PBDEs[28] via cytochrome P450-mediated hydroxylation in the liver. Such oxidations generally helps organisms make OH-PBDEs more water-soluble and thus excreted more easily as O-conjugates. Another biotransformation process is also possible via bacterial O-methylation of OH-PBDEs,[30] while it has been reported that microsomal demethylation of natural MeO-PBDEs to the corresponding OH-PBDEs may occur in the mammalian liver[37] (Figure 5). Further studies are necessary to elucidate the transformation and biomagnification processes of OH- and MeO-PBDEs in the environment.

■ ASSOCIATED CONTENT

Supporting Information. GC-EI-MS of peaks **a**−**d** in Figure 1, triBDD or methoxy-triBDD, hydroxy-methoxy analogues of tetraBDE, and pentaBDE; peaks 1, 4, and 5 in Figure 2; and SIM chromatograms of the neutral fractions from several fish materials in the western Pacific. This material is available free of charge via the Internet at http://pubs.acs.org.

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Analysis of perfluoroalkyl carboxylates in vacuum cleaner dust samples in Japan

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ABSTRACT

Perfluorooctanoic acid (PFOA) has long been an environmental contaminant of concern owing to its potential health risk. However, exposure to perfluorinated carboxylic acids (PFCAs) other than PFOA is not well understood. In this study, we investigated the concentrations of PFCAs in vacuum cleaner dust in Japan to measure the PFCAs contamination in an indoor environment. Most of the 77 samples contained PFCAs with 6–13 carbon atoms. The median concentration of perfluorononanoic acid (PFNA, 23.2 ng g^{-1}) was highest among PFCAs, followed by PFOA (20.8 ng g^{-1}) and perfluoroundecanoic acid (PFUnDA, 12.9 ng g^{-1}). The 90th percentile concentrations of PFNA, PFUnDA and perfluorotridecanoic acid (PFTrDA) were 948, 283 and 110 ng g^{-1}, respectively, and these were detected at greater concentrations than neighboring, even-numbered PFCAs. The proportion of long-chain PFCAs in vacuum cleaner dust from Japan was relatively higher than those reported for other countries. Factor analysis showed three independent factors. Odd-numbered long chain PFCAs (PFNA, PFUnDA and PFTrDA), which can correspond to factor 1, were major components of PFCAs in vacuum cleaner dust. Short chain PFCAs (factor 2) and even numbered long chain PFCAs (factor 3) were also statistically separated. These findings suggest that there are several sources of PFCAs with different origins in indoor environment. Further investigations into the origins of PFCAs are needed to evaluate indoor contamination with PFCAs.

1. Introduction

Perfluorinated alkyl acids such as perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) have been detected in various media in the environment, including wildlife and humans (Houde et al., 2006). In 2002, a major manufacturer, 3M Company, phased out PFOS production (Renner, 2001). Since then, several studies have demonstrated that PFOS and PFOA induce developmental toxicities in animals and humans (Lau et al., 2007; Steenland et al., 2010). Although PFOA was a major component of perfluoroalkyl carboxylates (PFCAs) emission, long chain PFCAs (perfluorononanoic acid (PFNA), perfluoroundecanoic acid (PFUnDA) and perfluorotridecanoic acid (PFTrDA)) have also been detected in discernible concentrations in samples collected from wildlife (Prevedouros et al., 2006; Furdui et al., 2008).

In human biomonitoring studies, PFOS was the major perfluoroalkyl acids (PFAAs) found in human serum. In addition, PFOA was found to be the most prevalent component of serum PFCAs in western countries, followed by PFNA, perfluorodecanoic acid (PFDA) and PFUnDA (Kärrman et al., 2007; Haug et al., 2009; Kato et al., 2009b). Our previous study of Japanese, Korean and Vietnamese adults showed that PFNA and PFUnDA were found in serum at concentrations generally similar to PFOA, and that these levels have continued to increase, even after 2002 (Harada et al., 2011). Factor analysis of PFCAs also revealed two major factors composed of PFNA, PFUnDA and PFTrDA (factor 1) and PFOA and perfluoroheptanoic acid (PFHpA) (factor 2) (Harada et al., 2011). However, the origin of these factors is still not known.

Long chain PFCAs have not been detected in food duplicate samples in Japan at concentrations greater than method detection limits (MDL: 0.1 ng g^{-1} for PFNA, 0.5 ng g^{-1} for PFDA and PFUnDA, respectively) (Kärrman et al., 2009). In addition, the predominance of odd numbered PFCAs has not been reported in aquatic systems in Japan (Murakami et al., 2008, 2009; Zushi et al., 2008; Raj Shivakoti et al., 2011). Fluorotelomer alcohols (FTOHs) are considered to be precursors of long chain PFCAs and have been detected in Japan and Western countries (Martin et al., 2002; Jahnke et al., 2007; Oono et al., 2008a,b; Mahmoud et al., 2009). Although atmospheric degradation of 8:2 FTOH has shown comparable yields of PFOA and PFNA (Ellis et al., 2004), the dominance of odd number PFCAs

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; PFAAs, perfluoroalkyl acids; IDLs, instrumental detection limits; LOD, limit of detection; MDLs, method detection limits; RSD, relative standard deviation; SD, standard deviation; GM, geometric mean; GSD, geometric standard deviation.

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in human serum has been observed only in East Asian countries (Harada et al., 2011). A review by Prevedouros et al. (2006) indicated that odd numbered PFCAs have been applied to fluoropolymer manufacturing aids and surfactants that were manufactured *via* oxidation of fluorotelomer olefins. Their application to commercial products might be an exposure source of long chain PFCAs in human serum. Given the indoor use of those products, a portion of them likely disperses and contaminates indoor dusts.

The primary goal of the present study was to investigate PFCAs in house dust. To achieve this goal, we investigated vacuum cleaner dust concentrations of PFCAs in Japan. To evaluate the potential factors influencing the PFCAs level, questionnaires regarding housing conditions and articles were collected. In addition, we conducted factor analysis to elucidate the potential compositions of PFCAs in indoor dust.

2. Materials and methods

2.1. Sample collection

To evaluate geographical differences in PFCAs in vacuum cleaner dust in Japan, we recruited 77 homes from Osaka, Kyoto, Wakayama and Toyama, Japan and conducted sampling from October to December, 2010. Osaka was selected to evaluate the effects of a local industrial source of PFOA (Saito et al., 2004; Morikawa et al., 2006; Niisoe et al., 2010). Vacuum cleaner dust samples were collected from the used bag of the household vacuum cleaner into a sealable polyethylene bag. Samples were shipped via an overnight delivery service to Kyoto University.

A 500 mg cleaner dust sample was taken out from the sealable polyethylene bag and hairs and plastic garbage were removed from the samples using forceps and a loupe. Samples were then sieved to remove materials greater than 150 μm in diameter and stored at −4 °C until analysis in the Kyoto University Human Specimen Bank (Koizumi et al., 2005, 2009). Information regarding the home condition, articles use and life habits was then collected by questionnaire (Table 1 and Supplemental 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 13 October 2010 (E960). Written informed consent was obtained from all participants.

2.2. Reagents

Methanol (LC–MS grade) and water (distilled LC–MS grade) were obtained from Kanto Chemicals (Tokyo, Japan). Ammonium hydroxide (25% in water) was purchased from Merck (Darmstadt, Germany). Benzyl bromide was purchased from Wako pure chemicals (Osaka, Japan). A mixture of $^{13}C_2$-labeled perfluorohexanoic acid (PFHxA), $^{13}C_2$-labeled PFOA, $^{13}C_4$-labeled PFOA, $^{13}C_5$-labeled PFNA, $^{13}C_2$-labeled PFDA, $^{13}C_2$-labeled PFUnDA and $^{13}C_2$-labeled perfluorododecanoic acid (PFDoDA) was obtained from Wellington Laboratories (Guelph, Ontario, Canada) and Perkin Elmer (Boston, MA).

2.3. Determination of PFCAs in vacuum cleaner dust

PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA and perfluorotetradecanoic acid (PFTeDA) were analyzed by gas chromatography/mass spectrometry (Agilent 6890GC/5973MSD, Agilent Technologies Japan, Ltd., Tokyo, Japan). Perfluorooctane sulfonamides were not included because main purpose in this study was to elucidate a pattern of PFCAs exposure in indoor environment. Vacuum cleaner dust samples were subjected to a clean-up procedure consisting of a weak anion exchange, solid-phase extraction. Briefly, approximately 500 mg of vacuum cleaner dust sample and internal standards (10 ng mixture of $^{13}C_2$-labeled PFHxA, $^{13}C_4$-labeled PFOA, $^{13}C_5$-labeled PFNA, $^{13}C_2$-labeled PFDA, $^{13}C_2$-labeled PFUnDA, and $^{13}C_2$-labeled PFDoDA) were put into a 50 mL polypropylene (PP) centrifugation tube and 20 mL of methanol were added. The samples were then vortexed and shaken on a vertical shaker for 30 min, after which they were placed in an ultrasonic bath for 15 min. Next, the samples were centrifuged (11 000g, 15 min), and the supernatant was then reduced to approximately 5 mL by rotary evaporation. Approximately 15 mL of water were subsequently added to the sample, and the solution was put through a WAX solid phase cartridge (6 cc, 150 mg 30 mm, Waters, Milford, MA, USA) that had been previously conditioned with 4 mL methanol and 4 mL water. Subsequent loading of the sample was followed by washing the sorbent with 4 mL 40% (v/v) methanol in water and a second wash using 8 mL methanol. The perfluorinated compounds were then eluted into a tube using 2 mL 2% (v/v) ammonium hydroxide in methanol. The solution was then dried under N_2, after which 0.25 mL of 100 mM benzyl bromide acetone and the performance standard $^{13}C_2$-PFOA were added. Next, the solution was transferred to an autosampler vial and heated for 1 h at 80 °C. Extracts were subsequently analyzed by GC/MS 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. Splitless injections (0.5 μL) were performed with the injector set to 220 °C, and the split was opened after 1.5 min. The initial oven temperature was 70 °C for 2 min, after which it was increased to 100 °C at 20 °C min^{-1}, and then to 280 °C at 30 °C min^{-1}. Ion fragments ($[M]^+$) were monitored and used for quantification (Supplemental Table 2).

Instrumental detection limits (IDL) were defined as the mass of the analyte producing a peak with a signal-to-noise ratio of 3, and ranged from 2 pg (PFTeDA) to 0.5 pg (other PFCAs) (Supplemental Table 2). All samples were quantified using a seven-point calibration curve (range: 0.5–50 000 ng mL^{-1} in methanol). Limit of detection (LOD) was defined as the lowest concentration with a relative standard deviation (RSD) of the relative response factors <15% ($n = 3$ for each concentration). The method detection limit (MDL) was defined as the blank response +3 standard deviations in procedural blank samples. The procedural blank levels using 0.5 mL distilled water were evaluated in every 11 samples. Since blank samples (0.5 mL distilled water) contained no detectable concentrations, MDL was considered to be equal to the LOD

Table 1
Study area and population.

Sampling site	Population density ($\times 10^3$ km^{-2})	n	Building age[a] (yr)	House type		Building Construction	
				Houses	Apartments	Timber	Concrete
Osaka	11.7	21	31.0 ± 27.3	10	11	7	14
Kyoto	1.7	20	30.3 ± 21.2	13	7	11	9
Wakayama	0.50	16	18.1 ± 14.2	14	2	13	3
Toyama	0.86	20	20.8 ± 9.7	19	1	15	5

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

corresponding to 2 ng g^{-1} for PFTeDA and 0.5 ng g^{-1} for the other PFCAs (Supplemental Table 2).

2.4. Quality assurance

Quantification was conducted using an internal standard dissolved in acetone. $^{13}C_2$-labeled PFHxA, $^{13}C_4$-labeled PFOA, $^{13}C_5$-labeled PFNA, $^{13}C_2$-labeled PFDA, $^{13}C_2$-labeled PFUnDA and $^{13}C_2$-labeled PFDoDA were used as the internal standard for PFCAs. PFHpA was quantified by $^{13}C_2$-PFHxA, PFTrDA, PFTeDA and $^{13}C_2$-PFDoDA. $^{13}C_2$-PFOA (10 ng) was added prior to derivatization for determination of amount of recovered ^{13}C-labeled PFHxA, PFOA, PFNA, PFDA, PFUnDA and PFDoDA. The results were corrected for recoveries. The recoveries were evaluated by five replicate fortifications (fortified by 10 times the original concentration of vacuum cleaner dust) of a vacuum cleaner dust sample with low contamination (Supplemental Table 2). To validate variations throughout analysis, duplicates of 5 ng-fortified cleaner dust samples were analyzed in a batch in each sampling sites ($n = 4$). They were compared and indicated good precision with RSDs $\leqslant$ 10.6% (Supplemental Table 2).

2.5. Statistical analysis

All statistical analyses were conducted using the JMP software (Version 4; SAS Institute Inc., Cary, NC). Values of $p < 0.05$ were considered to indicate statistical significance. Concentrations lower than the detection limit were considered to be equal to half of the detection limit for statistical analyses. Vacuum cleaner dust levels of PFCAs were assumed to be distributed lognormally because they displayed right-skewed patterns and had geometric means comparable to the medians. Statistical analyses were conducted after logarithmic transformation of the vacuum cleaner dust concentrations. Differences between mean values were tested by Tukey–Kramer's honestly significant difference (HSD) test when statistical tests by ANOVA were significant. Correlations were 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* a correlation matrix. Eigenvectors were employed through analysis, and the eigenvalues which account for the more than 80% of variations were taken into account. 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. PFCAs concentrations in vacuum cleaner dust

The descriptive statistics for PFCAs are presented in Table 2. Most samples contained PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA and PFTrDA. PFTeDA was less frequently observed at concentrations above the MDL. The median concentration of PFNA (23.2 ng g^{-1}) was highest among PFCAs, followed by that of PFOA (20.8 ng g^{-1}), PFUnDA (12.9 ng g^{-1}), and PFHxA (9.2 ng g^{-1}). Odd numbered long chain PFCAs showed large variations in concentrations, with geometric standard deviations ranging from 6.1 to 7.2. The 90th percentile concentrations of PFNA, PFUnDA and PFTrDA were 948, 283 and 110 ng g^{-1}, respectively, and these were detected at higher concentrations than neighboring, even-numbered PFCAs (PFDA, PFDoDA and PFTeDA).

The geometric mean (GM) of PFOA of dust samples was significantly higher in Osaka than those in Kyoto, Wakayama and Toyama ($p < 0.05$). The GM of PFHpA was also higher in samples from Osaka and Kyoto than those from Toyama ($p < 0.05$). Concentrations of PFHxA in dust samples were higher in Osaka than in Kyoto and Wakayama ($p < 0.05$). In contrast, there was no significant difference in the GMs of PFNA, PFDA, PFUnDA and PFTrDA among the four sampling sites ($p > 0.05$). Samples from Wakayama showed a higher GM of PFDoDA ($p < 0.05$). The mean proportions of PFOA in the total PFCAs were 33.0% ± 22.5%, 21.7% ± 14.7%, 12.3% ± 8.4% and 18.2% ± 12.2% in Osaka, Kyoto, Wakayama and Toyama, respectively.

3.2. Correlations among PFCAs levels and factor analysis

The nonparametric correlation coefficients among the PFCAs in the 77 samples are listed in Table 3. PFHpA was more highly correlated with PFOA and PFDA among the PFCAs ($\rho = 0.822$ and 0.544, respectively). PFOA was also significantly correlated with PFNA, PFDA and PFUnDA, although the highest ρ coefficient was 0.429 with PFDA. Among the long chain PFCAs, PFUnDA was strongly associated with PFNA and PFTrDA ($\rho = 0.913$ and 0.888, respectively). PFDoDA also showed high correlation coefficients with PFDA, PFTrDA and PFTeDA ($\rho = 0.719$, 0.554 and 0.535, respectively).

Factor analysis was applied to delineate the relationships among PFCA concentrations. The contributions of factors 1, 2 and 3 to the total variance were 47.7%, 19.7% and 14.5% (with an eigenvalue >1), respectively (Table 4). After varimax rotation, the first factor had greater correlations with odd numbered longer-chain PFCAs (PFNA, PFUnDA, PFTrDA) than short chain and even-numbered long chain PFCAs. Factor 1 represents odd numbered longer chain PFCAs. The second factor had a greater correlation with short-chain PFCAs (PFHxA, PFHpA and PFOA) than with other PFCAs. The second factor may represent PFOA and other short chain PFCAs. The third was negatively correlated with even numbered long-chain PFCAs (PFDA, PFDoDA and PFTeDA). The third factor represents even numbered long chain PFCAs.

Factor scores were compared among the four sampling sites (Table 4). There was no significant difference in factor 1 for samples from each site ($p > 0.05$), suggesting a common emission source of odd numbered longer chain PFCAs in Japan. In contrast, samples collected from Osaka had the highest factor 2 score ($p < 0.05$), suggesting a local emission source in Osaka. Factor 3 had the lowest score in Wakayama ($p < 0.05$), suggesting a possible local emission source specific to Wakayama.

3.3. Association between house conditions and PFCAs factors

To evaluate the influence of the participant's characteristics on PFCAs concentrations in vacuum cleaner dust samples, Pearson's correlation or ANOVA were conducted for the three potential factors (Supplemental Table 1). Only the number of household members was positively correlated with factor 1 (odd-numbered longer chain PFCAs, $p < 0.05$). Samples from apartments and concrete buildings showed higher factor 2 scores (PFOA and other short chain PFCAs, $p < 0.05$). The water repellent use group also showed higher factor 2 scores than the non-use group ($p < 0.05$). The score of factor 3 (even numbered long chain PFCAs) was higher in dust samples from air cleaning device users and lower in those from PTFE cookware users ($p < 0.05$). Frequent cleaning of the house also resulted in a higher factor 3 score ($p < 0.05$). To investigate potential confounding between sampling sites and house condition, analysis of covariance was conducted. After adjusted with sampling sites, house types and building construction did not indicate significant differences on factor 2 (Supplemental Table 1). Effect of frequency of cleaning on factor 3 was also decreased and statistical significance was disappeared. The number of household members,

Table 2
Concentrations of PFCAs in vacuum cleaner dust samples

Sampling site	n	Concentration (ng g^{-1})								
			PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	PFTeDA
Total	77	n > MDL (%)	66(85.7)	76(98.7)	77(100.0)	77(100.0)	77(100.0)	77(100.0)	73(94.8)	74(96.1)	10(13.0)
		median	9.2(0.5–7140)	3.7(0.5–81.2)	20.8(3.2–340)	23.2(2.0–37400)	7.3(1.4–201)	12.9(2.1–19900)	4.0(2.1–167)	5.5(<1–7010)	<2(<2–81.2)
		mean	1.21 ± 817	6.2 ± 10.1	42.3 ± 53.4	783 ± 4313	17.1 ± 29.2	394 ± 2230	8.4 ± 20.2	155 ± 833	3.1 ± 9.8
		GM(GSD)	7.0(7.0)	3.7(2.6)	24.4(2.8)	41.6(7.2)	8.8(2.8)	23.3(6.1)	4.1(2.8)	9.2(6.3)	1.3(2.3)
		P90	55	11	112	948	43	283	15	110	4
Osaka	21	n > MDL (%)	19(90.5)	21(100)	21(100)	21(100)	21(100)	21(100)	19(90.5)	19(90.5)	4(19.0)
		median	27.5(<0.5–7140)	5.5(1.1–81.2)	78.2(9.7–340)	28.8(3.3–37300)	6.5(2.2–93.3)	15.7(4.1–19900)	2.7(<1–59.2)	8.0(<1–701)	<2(<2–23.6)
		mean	415 ± 1550	10.4 ± 17.0	94.0 ± 73.0	2070 ± 8160	17.5 ± 26.5	1040 ± 4330	7.2 ± 12.7	368 ± 1530	2.6 ± 5.0
		GM(GSD)	23.6(10.8)^{A}	6.0(26)^{A}	72.0(2.2)^{A}	54.4(8.6)	8.7(30)	27.6(7.6)	3.6(3.1)^{AB}	10.3(8.7)	<2
		P90	720	21	184	4099	80	1142	17	388	6
Kyoto	20	n > MDL (%)	14(70.0)	20(100)	20(100)	20(100)	20(100)	20(100)	18(90.0)	19(95.0)	2(10.0)
		median	8.2(<0.5–27.7)	3.9(1.4–23.3)	21.6(5.3–133)	22.7(5.2–2830)	5.4(1.5–86.1)	8.9(2.1–574)	2.1 (<1–35.2)	28(<1-125)	<2(<2–14.0)
		mean	8.2 ± 7.5	5.7 ± 4.8	28.7 ± 28.4	338 ± 740	11.5 ± 18.4	82.6 ± 164	4.9 ± 8.3	19.4 ± 35.9	<2
		GM(GSD)	3.3(6.1)^{BC}	4.6(1.9)^{A}	21.8(2.0)^{B}	49.9(7.0)	6.9(25)	17.5(5.8)	2.5(2.8)^{B}	5.1(5.0)	<2
		P90	19	9	70	1804	22	470	19	101	3
Wakayama	16	n > MDL (%)	13(81.3)	16(100)	16(100)	16(100)	16(100)	16(100)	16(100)	16(100)	3(18.8)
		median	3.0(<0.5–10.5)	2.8(1.1–29.6)	13.2(3.2–63.2)	29.0(2.0-4580)	11.3(1.4-201)	21.6(3.7-3500)	6.0(2.1-167)	7.8(2.9-2130)	<2(<2-81.2)
		mean	3.3 ± 2.9	4.8 ± 6.8	19.8 ± 19.0	426 ± 1160	30.1 ± 49.3	320 ± 879	17.6 ± 40.1	179 ± 531	6.9 ± 20.0
		GM(GSD)	2.0(3.4)^{C}	3.3(2.1)^{AB}	13.7(2.4)^{B}	43.7(8.1)	14.2(3.3)	38.6(6.7)	7.9(2.7)^{A}	18.3(6.8)	<2
		P90	9	14	63	2343	107	1715	64	935	33
Toyama	20	n > MDL (%)	20(100.0)	19(95.0)	20(100)	20(100)	20(100)	20(100)	20(100)	20(100)	1(5.0)
		median	12.4(1.8–54.3)	2.1(<0.5–14.8)	12.1(4.1–114)	15.3(2.7–1580)	6.5(2.4–64.2)	11.0(3.6–1040)	4.1(1.5–26.1)	5.1(1.7–603)	<2(<2–18.8)
		mean	17.4 ± 15.2	3.3 ± 3.4	19.6 ± 24.1	161 ± 380	11.8 ± 14.4	88.5 ± 235	6.0 ± 5.9	45.6 ± 134	<2
		GM(GSD)	11.3(2.8)^{AB}	2.1(3.0)^{B}	13.8(2.2)^{B}	25.3(5.9)	7.7(23)	17.2(4.8)	4.4(2.1)^{AB}	8.6(4.6)	<2
		P90	43	8	41	658	28	216	14	104	<2

MDL: method detection limit; GM: geometric mean; GSD: geometric standard deviation; P90 90th percentile value
The geometric means within the same columns without bearing the same superscripts differ significantly ($p < 0.05$).
The geometric means within the same columns bearing the same superscripts or without superscripts do not differ significantly ($p > 0.05$).

Table 3
Correlation between PFCAs with different chain lengths.

Variables	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	PFTeDA
PFHxA	–								
PFHpA	0.070	–							
PFOA	0.296**	0.822***	–						
PFNA	−0.018	0.470***	0.382***	–					
PFDA	−0.099	0.544***	0.429***	0.421***	–				
PFUnDA	−0.049	0.347**	0.288*	0.913***	0.507***	–			
PFDoDA	−0.156	0.209	0.185	0.181	0.719***	0.397***	–		
PFTrDA	−0.065	0.169	0.165	0.712***	0.411***	0.888***	0.554***	–	
PFTeDA	−0.151	0.344**	0.352**	0.271*	0.489***	0.312**	0.535***	0.331**	–

Numbers indicate Spearman's rank correlation coefficients (ρ).
* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

Table 4
Factor analysis among PFCAs

	Initial solution			Varimax rotated		
	F1	F2	F3	F1	F2	F3
Eigenvalue	4.29	1.77	1.31			
Contribution (%)	47.7	19.7	14.5			
Eigenvector						
PFHxA	−0.074	0.463	0.244	−0.183	**0.579**	0.336
PFHpA	0.269	0.490	0.092	0.203	**0.804**	−0.243
PFOA	0.255	0.559	0.088	0.145	**0.878**	−0.224
PFNA	0.391	−0.142	0.459	**0.956**	0.203	−0.105
PFDA	0.377	0.211	−0.305	0.239	0.437	**−0.750**
PFUnDA	0.419	−0.213	0.340	**0.955**	0.104	−0.252
PFDoDA	0.339	−0.125	−0.504	0.222	−0.051	**−0.894**
PFTrDA	0.397	−0.327	0.219	**0.899**	−0.080	−0.336
PFTeDA	0.339	−0.008	−0.450	0.199	0.106	**−0.841**
				Factor score		
				(Mean ± standard deviation)		
		Osaka		0.06 ± 1.02	0.89 ± 1.09^{A}	0.18 ± 0.91^{AB}
		Kyoto		−0.03 ± 1.05	-0.02 ± 0.65^{B}	0.26 ± 0.94^{A}
		Wakayama		0.14 ± 1.08	-0.60 ± 0.64^{B}	-0.64 ± 1.24^{B}
		Toyama		−0.15 ± 0.91	-0.44 ± 0.79^{B}	0.06 ± 0.75^{AB}

The factors in bold indicate the greater correlations.The factor scores within the same columns without bearing the same superscripts differ significantly ($p < 0.05$). The facotr scores bearing the same superscripts or without superscripts do not differ significantly ($p > 0.05$). F1: 1st factor; F2: 2nd factor: F3: 3rd factor

air cleaning device use, water repellent use and PTFE cookware use remained significant factors after adjusted by sampling sites.

4. Discussion

In the present study, we extracted three major fingerprints (factors 1–3) from mixtures of different PFCAs in vacuum cleaner dust samples collected in Japan. Odd-numbered long chain PFCAs (PFNA, PFUnDA and PFTrDA), which corresponded to factor 1, were likely associated with chemical production processes, were the major components of PFCAs in vacuum cleaner dust. Factor 2, which corresponds to short chain PFCAs, was likely associated with a local emission source of PFOA in Osaka, while even numbered long chain PFCAs (factor 3) were likely associated with another local emission source in Wakayama, were statistically identified. Although these findings suggest that there are several sources of PFCAs with different origins, they also suggest that odd numbered long chain PFCAs are ubiquitous in indoor environments in Japan.

There are three major chemical engineering processes used to produce PFCAs (Supplemental Fig. 1) (Prevedouros et al., 2006). In the first process, fluorotelomer olefine is used as a starting compound and is then oxidized to produce PFCAs. In this process, odd-numbered PFCAs will be yielded predominately (Daikin Industries, 1998). In contrast, the fluorotelomer iodide oxidation process will yield even numbered PFCAs predominately (Tosoh Corporation, 1990). The electrochemical fluorination process was primarily employed by the 3M Company and yielded PFOA (3M Company Technical Bulletin, 1995).

Although PFOA is considered to be a major component among PFCAs, PFCAs of longer chain lengths than PFOA were frequently detected in vacuum cleaner dust samples in this study. We have also reported that the concentrations of odd numbered PFCAs in human serum have been increasing in Japan, Korea and Vietnam (Harada et al., 2011). The proportion of odd-numbered long-chain PFCAs in serum was estimated to be one order of magnitude higher when compared with reports in western countries (Harada et al., 2011). Importantly, because the serum and dust samples have similar odd-numbered long chain PFCAs profile, long chain PFCAs have not been detected in food duplicate samples in Japan (Kärrman et al., 2009) and the modern individual spends much time indoor, these suggest that dust exposure in indoor may be an important source for the human body burden of PFCAs in Asian. Additionally, the fluorotelomer olefine oxidation process was used primarily to produce the odd numbered PFCAs in Japan (Daikin Industries, 1998; Prevedouros et al., 2006). Collectively, the results of the present study and previous studies indicate the predominance of

Table 5
Comparison of concentrations of PFCAs in dust with reported data

Sampling site	Year	*n*		Concentration (ng g^{-1})									References
				PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	PFTeDA	
Japan													
Osaka	2010	21	median	27.5	5.5	78.2	28.8	6.5	15.7	2.7	8.0	<2	This study
Kyoto	2010	20	median	8.2	3.9	21.6	22.7	5.4	8.9	2.1	28	<2	This study
Wakayama	2010	16	median	3.0	2.8	13.2	29.0	11.3	21.6	6.0	78	<2	This study
Toyama	2010	20	median	12.4	2.1	12.1	15.3	6.5	11.0	4.1	5.1	<2	This study
Osaka	2003	16	median	–	–	165	–	–	–	–	–	–	Moriwaki et al. (2003)
Tokyo	2006	20	median	–	–	33.5	13.5	–	–	–	–	–	Katsumata et al. (2006)
China													
Nanchang	2009	5	mean		<0.17	1.4	0.66	<0.18	<0.17	<0.18	–	–	Zhang et al. (2010)
Shanghai	2009	5	mean		43.1	718	2.95	2.72	1.12	<0.18	–	–	Zhang et al. (2010)
Beijing	2009	14	mean		5.09	25.2	3	3.52	0.7	0.68	–	–	Zhang et al. (2010)
Tianjin	2009	4	mean		194	355	7.32	7.85	1.62	3.28	–	–	Zhang et al. (2010)
Kazahkstan													
Almaty and Astana	2007–2009	9	median	–	–	<0.98	–	–	–	–	–	–	Goosey and Harrad, 2011
Thailand													
Bangkok and Nakhonsrithammarat	2007–2009	20	median			18							Goosey and Harrad (2011)
Australia													
Brisbane, Newcastle and Sydney	2007–2009	20	median		–	120							Goosey and Harrad (2011)
USA													
Ohio and North Carolina	2000–2001	112	median	54.2	50.2	142	7.99	6.65	7.57	7.78	–	–	Strynar and Lindstrom, 2008
Colorado	2007–2009	10	median			240							Goosey and Harrad (2011)
Canada													
Ottawa	2002–2003	67	median			19.7							Kubwabo et al. (2005)
Toronto	2007–200S	20	median	–	–	69	–	–	–	–	–	–	Goosey and Harrad (2011)
Vancouver	2007–2008	132	mean		168	97	26	8.4	7.8	6.3		7.3	Shoeib et al. (2011)
UK													
Birmingham	2007–2009	45	median			190							Goosey and Harrad (2011)
Norway													
Oslo	2008	41	median	28	94	18	23	4.1		19	6.8	3.3	Haug et al. (2011)
Sweden													
Stockholm	2006–2007	38	median			93							Björklund et al. (2009)
Belgium													
Flanders	2008	45	median	0.3		0.7	0.1	02					D'Hollander et al. (2010)
Germany													
Augsberg and Michelstadt	2007–2009	10	median			300							Goosey and Harrad (2011)
France													
Annecy	2007–2009	10	median	–	–	31	–	–	–	–	–	–	Goosey and Harrad (2011)
Germany, UK, Australia and USA	2004	39	median	<2.6	97.3	96.5	<2.6	<2.6	<2.6	<2.6	-	-	Kato et al. (2009a)