PFOA and PFOS have been found globally in a variety of living organisms, including humans and wildlife [6, 7]. Many studies have revealed that these compounds have various toxicities toward living organisms, including humans [8–11]. PFOS has been regulated at various levels by governments, including those of the USA [12], Canada [13], and the European Union (EU) [14]. In the Stockholm Convention on Persistent Organic Pollutants (POPs), the third meeting of the POPs review committee decided to recommend PFOS for listing in Annex A or B of the convention [4]. Following the restriction of PFOS marketing in the EU, the issue of whether PFOA should be included in Directive 76/769/EEC has been discussed. The Environmental Protection Agency of the USA launched a stewardship program, and manufacturers have committed to reducing PFOA emissions [15].

Recent studies have revealed a unique situation in Japan, namely that PFOA contamination has progressed more profoundly than PFOS contamination [16, 17]. In agreement with these observations, serum concentrations in Japanese, albeit limited to subpopulations of Japan, are reported to be higher than those in US populations, while the opposite is true for PFOS [18, 19].

The present review has two main aims. First, we review the environmental and biological monitoring of PFOS and PFOA in Japan. Second, we characterize the toxicokinetics of PFOS and PFOA. We have compared data obtained in Japan with those obtained in other countries as required to clarify the unique situation in Japan.

Distributions of PFOA and PFOS in the water environment in Japan

Several studies have reported the concentrations of PFOA and PFOS in surface water in Japan [17, 20–23], and the data are summarized in Table 1.

The geometric mean concentrations of PFOA in five regions were within the range of 1–3 ng/l, except for the Kansai region. In the Kansai region, the PFOA concentrations in surface water were much higher than those in other regions [17, 21]. A systematic investigation of the Kanzaki River system by our group revealed that there is a single source of PFOA within the Ai River [17], which was confirmed by Nguyen et al. [21]. The concentrations of PFOA in other countries are also shown in Table 1 [24–27]. These values are relatively higher than the values in Japan, excluding the Kansai region. Local intense contaminations were also observed in other countries, for example, the Tennessee River near Decatur, the Ruhr River, and Etobicoke Creek. The identified sources could be related to fluorochemical manufacturers, sewage treatment plant effluents, and fire-fighting foam [17, 24, 25].

The current surface water PFOS contaminations are shown in Table 1. The PFOS concentrations in Japan were relatively high in the Kanto and Kansai regions [17, 22]. Sewage treatment plant effluents exhibited high concentrations of PFOS as well as PFOA. High concentrations of PFOS were found in wastewater around airports [17]. However, detailed information regarding the types of fire-fighting foams used was not available.

The concentrations of PFOS and PFOA in drinking water in Japan have been reported [28, 29]. The levels of PFOA were extremely high in the Kansai region, especially Osaka [28], where the water supply is mainly derived from the Yodo River. On the other hand, the PFOS levels in tap water were higher in the Kanto region than in other regions [29]. It is therefore possible that the serum levels of these compounds in residents may be proportional to the levels in tap waters.

Distributions of PFOA and PFOS in outdoor air and indoor dust in Japan

There are limited numbers of reports regarding airborne and indoor levels of PFOA and PFOS. Harada et al. [30] showed that the concentrations of PFOA detected within urban atmospheric particles were 50-fold higher than those of PFOS. The amounts of PFOA and PFOS in the respirable fraction (1.1–11.4 μm) ranged from 58.3 to 89.8% of the total amounts [31]. The levels of PFOS and PFOA were significantly higher in the urban atmosphere of Oyamazaki than in the suburban atmospheres of Morioka and Fukuchiyama [30, 32]. Across Japan, there was a tendency for PFOA to be the predominant contaminant of outdoor air, particularly in Osaka [33]. Boulanger et al. [34] reported that the mean concentration of PFOS in particulate-phase air samples was 6.4 pg/m^3 (SD 3.3) in the Great Lakes. In Manchester, PFOA and PFOS were both detected at relatively high concentrations (341 and 46 pg/m^3, respectively) [35].

The concentrations in indoor dust in Japan ranged from 18 to 3,700 ng/g dust for PFOA and from 7 to 2,500 ng/g dust for PFOS [36, 37]. Compared with outdoor dust, the PFOS levels in indoor dust were comparable, but there are few reports regarding the PFOA levels in indoor dust (Table 2). A study in the USA reported that indoor dust contained higher levels of PFOS than PFOA [38]. The variations in the proportions of various fluorochemicals may reflect the source signatures caused by the use of different composites during the application or manufacturing process [39] (Table 3).

The sources of PFOA in the environment remain unclear. However, degradation of fluorotelomer alcohols (FTOHs) with atmospheric lifetimes of approximately

Table 1 Levels of PFOA and PFOS in water in Japan and several other countries

Area	*n*	PFOA (ng/l)		PFOS (ng/l)		Ref.
		GM (GSD)	Range	GM (GSD)	Range	
River						
Hokkaido	1		0.4		1.9	[17]
Tohoku	15	1.1 (2.7)	0.1–4.2	1.2 (2.5)	0.3–4.6	[17]
Kanto	14	2.8 (3.6)	0.3–15.1	3.7 (3.9)	0.3–31.4	[17]
Kanto (Tsurumi River)	10		11.2–19.8		17.1–612	[22]
Chubu	17	2.5 (2.2)	0.3–16.3	1.1 (2.4)	0.3–6.0	[17]
Kansai	8	21.2 (6.2)	2.1–456.4	5.7 (3.6)	0.8–37.3	[17]
Kansai (Kanzaki River)	52		4.5–67,000		1.5–526	[17]
Kansai (Yodo River)	33	60.5 (4.0)	6–2568	7.1 (4.2)	0.8–123	[21]
Chugoku	9	1.5 (2.3)	0.5–8.1	1.0 (3.4)	0.4–25.1	[17]
Shikoku	7	3.0 (2.1)	1.4–13.8	1.1 (4.7)	0.2–14.9	[17]
Kyushu	8	1.3 (2.4)	0.2–3.3	0.7 (1.9)	0.3–1.7	[17]
Coastal water						
Hokkaido	1		1.9		2.1	[17]
Hokkaido	1				<2.5	[20]
Tohoku	2	2.0 (1.1)	2.1–2.1	1.0 (1.9)	0.6–0.9	[17]
Kanto (Funabashi)	1		32.2		2.6	[17]
Kanto	4			26	8–59	[20]
Kanto	3	166 (1.1)	154–192	20.1 (1.5)	12.7–25.4	[23]
Chubu (Toyohashi)	1		11.5		0.7	[17]
Kansai (Koshien Hama)	1		448		27.69	[17]
Kansai	3			8.7	<4–21	[20]
Chugoku	4				<4	[17]
Kyushu	5			4.8	<9–11	[20]
Okinawa	4				<2.5	[20]
Tap water						
Tohoku	15	0.3 (2.3)	0.01–1.0	0.2 (1.7)	0.1–0.5	[17]
Kanto (Tokyo)	19	<5	<5–25	6.4	<5–37	[29]
Kansai	15	15.3 (2.3)	4.9–42.2	3.8 (3.6)	0.3–12.7	[17]
Kansai (Osaka)	14	31	7.9–110	3.8	0.3–20	[28]
River						
Tennessee, USA	40	366 (1.5)	140–498	55.1 (2.0)	16.8–144	[24]
Etobicoke Creek, Canada	13		$11\text{–}1.1 \times 10^4$		ND-2.2×10^6	[25]
Cape Fear Drainage Basin, USA	80	16.2	<0.05–287	20.0	<0.05–132	[26]
Rhine River, Germany	38	4.9 (2.9)	2–48	6.5 (1.9)	2–26	[27]
Ruhr River, Germany	22	102 (5.0)	9–3640	11.7 (2.7)	4–193	[27]

GM geometric mean, *GSD* geometric standard deviation, *ND* not detected

10–20 days is speculated to be a source of PFOA [40]. FTOHs are currently produced and used as intermediates for the synthesis of coatings, polymers, inks, adhesives, waxes, and so on. Oono et al. [41, 42] reported that the airborne levels of several FTOHs were significantly higher in the Kyoto-Osaka area than in other areas. Taken together, the higher levels of airborne PFOA in the Kyoto-Osaka area may be caused by the high levels of FTOHs in the air.

Levels of PFOA and PFOS in food and dietary intakes

PFOA and PFOS concentrations in food samples have been reported for food duplicates [33, 43] and total diet studies (TDSs) [44]. The estimated daily dietary intakes of PFOA and PFOS were within the same ranges in Japan and other countries. Although no geographical differences in the dietary intakes were obvious, the serum levels of PFOA were higher in the Osaka area [33, 45]. A TDS in Canada showed

Table 2 PFOA and PFOS levels in outdoor air and indoor dust in Japan and several other countries

Sampling site		*n*	Units	PFOA			PFOS			Ref.
				GM	GSD	Range	GM	GSD	Range	
Japan	Oyamazaki Town (on a highway) 2001/April 2002/March	12	pg/m^3 air				5.3	1.2	2.3–21.8	[32]
			ng/g dust				97.4	1.2	38.0–427.4	
		12	pg/m^3 air	263	2.4	71.8–919.4	5.2	1.4	2.5–9.8	[30]
			ng/g dust	3,413	2.4	469–9049	72.2	1.8	19.7–168.0	
	Fukuchiyama City (on a local road) 2001/April 2002/March	12	pg/m^3 air				0.6	1.3	ND-2.1	[32]
			ng/g dust				19.2	1.2	ND-60.6	
	Morioka City (on a local road) 2003/July	8	pg/m^3 air	2.0	1.2	1.6–2.6	0.7	1.4	0.5–1.2	[30]
	Japan (20 sites) 2004	20	pg/m^3 air	9.0		6.0–2500	1.8		<0.1–30	[33]
	Indoor dust (general home)	16	ng/g dust	178	2.6	69.0–3700	39.5	3.9	11.0–2500	[37]
	Indoor dust (0.075–1 mm) (general home)	20	ng/g dust	36.1	1.5	18–89	19.9	1.6	7–41	[36]
Other	Lake Ontario (over a lake)	8	pg/m^3 air				6.4	3.3		[34]
	Manchester, UK 2003/July	2	pg/m^3 air	Mean 341			Mean 46			[35]
	Indoor dust, Ohio and North Carolina (general home)	102	ng/g dust	Median 142		<10–1960	Median 201		<8.9–12,100	[38]

GM geometric mean, *GSD* geometric standard deviation, *ND* not detected

Table 3 PFOA and PFOS levels in food samples and their daily intakes

Sampling site	*n*	Units	PFOA			PFOS			Ref.
			GM	GSD	Range	GM	GSD	Range	
Ten sites in Japan	50	ng/g wet weight	<0.01		<0.01–0.024	0.012	2.6	<0.0017–0.12	[33]
Osaka	10	ng/day	61.4	1.7	22.7–124	76.3	1.6	32.1–180	[45]
		ng/g wet weight	0.022	1.7	0.008–0.040	0.027	1.5	0.015–0.057	
Miyagi	10	ng/day	44.4	1.5	29.0–90.4	61.5	2.5	18.5–267	[45]
		ng/g wet weight	0.019	1.3	0.012–0.031	0.026	2.3	0.008–0.087	
			Median	Average	Range	Median	Average	Range	
Canada		ng/day		70			110		[44]
Germany	31	ng/day	169		91.9–839	90.4		47.7–371	[43]

GM geometric mean, *GSD* geometric standard deviation

that dietary intake of PFOS was mainly derived from beef and fish, while PFOA originated from beef and microwave popcorn [44]. Owing to PFOS bioaccumulation in the environment, fish seem to represent important routes of exposure [46]. In addition, several food packaging coatings for oil- and moisture-resistance are made from fluorochemicals that may degrade into PFOA and PFOS [47].

Estimated daily intakes of PFOA and PFOS in Japanese

Exposure levels to PFOA and PFOS have been estimated using their concentrations in indoor dust, outdoor air, tap water, consumed items, and diet. It was estimated that the adult intake of indoor dust is 50 mg/day [48], the adult intake of tap water is 1.3 l/day, and adult humans inspire 13.3 m^3 of air/day; 69 and 74% of particles in air are respirable for PFOA and PFOS, respectively, and PFOA and PFOS in each medium are completely absorbed into the body.

The estimated exposure through food was predominant for both PFOA and PFOS (Table 4). Among the estimates, exposure via food consumption was the major source, followed by tap water and indoor dust. Exposure via tap water was more intense in the Kansai region than in the Tohoku region. Information regarding exposure levels via

Table 4 Estimates of adult exposures (ng/day) to PFOA and PFOS

Source	Kansai		Tohoku		Notes	Ref.
	PFOA	PFOS	PFOA	PFOS		
Water	19.9	4.9	0.4	0.3	Calculated from tap water concentrations (GM) for Kansai and Tohoku	[17]
Indoor dust	8.9	2.0	8.9	2.0	Calculated from vacuum cleaner dust concentrations (median) for Osaka	[37]
Ambient air	2.4	0.1	0.02	0.01	Calculated from airborne dust concentrations (GM) for Kyoto and Iwate	[30]
Food	61.4	76.3	44.4	61.5	Estimated from food duplicate concentrations (GM) for Osaka and Miyagi	[45]
Total	92.6	83.3	53.7	63.8		

GM geometric mean

indoor dust and food is still insufficient. Moreover, exposure levels to precursors of PFOA and PFOS have not been evaluated. Even if these estimates for PFOA and PFOS exposure are uncertain, they play important roles in allowing speculation for sources of exposure that may lead to regional differences in serum levels.

Compared with intakes of these compounds, analyses of 24-h pooled urine from residents in Kyoto revealed levels of 17.6 and 13.3 ng/day for PFOA and PFOS, respectively [49]. Although fecal excretion of these chemicals remains unclear, such limited excretion in urine was in clear contrast to the case for rodents and monkeys [49], suggesting unique pharmacokinetic behaviors in humans.

Serum levels of PFOA and PFOS in Japanese

Figure 1 shows the serum concentrations of PFOA and PFOS in Japanese [50]. There were significant geographical differences in PFOA and PFOS serum concentrations for both males and females. Residents belonging to the Kansai region (Kyoto, Osaka, and Hyogo) exhibited significantly higher serum PFOA levels. Serum PFOS levels in the Kansai region were significantly higher than those in the Tohoku and Chubu regions (Akita, Miyagi, Gifu, and Fukui) and comparable to those in Yamaguchi, Kochi, and Okinawa. The serum PFOS and PFOA levels in other countries are shown in Fig. 1. The serum PFOS levels were higher in the USA than in Japan and Europe, while the serum PFOA levels were comparable among the USA, Europe, and Japan, except for the Kansai region [19, 51, 52].

Several factors influencing the serum levels of these compounds have been reported. Sex-related differences in the serum concentrations of PFOS and PFOA were observed and the concentrations of PFOS and PFOA were higher in males than in females [49, 51, 52]. There were positive correlations between age and PFOS and PFOA levels only in females [49]. Multiparous women had lower PFOS and PFOA levels than nulliparous women [10]. With regard to ethnicities, Mexican Americans had lower levels than non-Hispanic blacks and whites in the USA. Higher education was associated with higher PFOS and PFOA levels [53].

Time trends of the serum levels of these compounds have been presented by several researchers (Fig. 2). Harada et al. [18] revealed that the serum concentrations increased 3-fold for PFOS and 14-fold for PFOA between 1977 and 2003 in Yokote in Miyagi prefecture. The PFOA concentrations in Kyoto increased by 4.4-fold from 1983 to 1999 [50]. In the USA, the PFOS and PFOA concentrations increased between 1974 and 1989, and reached plateau levels in 1989 [54]. In China, the serum levels of both PFOA and PFOS have increased significantly over recent years and reached the corresponding levels in Japan [55]. After 3M Company phased out PFOA and PFOS production, decreases in the PFOA and PFOS concentrations were observed in the USA [56]. It should be confirmed whether similar decreases also have been observed in other countries.

Toxicokinetics of PFOA and PFOS

A recent study revealed interspecies differences in the pharmacokinetics of PFOA and PFOS. The mean serum half-lives of PFOA and PFOS in humans were 3.5 and 5.4 years, respectively [57]. These long half-lives explain why PFOA and PFOS tend to accumulate in humans. In contrast, the half-lives in experimental animals were orders of magnitudes shorter than those in humans. The serum half-lives of PFOA in Wistar rats were reported to be 5.68 days for males and 0.08 days for females [58], whereas those in primates were 5.6 days for males and

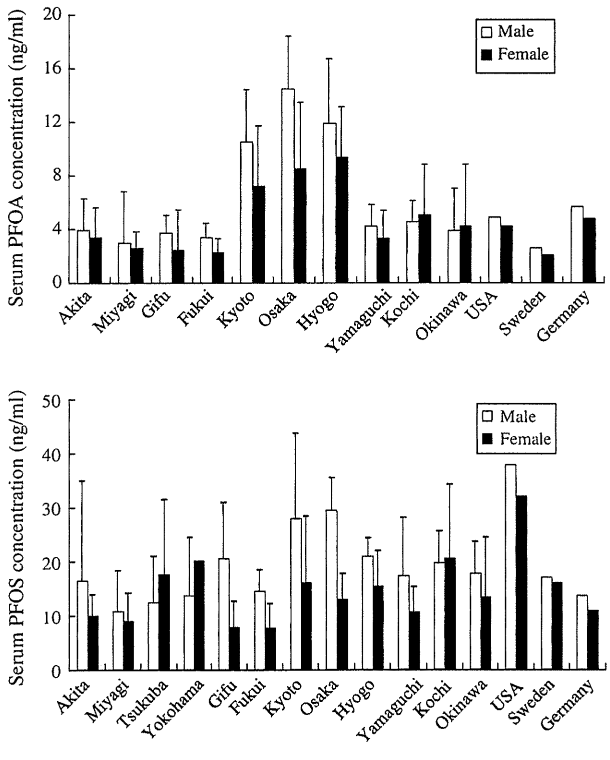

Fig. 1 Human serum concentrations of PFOA and PFOS in Japan and several other countries collected from 2000 to 2005. The data for Tsukuba, Yokohama, and other sites in Japan are taken from Taniyasu et al. [20] and Harada et al. [18, 50]. The data are geometric means and geometric standard errors in Japan. For the USA and Sweden, the data are geometric means reported by Olsen et al. [19] and Kärrman et al. [52], respectively. For Germany, the data are medians reported by Fromme et al. [51]

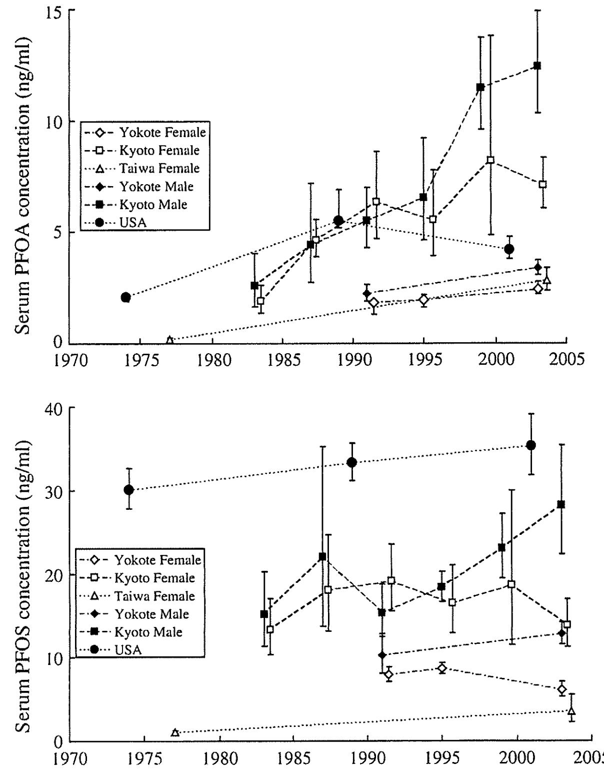

Fig. 2 Time trends in PFOA and PFOS serum levels in Japan [18, 50] and the USA [54]. Data are geometric means and geometric standard errors

2.7 days for females in Japanese macaques, and approximately 1 month for both sexes in cynomolgus monkeys [59, 60]. The serum half-lives of PFOS were longer than those of PFOA, comprising more than 89 days in male CR:CD rats [61], and approximately 100 days in both male and female cynomolgus monkeys [62].

In Cr:CD rats, intravenously administered PFOA and PFOS were excreted via the urine (67 and 18%, respectively) and feces (4.4 and 8.0%, respectively) [63, 64]. However, the serum clearances of PFOA via urine in humans were 300–1,000-fold lower than those in Wistar rats and Japanese macaques (Table 5) [49]. A critical role of the resorption process was supposed as a determinant for the large species differences in renal excretion of PFOA [65]. Several organic anion transporters (OATs) have been investigated. Nakagawa et al. [66] found that OAT1 (Slc22a6) and OAT3 (Slc22a8) mediated transport of PFOA in both humans and rats, while OAT2 (Slc22a7) did not. Rat Oatp1 (Slc21a1) had transport activity for PFOA, which may be involved in the resorption process and cause sex-related differences in rats, although no human ortholog for rat Oatp1 exists [67]. There have been few studies regarding the species difference in urinary excretion of these compounds, and further studies are warranted in this area.

Biliary excretion of PFOA and PFOS has emerged as a major elimination route in humans owing to the poor urinary excretion. PFOA and PFOS were detected in human bile samples at similarly high levels to those detected in serum samples [68]. The ratio of PFOS concentrations (bile/serum: 0.60) was significantly higher than that for PFOA concentrations (bile/serum: 0.21). The biliary excretion rates of PFOA and PFOS in humans were estimated to be 1.06 and 2.98 ml/(kg day), respectively (Table 5). Although elimination of PFOA and PFOS in feces remains unexplored, available evidence in rats suggests that trace amounts were excreted via this route owing to enterohepatic circulation of these chemicals [63]. Such enterohepatic circulation of PFOS and PFOA may account for their long half-lives in humans.

PFOS and PFOA have been detected in cerebrospinal fluid (CSF) in humans [68]. The PFOA and PFOS levels in CSF were approximately 1% of the corresponding levels in serum, suggesting that PFOA and PFOS cannot freely pass through the blood–brain barrier. The reported brain-to-

Table 5 Estimated clearances of PFOA and PFOS in rats, monkeys, and humans

			Serum half-life (days)	Total clearance [ml/(day kg)]	Urinary excretion [ml/(day kg)]	Biliary excretion [ml/(day kg)]	Menstrual bleeding [ml/(day kg)]
PFOA	Wistar rats[a]	Male	5.63	50.4	46.1	3.30	NA
		Female	0.08	2233	1054	3.52	NA
	Cynomolgus monkeys[b]	Male	20.9	6.0	NA	NA	NA
		Female	32.6	4.2	NA	NA	NA
	Japanese macaques[c]	Male	5.6	37.1	15	NA	NA
		Female	2.7	77.0	32	NA	NA
	Humans[d]		1387	0.070	0.030	1.06	0.028[g]
PFOS	CR:CD rats[e]	Male	>89	NA	NA	NA	NA
	Cynomolgus monkeys[f]	Male	132	NA	NA	NA	NA
		Female	110	NA	NA	NA	NA
	Humans[d]		1971	0.077	0.015	2.98	0.028[g]

NA not available

[a] Half-lives, total clearance, and urinary excretion were reported by Kudo et al. [58]. Biliary excretion was estimated from a report by Vanden Heuvel et al. [64]

[b] Half-lives in an intravenous study were reported by Butenhoff et al. [60]. Total clearances were calculated based on volume distributions of 181 and 198 ml/kg for males and females, respectively

[c] Half-lives and renal clearance were reported by Kudo and Kawashima [59]. Total clearances were calculated based on a volume distribution of 300 ml/kg

[d] Half-lives in retired workers were reported by Olsen et al. [57]. Urinary and biliary excretions were reported by Harada et al. [49, 68]. Total clearance was calculated based on volume distributions of 220 ml/kg for PFOS and 140 ml/kg for PFOA [65]

[e] Cited from an intravenous study by Gibson and Johnson [61]

[f] Cited from a report by Noker and Gorman [62]

[g] Menstrual serum loss was assumed to be 42 ml/month

blood ratios of 0.17 for PFOA and 0.26 for PFOS in cadavers were consistent with low partition to CSF [69]. However, the occurrence of these chemicals in CSF raises concerns that the central nervous system may be one of the target organs of PFOA and PFOS toxicities.

Sex-related differences in elimination

The sex-related differences in the renal clearances of PFOA in rats suggest that hormone-regulated elimination is probably involved to a certain extent in this species [58]. The expressions of OATs are known to be regulated by sex steroids and/or growth hormones in rodents, but not in humans [70].

Sex-related differences in the serum levels of these compounds were reported in humans, but there was no difference in renal clearance between males and females [49]. The observed difference may be explained by female-specific excretion routes, such as menstrual blood loss, lactation, and direct maternal-fetal transfer. Menopausal females had significantly higher serum concentrations than menstrual females in a 20–50-year age group [49]. With regard to excretion through lactation, PFOS and PFOA were detected in breast milk samples [71]. The mean ratios between the milk and serum concentrations were 0.01:1 for PFOS and 0.02:1 for PFOA, resulting in clearances of 6–12 ml/day. Decreases in the concentrations of PFOS and PFOA were reported between the first and second trimesters [8]. Several researchers have reported the concentrations of PFOA and PFOS in maternal and fetal cord serum samples [72]. In addition, PFOS and PFOA concentrations decreased with increased parity of mothers [10], implying that maternal-fetal transfer may reduce the maternal stores. Moreover, it is possible that hormonal changes in body composition or alterations of protein-binding affinity may affect the distribution and elimination of PFOS and PFOA [73, 74].

Toxicology of PFOA and PFOS

Epidemiological studies

There have been a number of reports on the health effects of PFOA and PFOS (Table 6). One epidemiological study conducted by 3M Company suggested an increase in prostate cancer mortality among workers exposed to PFOA [11]. Another study conducted by 3M Company revealed an increased mortality from bladder cancer among workers

Table 6 Epidemiology and in vivo toxicities of PFOA and PFOS

Species	Humans	Monkeys	Rodents
PFOA			
Carcinogenicity	Prostate cancer mortality [11]		Liver tumors, pancreatic acinar cell tumors, Leydig cell tumors [79]
Hepatotoxicities	Slight increases in total cholesterol, low-density lipoprotein, very low-density lipoprotein, gamma glutamyl aminotransferase, and aspartate aminotransferase [77]	Hepatomegaly accompanied by mitochondrial proliferation, no peroxisome proliferation [85]	Peroxisome proliferation, increased hepatocyte hypertrophy, increased labeling index [83]
Developmental toxicities	Decreased birth weight [8, 10]		Early pregnancy loss, increased neonatal mortality, delayed eye opening, growth deficits, altered pubertal maturation [87, 91]
Behavioral and neurotoxicities		Decreases in food consumption and body weight [85]	Decreased food intake, reduced habituation and hyperactivity, hypoactive response to nicotine [94, 96]
Other	Higher prevalence of angina, myocardial infarction, stroke, chronic bronchitis, shortness of breath on stairs, asthma [78]		
PFOS			
Carcinogenicity	Bladder cancer mortality [9]		Hepatocellular adenoma, thyroid follicular cell adenoma [80]
Hepatotoxicities	Possible increase in cholesterol, decrease in high-density cholesterol, initial decrease and subsequent increase in total bilirubin [76]	Decreased body weights, increased liver weights, lowered serum total cholesterol levels, lowered estradiol levels, no peroxisome proliferation [86]	Peroxisome proliferation, mild increase in hepatic palmitoyl CoA oxidase [80]
Developmental toxicities	Decreased birth weight, ponderal index, and head circumference [10]		Increased relative liver weight of pups, delayed eye opening, neonatal death due to intracranial blood vessel dilatation and lung atelectasis, decreased natural killer cell function in male pups [88–90]
Behavioral and neurotoxicities		Decreases in food consumption [86]	Decreased food intake [95], reduced habituation and hyperactivity, hypoactive response to nicotine [96]

exposed to PFOS [9]. Hepatic, lipid, and thyroid parameters, which are known toxicological effects in rodents, showed inconsistent associations with serum levels of PFOS and PFOA in fluorochemical workers [75–77]. Besides hepatotoxicity, higher prevalences of cardiovascular and respiratory diseases were reported in plaintiffs or potential plaintiffs in a lawsuit, which might be biased [78]. Two epidemiological studies in the USA and Denmark showed an inverse correlation between PFC concentrations (cord or maternal blood) and birth weight [8, 10]. In the populations examined in these studies, the PFC levels were much lower than those in animal experiments, which may suggest species differences in susceptibility to PFCs.

Hepatotoxicity and molecular targets of PFOA and PFOS

Although these chemicals were found to be carcinogens for rodents [79, 80], they were not genotoxic in umu tests [81]. It should be further investigated whether the hepatocarcinogenic potencies are in proportion to the degrees of induction of peroxisome proliferator-activated receptor-alpha (PPARα), to which these chemicals bind as ligands [82]. In PPARα-null mice, hepatomegaly induced by PFOA was still observed, suggesting that another mode of action exists [83], such as constitutive activated/androstane receptor actions [84].

The expression and activation of PPARα differ between humans and rodents. In monkeys, PFOA and PFOS caused hepatomegaly, but did not lead to peroxisome proliferation [85, 86]. Therefore, results generated in rodents cannot be simply extrapolated to humans. In addition, a number of studies have revealed that the toxicological target organs of PFOS and PFOA may differ between humans and rodents, such as developmental toxicities in humans and carcinogenicity and liver toxicity in rodents.

Developmental toxicity

The reproductive and developmental toxicities of these chemicals toward humans are of particular concern [87]. Prenatal as well as postnatal toxicities of PFOA and PFOS were observed in rats and mice, including increased liver weights, growth lags, delayed development, and suppressed immune functions [88–90]. PFOA had significant effects on fetal growth and development in males, but much lesser effects in females. The difference in sensitivity was presumed to be due to the sex-related difference in PFOA elimination. Some developmental toxicities of PFOA, such as delayed eye opening and deficits in postnatal weight gain, were diminished in PPARα-null mice [91], whereas PFOS-induced neonatal lethality and delayed eye opening are not dependent on PPARα [92].

Neurotoxicity

PFOS may have effects on the neuroendocrine system in rats and mice. Increased corticosterone concentrations in serum and norepinephrine in the hypothalamus were induced by PFOS in mice, indicating that PFOS stimulates the stress axis [93]. Observed decreases in food intake caused by PFOA and PFOS were mediated via the activation of hypothalamic urocortin 1 and 2, respectively [94, 95]. PFOS exposure also induced behavioral effects in mice, such as anxiety and spatial memory loss [96]. These observations suggested neurotoxic effects of PFOA and PFOS, although the target molecules in the central nervous system remain unclear.

PFOS and PFOA were reported to exhibit electrophysiological effects on action potentials and currents in isolated guinea-pig ventricular myocytes, cerebellar Purkinje cells, and protozoa [97–99]. In addition to excitable cells, PFOS activated voltage-dependent Ca^{2+} channels (VDCCs) and increased intracellular Ca^{2+} concentrations in non-excitable tracheal cells [100]. PFOS also inhibited neurite growth and suppressed synaptogenesis in cultured hippocampal neurons through VDCCs [101]. The mechanisms of these effects are hypothesized to involve incorporation of PFOS and PFOA into the outer cell membrane, which would decrease the steepness of the transmembrane potential gradient and result in hyperpolarizing shifts of both the activation and inactivation of voltage-gated ionic channels.

Conclusions

Perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), and related compounds have been used for many applications. The chemical stability of perfluorinated alkyl chains results in their persistence in the environment and organisms.

These compounds have been detected in various areas of Japan. In the Kansai region, environmental contamination by PFOA and related compounds was more intense than in other regions. Serum PFOA levels have been increasing insidiously throughout the last 20 years in Kyoto residents, and also in northern Japan. The geographic heterogeneity in the exposure intensities of PFOS and PFOA is likely, at least in part, to be associated with industrial activities. Although the estimated daily intakes of PFOS and PFOA remain somewhat uncertain, intake from drinking water is considered to represent a major component and could explain the regional differences in Japan. If this is indeed the case, identification of the sources and appropriate control of the release of PFCs urgently require discussion.

There are large interspecies differences in the toxicokinetics of these compounds. In particular, their poor renal clearances and long half-lives in humans suggest uncertainty regarding exposure assessment and extrapolation of test dosages. These qualitative differences may involve transporters in various organs.

The toxicological susceptibilities of humans to PFCs may also be higher than those of rodents. Epidemiological studies on birth weight in the general population revealed inverse correlations with PFOA and PFOS levels, which were 100–1,000-fold less than those in animal experiments. Although human PPARα has relatively low activity in comparison to rodent PPARα, other molecular targets of PFOS and PFOA may exist.

PFOA and PFOS are now under control and regulation in various countries. Monitoring of these compounds should be continued to evaluate measurements of PFCs. In closing, there are growing concerns regarding the developmental toxicities of these compounds toward human fetuses, particularly in the Kansai region. Further studies regarding the issue of whether adverse developmental effects occur are urgently required.

Acknowledgments This study was supported by grants-in-aid from the Japan Society for the Promotion of Science (17-1910 and 19890107 to K.H.H.) and a grant-in-aid for Health Science Research from the Ministry of Health, Labor and Welfare of Japan (H15-Chemistry-004 to A.K.).

References

1. Kissa E. Fluorinated surfactants and repellents. 2nd edn. New York: Marcel Dekker; 2001.
2. Hogue C. Government & policy—perfluorinated pollutant puzzle. Chem Engl News. 2004;82:17–19,59.
3. Prevedouros K, Cousins IT, Buck RC, Korzeniowski SH. Sources, fate and transport of perfluorocarboxylates. Environ Sci Technol. 2006;40:32–44.
4. UNEP, 2007. POPRC3: development of risk management evaluation. UNEP/POPS/POPRC.3/20. http://chm.pops.int/Portals/0/Repository/poprc3/UNEP-POPS-POPRC.3-POPRC-3-5.English.PDF.
5. Wood A, Clarin W. 3M to phase out PFOS. Chem Week. 2000;162:9–9.
6. Kannan K, Koistinen J, Beckmen K, Evans T, Gorzelany JF, Hansen KJ, et al. Accumulation of perfluorooctane sulfonate in marine mammals. Environ Sci Technol. 2001;35:1593–8.
7. Hansen KJ, Clemen LA, Ellefson ME, Johnson HO. Compound-specific, quantitative characterization of organic fluorochemicals in biological matrices. Environ Sci Technol. 2001;35:766–70.
8. Fei CY, McLaughlin JK, Tarone RE, Olsen J. Perfluorinated chemicals and fetal growth: a study within the Danish National Birth Cohort. Environ Health Perspect. 2007;115:1677–82.
9. Alexander BH, Olsen GW, Burris JM, Mandel JH, Mandel JS. Mortality of employees of a perfluorooctanesulphonyl fluoride manufacturing facility. Occup Environ Med. 2003;60:722–9.
10. Apelberg BJ, Witter FR, Herbstman JB, Calafat AM, Halden RU, Needham LL, et al. Cord serum concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in relation to weight and size at birth. Environ Health Perspect. 2007;115:1670–6.
11. Gilliland FD, Mandel JS. Mortality among employees of a perfluorooctanoic acid production plant. J Occup Med. 1993; 35:950–4.
12. USEPA. Perfluoroalkyl sulfonates, proposed significant new use rule. Federal Register. 2006;71:12311–24.
13. Canada. Order Adding Toxic Substances to Schedule 1 to the Canadian Environmental Protection Act, 1999. Canada Gazette Part II. 2006;140:2147–53.
14. European Commission. Directive 2006/122/EC amending for Directive 76/769/EEC. Off J Eur Commun 2006;L 372/32, 27 December 2006.
15. USEPA, Risk profile on perfluorooctane sulfonate. UNEP/POPS/POPRC.2/17/Add.5 http://www.pops.int/documents/meetings/poprc_2/meeting_docs/report/default.htm.
16. Harada K, Saito N, Sasaki K, Inoue K, Koizumi A. Perfluorooctane sulfonate contamination of drinking water in the Tama River, Japan: estimated effects on resident serum levels. Bull Environ Contam Toxicol. 2003;71:31–6.
17. Saito N, Harada K, Inoue K, Sasaki K, Yoshinaga T, Koizumi A. Perfluorooctanoate and perfluorooctane sulfonate concentrations in surface water in Japan. J Occup Health. 2004;46:49–59.
18. Harada K, Saito N, Inoue K, Yoshinaga T, Watanabe T, Sasaki S, et al. The influence of time, sex and geographic factors on levels of perfluorooctane sulfonate and perfluorooctanoate in human serum over the last 25 years. J Occup Health. 2004; 46:141–7.
19. Olsen GW, Church TR, Miller JP, Burris JM, Hansen KJ, Lundberg JK, et al. Perfluorooctanesulfonate and other fluorochemicals in the serum of American Red Cross adult blood donors. Environ Health Perspect. 2003;111:1892–901.
20. Taniyasu S, Kannan K, Horii Y, Hanari N, Yamashita N. A survey of perfluorooctane sulfonate and related perfluorinated organic compounds in water, fish, birds, and humans from Japan. Environ Sci Technol. 2003;37:2634–9.
21. Nguyen PHL, Fujii S, Tanaka S, Nozoe M, Tanaka H. Contamination of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in surface water of the Yodo River basin (Japan). Desalination. 2008;226:338–47.
22. Zushi Y, Takeda T, Masunaga S. Existence of nonpoint source of perfluorinated compounds and their loads in the Tsurumi River basin, Japan. Chemosphere. 2008;71:1566–73.
23. Yamashita N, Kannan K, Taniyasu S, Horii Y, Okazawa T, Petrick G, et al. Analysis of perfluorinated acids at parts-per-quadrillion levels in seawater using liquid chromatography-tandem mass spectrometry. Environ Sci Technol. 2004; 38:5522–8.
24. Hansen KJ, Johnson HO, Eldridge JS, Butenhoff JL, Dick LA. Quantitative characterization of trace levels of PFOS and PFOA in the Tennessee River. Environ Sci Technol. 2002;36:1681–5.
25. Moody CA, Martin JW, Kwan WC, Muir DC, Mabury SA. Monitoring perfluorinated surfactants in biota and surface water samples following an accidental release of fire-fighting foam into Etobicoke Creek. Environ Sci Technol. 2002;36:545–51.
26. Nakayama S, Strynar MJ, Helfant L, Egeghy P, Ye X, Lindstrom AB. Perfluorinated compounds in the Cape Fear Drainage Basin in North Carolina. Environ Sci Technol. 2007;41:5271–6.
27. Skutlarek D, Exner M, Farber H. Perfluorinated surfactants in surface and drinking water. Environ Sci Pollut R. 2006;13:299–307.
28. Osaka Prefecture, Monitoring of perfluorooctanoic acid and perfluorooctane sulfonate in river. 2007. http://www.epcc.pref.osaka.jp/press/h19/0831_2/.
29. Mitsuya R, Sometani A, Hosoda N, Matsuzaki T, Ohara K. A survey of PFOS and PFOA and its removal during water

treatment. Abstracts of 58th annual meeting of Japan Water Works Association. 2007; 554–5.
30. Harada K, Nakanishi S, Saito N, Tsutsui T, Koizumi A. Airborne perfluorooctanoate may be a substantial source contamination in Kyoto area, Japan. Bull Environ Contam Toxicol. 2005;74:64–9.
31. Harada K, Nakanishi S, Sasaki K, Furuyama K, Nakayama S, Saito N, et al. Particle size distribution and respiratory deposition estimates of airborne perfluorooctanoate and perfluorooctanesulfonate in Kyoto area, Japan. Bull Environ Contam Toxicol. 2006;76:306–10.
32. Sasaki K, Harada K, Saito N, Tsutsui T, Nakanishi S, Tsuzuki H, et al. Impact of airborne perfluorooctane sulfonate on the human body burden and the ecological system. Bull Environ Contam Toxicol. 2003;71:408–13.
33. Ministry of Environment, Chemicals in the Environment FY2004, 2006. http://www.env.go.jp/chemi/kurohon/2005/http2005d/d_section02_01.html.
34. Boulanger B, Peck AM, Schnoor JL, Hornbuckle KC. Mass budget of perfluorooctane surfactants in Lake Ontario. Environ Sci Technol. 2005;39:74–9.
35. Barber JL, Berger U, Chaemfa C, Huber S, Jahnke A, Temme C, et al. Analysis of per- and polyfluorinated alkyl substances in air samples from Northwest Europe. J Environ Monit. 2007;9:530–41.
36. Katsumata T, Nakata A, Iwasaki Y, Ito R, Saito K, Nakazawa H. Determination of perfluorochemicals in house-dust by LC/MS/MS after supercritical fluid extraction. Bunseki Kagaku. 2006; 55:955–61.
37. Moriwaki H, Takatah Y, Arakawa R. Concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) in vacuum cleaner dust collected in Japanese homes. J Environ Monit. 2003;5:753–7.
38. Strynar MJ, Lindstrom AB. Perfluorinated compounds in house dust from Ohio and North Carolina, USA. Environ Sci Technol. 2008;42:3751–6.
39. Dinglasan-Panlilio MJ, Mabury SA. Significant residual fluorinated alcohols present in various fluorinated materials. Environ Sci Technol. 2006;40:1447–53.
40. Ellis DA, Martin JW, Mabury SA, Hurley MD, Andersen MPS, Wallington TJ. Atmospheric lifetime of fluorotelomer alcohols. Environ Sci Technol. 2003;37:3816–20.
41. Oono S, Harada KH, Mahmoud M, Inoue K, Koizumi A. Survey of airborne polyfluorinated telomers in Japan. Chemosphere. 2008;73:932–7.
42. Oono S, Matsubara E, Harada KH, Takagi S, Hamada S, Asakawa A, et al. Survey of airborne polyfluorinated telomers in Keihan area, Japan. Bull Environ Contam Toxicol. 2008;80:102–6.
43. Fromme H, Schlummer M, Moller A, Gruber L, Wolz G, Ungewiss J, et al. Exposure of an adult population to perfluorinated substances using duplicate diet portions and biomonitoring data. Environ Sci Technol. 2007;41:7928–33.
44. Tittlemier SA, Pepper K, Seymour C, Moisey J, Bronson R, Cao XL, et al. Dietary exposure of Canadians to perfluorinated carboxylates and perfluorooctane sulfonate via consumption of meat, fish, fast foods, and food items prepared in their packaging. J Agric Food Chem. 2007;55:3203–10.
45. Karrman A, Harada KH, Inoue K, Takasuga T, Ohi E, Koizumi A. Analysis of perfluorochemicals (PFCs) in diet duplicates and serum from Japan. DIOXIN 2007, Tokyo, Japan.
46. Falandysz J, Taniyasu S, Gulkowska A, Yamashita N, Schulte-Oehlmann U. Is fish a major source of fluorinated surfactants and repellents in humans living on the Baltic Coast? Environ Sci Technol. 2006;40:748–51.
47. Begley TH, White K, Honigfort P, Twaroski ML, Neches R, Walker RA. Perfluorochemicals: potential sources of and migration from food packaging. Food Addit Contam. 2005;22:1023–31.
48. Aung NN, Yoshinaga J, Takahashi J-I. Exposure Assessment of Lead among Japanese Children. Environ Health Prev Med. 2004;9:257–61.
49. Harada K, Inoue K, Morikawa A, Yoshinaga T, Saito N, Koizumi A. Renal clearance of perfluorooctane sulfonate and perfluorooctanoate in humans and their species-specific excretion. Environ Res. 2005;99:253–61.
50. Harada K, Koizumi A, Saito N, Inoue K, Yoshinaga T, Date C, et al. Historical and geographical aspects of the increasing perfluorooctanoate and perfluorooctane sulfonate contamination in human serum in Japan. Chemosphere. 2007;66:293–301.
51. Fromme H, Midasch O, Twardella D, Angerer J, Boehmer S, Liebl B. Occurrence of perfluorinated substances in an adult German population in southern Bavaria. Int Arch Occup Environ Health. 2007;80:313–9.
52. Kärrman A, Mueller JF, van Bavel B, Harden F, Toms LM, Lindstrom G. Levels of 12 perfluorinated chemicals in pooled australian serum, collected 2002–2003, in relation to age, gender, and region. Environ Sci Technol. 2006;40:3742–8.
53. Calafat AM, Kuklenyik Z, Caudill SP, Reidy JA, Needham LL. Perfluorochemicals in pooled serum samples from USA residents in 2001 and 2002. Environ Sci Technol. 2006;40:2128–34.
54. Olsen GW, Huang HY, Helzlsouer KJ, Hansen KJ, Butenhoff JL, Mandel JH. Historical comparison of perfluorooctanesulfonate, perfluorooctanoate, and other fluorochemicals in human blood. Environ Health Perspect. 2005;113:539–45.
55. Jin Y, Saito N, Harada KH, Inoue K, Koizumi A. Historical trends in human serum levels of perfluorooctanoate and perfluorooctane sulfonate in Shenyang, China. Tohoku J Exp Med. 2007;212:63–70.
56. Olsen GW, Mair DC, Reagen WK, Ellefson ME, Ehresman DJ, Butenhoff JL, et al. Preliminary evidence of a decline in perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) concentrations in American Red Cross Blood Donors. Chemosphere. 2007;68:105–11.
57. Olsen GW, Burris JM, Ehresman DJ, Froehlich JW, Seacat AM, Butenhoff JL, et al. Half-life of serum elimination of perfluorooctanesulfonate, perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. Environ Health Perspect. 2007;115:1298–305.
58. Kudo N, Katakura M, Sato Y, Kawashima Y. Sex hormone-regulated renal transport of perfluorooctanoic acid. Chem Biol Interact. 2002;139:301–16.
59. Kudo N, Kawashima Y, The study of excretion pathway and long-term persistency of fluorinated fatty acids in primates. In: Annual reports of the Primate Research Institute, Kyoto University, Aichi, Vol. 31;2001
60. Butenhoff JL, Kennedy GL Jr, Hinderliter PM, Lieder PH, Jung R, Hansen KJ, et al. Pharmacokinetics of perfluorooctanoate in cynomolgus monkeys. Toxicol Sci. 2004;82:394–406.
61. Gibson SJ, Johnson JD, Absorption of FC–95-^{14}C in rats after a single oral dose. Riker Laboratories, Inc., Subsidiary of 3 M, St. Paul, MN, USEPA Public Docket AR-226-0007.1979.
62. Noker PE, Gorman GS. A pharmacokinetic study of potassium perfluorooctanesulfonate in the Cynomolgus monkey. USEPA public docket, administrative record AR-226-1356. 2003.
63. Johnson JD, Gibson SJ, Ober RE. Cholestyramine-enhanced fecal elimination of carbon-14 in rats after administration of ammonium [^{14}C]perfluorooctanoate or potassium [^{14}C]perfluorooctanesulfonate. Fundam Appl Toxicol. 1984;4:972–6.
64. Vanden Heuvel JP, Kuslikis BI, Van Rafelghem MJ, Peterson RE. Tissue distribution, metabolism, and elimination of perfluorooctanoic acid in male and female rats. J Biochem Toxicol. 1991;6:83–92.

65. Andersen ME, Clewell HJ 3rd, Tan YM, Butenhoff JL, Olsen GW. Pharmacokinetic modeling of saturable, renal resorption of perfluoroalkylacids in monkeys—probing the determinants of long plasma half-lives. Toxicology. 2006;227:156–64.
66. Nakagawa H, Hirata T, Terada T, Jutabha P, Miura D, Harada KH, et al. Roles of organic anion transporters in the renal excretion of perfluorooctanoic acid. Basic Clin Pharmacol Toxicol. 2008;103:1–8.
67. Katakura M, Kudo N, Tsuda T, Hibino Y, Mitsumoto A, Kawashima Y. Rat organic anion transporter 3 and organic anion transporting polypeptide 1 mediate perfluorooctanoic acid transport. J Health Sci. 2007;53:77–83.
68. Harada KH, Hashida S, Kaneko T, Takenaka K, Minata M, Inoue K, et al. Biliary excretion and cerebrospinal fluid partition of perfluorooctanoate and perfluorooctane sulfonate in humans. Environ Toxicol Pharmacol. 2007;24:134–9.
69. Maestri L, Negri S, Ferrari M, Ghittori S, Fabris F, Danesino P, et al. Determination of perfluorooctanoic acid and perfluorooctanesulfonate in human tissues by liquid chromatography/single quadrupole mass spectrometry. Rapid Commun Mass Spectrom. 2006;20:2728–34.
70. Buist SC, Cherrington NJ, Choudhuri S, Hartley DP, Klaassen CD. Gender-specific and developmental influences on the expression of rat organic anion transporters. J Pharmacol Exp Ther. 2002;301:145–51.
71. Karrman A, Ericson I, van Bavel B, Darnerud PO, Aune M, Glynn A, et al. Exposure of perfluorinated chemicals through lactation: levels of matched human milk and serum and a temporal trend, 1996–2004, in Sweden. Environ Health Perspect. 2007;115:226–30.
72. Inoue K, Okada F, Ito R, Kato S, Sasaki S, Nakajima S, et al. Perfluorooctane sulfonate (PFOS) and related perfluorinated compounds in human maternal and cord blood samples: assessment of PFOS exposure in a susceptible population during pregnancy. Environ Health Perspect. 2004;112:1204–7.
73. Han X, Snow TA, Kemper RA, Jepson GW. Binding of perfluorooctanoic acid to rat and human plasma proteins. Chem Res Toxicol. 2003;16:775–81.
74. Jones PD, Hu W, De Coen W, Newsted JL, Giesy JP. Binding of perfluorinated fatty acids to serum proteins. Environ Toxicol Chem. 2003;22:2639–49.
75. Olsen GW, Burris JM, Burlew MM, Mandel JH. Epidemiologic assessment of worker serum perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) concentrations and medical surveillance examinations. J Occup Environ Med. 2003;45:260–70.
76. Olsen GW, Burris JM, Mandel JH, Zobel LR. Serum perfluorooctane sulfonate and hepatic and lipid clinical chemistry tests in fluorochemical production employees. J Occup Environ Med. 1999;41:799–806.
77. Sakr CJ, Kreckmann KH, Green JW, Gillies PJ, Reynolds JL, Leonard RC. Cross-sectional study of lipids and liver enzymes related to a serum biomarker of exposure (ammonium perfluorooctanoate or APFO) as part of a general health survey in a cohort of occupationally exposed workers. J Occup Environ Med. 2007;49:1086–96.
78. Anderson-Mahoney P, Kotlerman J, Takhar H, Gray D, Dahlgren J. Self-reported health effects among community residents exposed to perfluorooctanoate. New Solut. 2008;18:129–43.
79. Abdellatif AG, Preat V, Taper HS, Roberfroid M. The modulation of rat liver carcinogenesis by perfluorooctanoic acid, a peroxisome proliferator. Toxicol Appl Pharmacol. 1991;111:530–7.
80. Seacat AM, Thomford PJ, Butenhoff JL. Terminal observations in Sprague Dawley rats after lifetime dietary exposure to potassium perfluorooclanesulfonate. Toxicologist. 2002;66:185.
81. Oda Y, Nakayama S, Harada KH, Koizumi A. Negative results of umu mutagenecity test of fluorotelomer alcohols and perfluorinated alkyl acids. Environ Health Prev Med. 2007;12:217–9.
82. Vanden Heuvel JP, Thompson JT, Frame SR, Gillies PJ. Differential activation of nuclear receptors by perfluorinated fatty acid analogs and natural fatty acids: a comparison of human, mouse, and rat peroxisome proliferator-activated receptor-alpha, -beta, and -gamma, liver X receptor-beta, and retinoid X receptor-alpha. Toxicol Sci. 2006;92:476–89.
83. Wolf DC, Moore T, Abbott BD, Rosen MB, Das KP, Zehr RD, et al. Comparative hepatic effects of perfluorooctanoic acid and WY 14, 643 in PPAR-alpha knockout and wild-type mice. Toxicol Pathol. 2008;36:632–9.
84. Rosen MB, Lee JS, Ren H, Vallanat B, Liu J, Waalkes MP, et al. Toxicogenomic dissection of the perfluorooctanoic acid transcript profile in mouse liver: evidence for the involvement of nuclear receptors PPAR alpha and CAR. Toxicol Sci. 2008;103:46–56.
85. Butenhoff J, Costa G, Elcombe C, Farrar D, Hansen K, Iwai H, et al. Toxicity of ammonium perfluorooctanoate in male cynomolgus monkeys after oral dosing for 6 months. Toxicol Sci. 2002;69:244–57.
86. Seacat AM, Thomford PJ, Hansen KJ, Olsen GW, Case MT, Butenhoff JL. Subchronic toxicity studies on perfluorooctanesulfonate potassium salt in cynomolgus monkeys. Toxicol Sci. 2002;68:249–64.
87. Lau C, Butenhoff JL, Rogers JM. The developmental toxicity of perfluoroalkyl acids and their derivatives. Toxicol Appl Pharmacol. 2004;198:231–41.
88. Lau C, Thibodeaux JR, Hanson RG, Rogers JM, Grey BE, Stanton ME, et al. Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. II: postnatal evaluation. Toxicol Sci. 2003;74:382–92.
89. Thibodeaux J, Hanson RG, Grey BE, Barbee BD, Richards JH, Butenhoff JL, et al. Maternal and developmental toxicity of perfluorooctane sulfonate (PFOS) in the mouse. Toxicol Sci. 2003;72:342.
90. Keil DE, Mehlmann T, Butterworth L, Peden-Adams MM. Gestational exposure to perfluorooctane sulfonate suppresses immune function in B6C3F1 mice. Toxicol Sci. 2008;103:77–85.
91. Abbott BD, Wolf CJ, Schmid JE, Das KP, Zehr RD, Helfant L, et al. Perfluorooctanoic acid-induced developmental toxicity in the mouse is dependent on expression of peroxisome proliferator-activated receptor-alpha. Toxicol Sci. 2007;98:571–81.
92. Abbott BD, Wolf CJ, Das KP, Zehr RD, Schmid JE, Lindstrom AB, et al. Developmental toxicity of perfluorooctane sulfonate (PFOS) is not dependent on expression of peroxisome proliferator activated receptor-alpha (PPARα) in the mouse. Reprod Toxicol. 2008.
93. Austin ME, Kasturi BS, Barber M, Kannan K, MohanKumar PS, MohanKumar SM. Neuroendocrine effects of perfluorooctane sulfonate in rats. Environ Health Perspect. 2003;111:1485–9.
94. Asakawa A, Toyoshima M, Fujimiya M, Harada K, Ataka K, Inoue K, et al. Perfluorooctane sulfonate influences feeding behavior and gut motility via the hypothalamus. Int J Mol Med. 2007;19:733–9.
95. Asakawa A, Toyoshima M, Harada KH, Fujimiya M, Inoue K, Koizumi A. The ubiquitous environmental pollutant perfluorooctanoic acid inhibits feeding behavior via peroxisome proliferator-activated receptor-alpha. Int J Mol Med. 2008;21:439–45.
96. Fuentes S, Vicens P, Colomina MT, Domingo JL. Behavioral effects in adult mice exposed to perfluorooctane sulfonate (PFOS). Toxicology. 2007;242:123–9.

97. Harada K, Xu F, Ono K, Iijima T, Koizumi A. Effects of PFOS and PFOA on L-type Ca^{2+} currents in guinea-pig ventricular myocytes. Biochem Biophys Res Commun. 2005;329:487–94.
98. Harada KH, Ishii TM, Takatsuka K, Koizumi A, Ohmori H. Effects of perfluorooctane sulfonate on action potentials and currents in cultured rat cerebellar Purkinje cells. Biochem Biophys Res Commun. 2006;351:240–5.
99. Matsubara E, Harada K, Inoue K, Koizumi A. Effects of perfluorinated amphiphiles on backward swimming in *Paramecium caudatum*. Biochem Biophys Res Commun. 2006;339:554–61.
100. Matsubara E, Nakahari T, Yoshida H, Kuroiwa T, Harada KH, Inoue K, et al. Effects of perfluorooctane sulfonate on tracheal ciliary beating frequency in mice. Toxicology. 2007;236:190–8.
101. Liao CY, Li XY, Wu B, Duan S, Jiang GB. Acute enhancement of synaptic transmission and chronic inhibition of synaptogenesis induced by perluorooctane sulfonate through mediation of voltage-dependent calcium channel. Environ Sci Technol. 2008;42:5335–41.

Environment International 35 (2009) 1072–1079

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Levels and regional trends of persistent organochlorines and polybrominated diphenyl ethers in Asian breast milk demonstrate POPs signatures unique to individual countries

Koichi Haraguchi [a,*], Akio Koizumi [b], Kayoko Inoue [b], Kouji H. Harada [b], Toshiaki Hitomi [b], Mutsuko Minata [b], Miyako Tanabe [b], Yoshihisa Kato [c], Eri Nishimura [a], Yoshiaki Yamamoto [d], Takao Watanabe [e], Katsunobu Takenaka [f], Shigeki Uehara [g], Hye-Ran Yang [h], Min-Young Kim [h], Chan-Seok Moon [i], Hae-Sook Kim [j], Peiyu Wang [k], Aiping Liu [k], Nguyen Ngoc Hung [l]

[a] *Daiichi College of Pharmaceutical Sciences, Fukuoka 815-8511, Japan*
[b] *Department of Health and Environmental Sciences, Kyoto University Graduate School of Medicine, Yoshida Kyoto 606-8501, Japan*
[c] *Kagawa School of Pharmaceutical Sciences, Tokushima Bunri University, Sanuki, Kagawa 769-2193, Japan*
[d] *Department of Obstetrics and Gynecology, Ijinkai Takeda General Hospital, Ishida Kyoto 601-1495, Japan*
[e] *Miyagi University of Education, Sendai 980-0845, Japan*
[f] *Department of Neurosurgery, Takayama Red Cross Hospital, Takayama 506-8550, Japan*
[g] *Department of Obstetrics and Gynecology, Tohoku Kosai Hospital, Sendai 980-0803, Japan*
[h] *Research Institute of Public Health and Environment, Seoul Metropolitan Government, Yangjae Seoul 137734, Republic of Korea*
[i] *Department of Industrial Health, Catholic University of Pusan, Keumjeong, Busan 609757, Republic of Korea*
[j] *College of Nursing Science, Kyung Hee University, Seoul 130701, Republic of Korea*
[k] *Department of Social Medicine and Health Education, School of Public Health, Peking University, Haidian Beijing 100083, PR China*
[l] *Department of Science and Technology, Hanoi Medical University, Ton That Tung, Hanoi, S.R. Vietnam*

ARTICLE INFO

Article history:
Received 2 March 2009
Accepted 7 June 2009
Available online 1 July 2009

Keywords:
Human milk
DDTs
Pesticides
PCBs
PBDEs
Asia
Exposure

ABSTRACT

Human breast milk samples collected in 2007–2008 from four countries, Vietnam (Hanoi), China (Beijing), Korea (Seoul) and Japan (Sendai, Kyoto and Takayama), were analyzed for persistent organic pollutants (POPs) such as dichlorodiphenyltrichloroethane and its metabolites (DDTs), chlordane-related compounds (CHLs), hexachlorocyclohexanes (HCHs), hexachlorobenzene (HCB), polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs). Comparing with previous surveys, the present study indicates that the DDTs in breast milk from China and Vietnam had gradually decreased during the last decade, but were still 5–10 times higher than those in other nations. The ratios of *p,p′*-DDE/*p,p′*-DDT and *o,p′*-DDT/*p,p′*-DDT were higher in Beijing than in the other countries, suggesting that there is less fresh intake of commercial DDT products and a possible exposure to dicofol in China. CHL and PCB levels were relatively higher in mothers from Japan, whereas β-HCH and HCB were more common in Chinese women. In Japan, it is suspected that mothers in the urban/coastal area (Sendai) were more continuously exposed to organochlorine pesticides (OCPs) than mothers in the rural/inland area (Takayama). In addition, OCP levels in primiparae were significantly higher than those in multiparae from Japan and Korea. These indicate that both parity and regional factors are major determinants of the levels of OCPs and PCBs in human milk. On the other hand, higher concentrations of PBDEs were observed in mothers' milk from Korea. The congener was dominated by BDE-47 (43–54%), followed by BDE-153 (23–33%) in all regions except for Beijing where BDE-28 (23%) was relatively abundant. In Japanese breast milk, regional and parity-dependent distributions were not observed for PBDEs. Among PBDE congeners, age-dependency was observed for BDE-153, which was negatively correlated ($p<0.05$) to the age of mothers in Kyoto (17 participants were housewives), while it increased with age in Sendai (10 participants were clerks). No such correlation was seen for BDE-47, indicating that BDE-47 was ingested and assimilated via different kinetics or routes from BDE-153 in Japan.

1. Introduction

Environmental contamination by synthetic halogenated compounds, including organochlorine pesticides (OCPs), has been a major concern over the past three decades, because of their persistence, long-range

* Corresponding author. Daiichi College of Pharmaceutical Sciences, 22-1 Tamagawa-cho, Minami-ku, Fukuoka 815-8511, Japan. Tel.: +81 09 541 0161; fax: +81 92 554 5698.
E-mail address: k-haraguti@daiichi-cps.ac.jp (K. Haraguchi).

0160-4120/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.envint.2009.06.003

transport, and long-term adverse effects on humans, animals and the environment. Most of the developed nations have already banned or restricted the production and usage of persistent organic pollutants (POPs) such as dichlorodiphenyltrichloroethane and its metabolites (DDTs), chlordane-related compounds (CHLs), hexachlorocyclohexanes (HCHs), hexachlorobenzene (HCB) and polychlorinated biphenyls (PCBs). However, recent studies in East-Asian countries have reported elevated concentrations of OCPs in various environmental media, suggesting that some of these OCPs are still being used (Wong et al., 2005). In Japan, although ban on the usage of CHLs was enforced in 1986, no reduction of CHLs was observed until 1998 (Konishi et al., 2001). In China, DDTs, HCHs and HCB decreased until 2000, but their levels in human milk appear to be still the highest among Asian nations (Kunisue et al., 2004; Wong et al., 2002). In Vietnam and Korea, there is little information on the current contamination trend of OCPs. Overall, it is likely that humans in Asian countries may still be exposed to relatively high levels of POPs, implying that the infants are continuously exposed to OCPs and PCBs via lactation (Wong et al., 2005).

Polybrominated diphenyl ethers (PBDEs) are flame retardants which are used as additives in polymeric materials for ensuring the fire safety of furniture, textiles and electronics. Due to their similar lipophilicity to PCBs, PBDEs have been detected in milk, serum and adipose tissue of humans from different countries throughout the world (Akutsu et al., 2003; Choi et al., 2003; Kalantzi et al., 2004; Koizumi et al., 2005; Inoue et al., 2006; She et al., 2007). Although the levels of PCBs have decreased in industrialized countries over the decades, PBDEs appear to be increasing or remaining at higher levels in North America (Johnson-Restrepo et al., 2007). In Asia, although several studies have shown that the levels of PBDEs in breast milk are still lower than those from Europe and USA, the source of PBDEs has changed during the last decade, depending on domestic demands (Choi et al., 2003; Watanabe and Sakai, 2003; Wang et al., 2008). PBDEs may enter humans through the food chain (Ohta et al., 2002; Wada et al., 2005), by ingestion of dust (Wu et al., 2007; Sjödin et al., 2008), by inhalation of some BDE congeners at home or at a job in the electronics and computer industries (Sjödin et al., 2001). Like PCBs, PBDEs are transferred via the placenta and breast milk from the mother to the offspring in humans (Guvenius et al., 2003; Kawashiro et al., 2008). The available data suggest that some of these congeners are potential thyroid disruptors (Meerts et al., 2000) and developmental neurotoxins (Costa and Giordano, 2007). Therefore, the presence of PBDEs in humans is of great concern to the healthy growth of fetuses and infants.

The present study was designed to compare differences in the concentrations of DDTs, CHLs, HCHs, HCB, PCBs and PBDEs in human milk samples among four Asian countries (Vietnam, China, Korea and Japan) as well as among three regions in Japan: an urban/coastal area (Sendai), an urban/inland area (Kyoto) and a rural/inland area (Takayama) (Fig. 1). One of the purposes of this study was to evaluate the regional trends and sources of POPs in human breast milk from four Asian countries as compared with the previous data from the other countries/regions. The other purpose of this study was to assess the relationships between POP contaminant levels and the parity/age of mothers from respective countries or regions.

2. Materials and methods

2.1. Sample collection

Human milk was obtained from the Kyoto University Human Specimen Bank (Koizumi et al., 2005). A total of 134 human milk samples were collected in 2007–2008 from volunteers living in Vietnam ($n=20$, Hanoi in Nov. 2007), China ($n=25$, Beijing in Dec. 2007), Korea ($n=29$, Seoul in Oct. 2007), and Japan ($n=20$, Sendai in Sept. 2007; $n=20$, Kyoto from Dec. 2007 to Apr. 2008 and $n=20$, Takayama from Sept. to Nov. 2007). Milk samples were

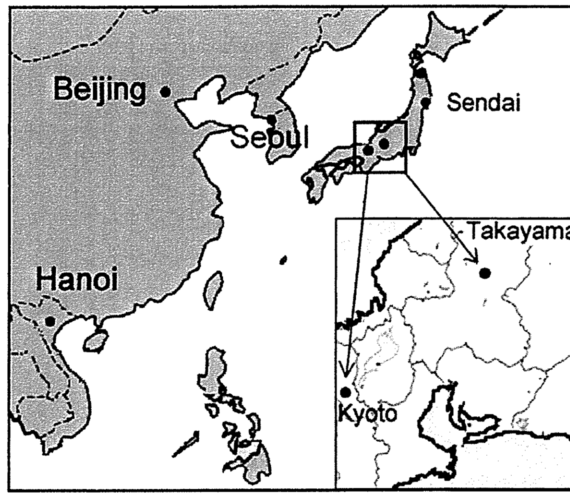

Fig. 1. Map of sampling locations.

collected manually during breast-feeding, 4–8 weeks after childbirth, either by the subjects themselves or with the assistance of midwives. The target volume was at least 10 mL from each mother during one sampling. The breast milk was kept frozen (−20 °C) in 15 mL polypropylene conical tubes. Five empty tubes were prepared for field blanks, and 10 distilled water tubes were prepared for operational blanks and tested for possible contamination. The Ethics Committee of Kyoto University approved the present study and appropriate written informed consent was obtained from all participants.

2.2. Chemicals

Two internal standards, $^{13}C_{12}$-labelled 2,3,4,5,6,3′,4′,5′-octachlorobiphenyl (CB-205, AccuStandard, Inc., CA, USA) and 4′-methoxy-3′,5′,2,4,6-pentachlorodiphenyl ether (4′-MeO-BDE121, donated by Dr. G. Marsh from Stockholm University) were used for the determination of OCPs and PBDEs. The analytes investigated were *p,p*′-dichlorodiphenyldichloroethylene (*p,p*′-DDE), *p,p*′-dichlorodiphenyldichloroethane (*p,p*′-DDD), *o,p*′-dichlorodiphenyltrichloroethane (*o,p*′-DDT), *p,p*′-dichlorodiphenyltrichloroethane (*p,p*′-DDT), oxychlordane, trans-nonachlor, cis-nonachlor, hexachlorocyclohexanes (α-, β- and γ-HCHs), and hexachlorobenzene (HCB). Persistent pesticide calibration solution (ES-5019, Cambridge Isotope Laboratories, Inc., MA, USA) was used for four point calibration and determination of OCPs. For determination of PCBs, a standard PCB mixture (C-SCA-06, AccuStandard, Inc., CT, USA) was used for identification, and 15 selected isomers were quantified. For the determination of PBDEs, native BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154 and BDE-187 (Wellington Laboratories, Inc., ON, Canada) were used as external standards. All solvents used were of pesticide grade quality.

2.3. Clean up procedure

Before extraction, a milk sample (10 mL) was fortified with two internal standards, ^{13}C-labelled CB-205 (2 ng) and 4′-MeO-BDE121 (0.2 ng). We extracted human milk (10 mL) twice using 15 mL of *n*-hexane, after adding 5 mL of 2% calcium oxalate solution, and 10 mL of ethanol and diethylether (1:1). The extract was washed with water and dried over sodium sulfate. After solvent evaporation, gravimetric lipid determination was performed. An aliquot of lipids (200–300 mg) was dissolved in *n*-hexane:dichloromethane (1:1) and subjected to gel permeation chromatography (400 × 45 mm, id, Bio-Beads S-X3, Bio-Rad Laboratories, CA, USA). Eluate containing

Table 1
GC/MS conditions and selected ions (m/z) for determination of POPs.

Carrier gas	Helium (head pressure of 3 psi)	
Injection mode	Splitless	
Column	HP-5MS (30% dimethylpolysiloxane, 30 m × 0.25 mm i.d. and 0.25 μm film thickness, J&W Scientific, CA, USA)	
Oven	70 °C (1.5 min), then 20 °C/min to 230 °C (0.5 min), and then 4 °C/min to 280 °C (5 min)	
Temperature	Injector (250 °C), transfer line (280 °C), and ion source (230 °C for EI, 150 °C for ECNI)	
Target ions — EI	235 (237)[a]	o,p′-DDT, o,p′-DDT, p,p′-DDD
	318 (316)	p,p′-DDE
	373 (375)	Oxychlordane
	409 (411)	trans- and cis-nonachlor
	219 (217)	HCHs (α, β, γ)
	284 (286)	HCB
	292 (294)	TetraCBs (#74)
	324 (326)	PentaCBs (#99, #101, #105, #118)
	362 (360)	HexaCBs (#138, #146, #149, #153, #156, #163)
	394 (396)	HeptaCBs (#170, #180, #183, #187)
	442 (444)	[^{13}C]CB-205 (IS)
Target ions — ECNI	79 (81)	PBDEs (28, 47, 99, 100, 153, 154, 183) 4′-MeO-BDE121 (IS)

[a] Confirmation ion.

lipophilic organohalogens was concentrated to dryness and dissolved in *n*-hexane (1 mL). The extract was then purified by silica gel chromatography (1 g, Wako gel S-1, Wako Pure Chemical Industries, Ltd., Osaka, Japan), by eluting with 15 mL of *n*-hexane:dichloromethane (88:12 v/v). This fraction was concentrated to 200 μL prior to gas chromatography–mass spectrometry (GC–MS) analysis.

2.4. Instruments and quantification

GC–MS analyses of the samples and reference standards were performed on an Agilent GC/MSD 5973i (Agilent technologies, CA, USA) equipped with a 6890 N gas chromatograph. GC/MS conditions and target ions for the determination of POPs are summarized in Table 1. DDTs, CHLs, HCHs, HCB and PCBs were analyzed by electron ionization (EI) mode, whereas PBDEs were analyzed by scanning for the negative bromine ion (isotopes m/z 79 and 81) formed by electron capture reactions at chemical ionization (ECNI) with methane as the reagent gas. All analytes were quantified by comparing the peak area of the particular compound in the sample extracts to that of the internal standard ($^{13}C_{12}$-labelled CB-205 for OCPs and PCBs; 4′-MeO-BDE121 for PBDEs).

2.5. Quality control

For quality assurance and control, a standard reference material (cod liver oil, NIST, SRM 1588b) was analyzed for selected OCPs, PCBs and PBDEs. Data from our laboratory were in good agreement with the certified values (range; 5.5–9.2% relative standard deviation, $n = 8$) for POPs. Therefore, no correction was made for the determinations. Quantification was done only if the sample level was at least twice the blank level. The limits of quantification (LOQ) for all target compounds were between 0.1 and 2.5 ng/g lipid. Procedural blanks processed in parallel to every batch of ten samples were negligible. The samples were kept in the dark during the extraction.

2.6. Statistic analysis

Statistical treatment of the obtained results was performed using the SPSS software (SPSS, version 14.0 for windows 2001, SPSS, Inc., IL, USA). The Mann–Whitney *U*-test was used to examine differences in POP concentrations between subjects grouped by parity. Spearman's rank correlation coefficient was used to measure the strength of the association between the mother's ages and POP concentrations. Probability values less than 0.05 were considered as statistically significant.

3. Results and discussion

3.1. Contamination trend

Age, occupation of the mothers and lipid contents of milk samples are listed in Table 2. The participants were on average about 4 years older in Japan and Korea (31 years old each) than in China and Vietnam (27 years old each). Lipid contents ranged from 3.1 to 4.5% and no regional differences were observed. Table 3 shows the lipid-normalized mean concentrations (ng/g lipid) of DDTs (4 congeners), CHLs (3 congeners), β-HCH and HCB, PCBs (2 congeners) and PBDEs (6 congeners) in mother's milk from six regions in four countries.

3.1.1. DDTs

The mean concentrations of DDTs were 1300 ng/g lipid ($n = 25$) in China and 1200 ng/g lipid ($n = 20$) in Vietnam, both of which were 5 to 7 times higher than values in Korea (180 ng/g lipid) and Japan (170 ng/g lipid). DDT composition was dominated by *p,p′*-DDE (94–96%), followed by *p,p′*-DDT (3–5%), and *p,p′*-DDD (1–2%) in most cases. The levels of *p,p′*-DDD were the highest in mothers from Hanoi, whereas the levels of *o,p′*-DDT were the highest in mothers from Beijing. Thus the ratios of *p,p′*-DDE/*p,p′*-DDT and *o,p′*-DDT/*p,p′*-DDT were significantly higher in Beijing than in the other regions (Table 3).

In China, DDT levels in breast milk had drastically declined from 7700 to 2000 ng/g lipid during the period 1983–1998 (Yu et al., 2006). The present data indicate that DDTs in mothers from Beijing have been gradually decreasing since 1998, but the levels were still 5–10 times higher than those from the developed nations (Table 4). We are aware that variation of DDT levels in human milk is large in China depending on whether it comes from an urban, rural, inland or coastal area (Wong et al., 2002; Kunisue et al., 2004) while possibly reflecting the historical or current use of DDTs. In particular, notably higher concentrations of DDTs have been observed in mothers from Guangzhou, Hong Kong, Dalian and Beijing (Table 4). In Vietnam, from the 2000 survey, DDT levels were reported to be 2100 ng/g lipid ($n = 42$) (Minh et al., 2004). The present study showed that the mean concentration of DDTs in mothers from Hanoi has decreased by approximately half in the last eight years similar to the case observed for mothers from Beijing. However, considering the half-lives (5–10 years) of DDTs in humans (Nakata et al., 2005), the technical product may have continued until recently for agricultural purposes and vector-borne disease eradication programs (Wong et al., 2005). In Korea and Japan, there was no temporal change in DDT levels during the last decade (Konishi et al., 2001; Kunisue et al., 2006), but regional variation was found ($p < 0.05$) between the coastal/urban city (Sendai) and the inland/rural city (Takayama) (Table 3). In mothers' milk from Seoul (urban), the levels were similar to those in Sendai (urban), and comparable to other reports from urban cities from Taiwan (Chao et al., 2006) and the UK (Kalantzi et al., 2004).

The concentration ratio of *p,p′*-DDE/*p,p′*-DDT is usually used as an indicator for the resident time of *p,p′*-DDT in the environment. The lower the ratio is, the more recent the exposure to the parent DDT (Wong et al., 2005). In the present study, the ratios in Beijing were higher than the ratios from the other regions, while the ratios in Korea were relatively low (Table 3). The high ratios and high *p,p′*-DDE levels may imply the historical application of technical DDTs in China. Alternatively, the elevated DDE levels

Table 2
Information on age, occupation of participants and lipid contents of milk samples from six regions.

Region	n	Age[a]	Participants (number)					Lipid (%)
			Housewife	Clerk	Others	Primiparae	Multiparae	
Hanoi	20	27 (23–32)[b]	6	7	7	20	0	3.3 (0.8–8.6)[b]
Beijing	25	27 (23–30)	0	10	15	25	0	4.3 (1.7–7.1)
Seoul	29	31 (22–37)	13	3	13	20	9	3.1 (1.4–5.3)
Sendai	20	32 (27–41)	3	10	7	14	6	3.4 (1.4–7.6)
Kyoto	20	30 (19–39)	17	0	3	11	9	3.2 (1.1–7.5)
Takayama	20	31 (26–43)	2	14	4	6	14	4.5 (1.4–8.2)

[a] Year.
[b] Range.

Table 3
Concentrations (ng/g lipid) of major POP congeners in human breast milk from four countries/regions.

Congener	Mean concentration, ng/g lipid (range) and ratio						
	Vietnam	China	Korea	Japan			
	Hanoi ($n=20$)	Beijing ($n=25$)	Seoul ($n=29$)	Sendai ($n=20$)	Kyoto ($n=20$)	Takayama ($n=20$)	Total ($n=60$)
p,p'-DDE	1200 (250–3000)	1250 (410–2800)	170 (35–570)	250 (60–960)	150 (43–540)	92 (19–270)	160 (19–960)
p,p'-DDD	9.4* (2.3–37)	5.7 (2.6–15)	2.0 (0.36–4.2)	1.7 (0.73–4.0)	1.5 (0.41–2.7)	1.1 (0.55–1.8)	1.4 (0.41–4.0)
o,p'-DDT	4.7 (0.40–15)	8.0** (2.3–41)	2.0 (0.14–5.9)	1.4 (0.10–4.4)	0.54 (nd–2.0)	0.62 (0.09–1.7)	0.84 (nd–4.4)
p,p'-DDT	56 (10–180)	38 (8.2–160)	10 (3.8–20)	6.9 (2.0–17)	6.4 (2.1–13)	4.0 (1.2–13)	5.8 (1.2–17)
ΣDDT	1200 (280–3300)	1300 (430–3000)	180 (49–580)	260 (65–970)	160 (49–550)	97 (23–180)	170 (23–970)
trans-Nonachlor	0.59 (0.28–0.98)	2.6 (0.86–7.3)	6.5 (2.9–12)	32 (5.4–100)	20 (3.5–39)	10 (2.9–20)	21 (2.9–100)
cis-Nonachlor	0.11 (nd–0.55)	0.71 (nd–2.7)	2.2 (0.69–4.4)	10 (2.3–29)	7.9 (0.70–15)	4.2 (1.4–6.2)	7.4 (0.70–29)
Oxychlordane	0.047 (nd–0.31)	0.49 (0.19–1.1)	5.1 (2.2–19)	4.8 (0.94–12)	3.3 (1.5–8.3)	3.0 (0.72–12)	3.7 (0.72–12)
ΣCHL	0.75 (0.28–1.7)	3.8 (1.4–11)	14 (6.4–31)	47* (8.6–140)	31 (11–58)	17 (6.3–27)	31** (8.6–140)
β-HCH	140 (6–3000)	570** (67–3000)	110 (17–830)	190 (9–1200)	77 (31–270)	49 (10–370)	140 (9–1200)
HCB	7.4 (4.2–13)	86** (48–150)	13 (8.1–21)	18 (6.4–31)	13 (4.5–25)	8.1 (2.7–14)	13 (8.1–21)
CB-118	11 (5.2–20)	6.4 (3.2–16)	4.5 (1.9–12)	10 (3.4–20)	7.8 (1.4–19)	5.0 (1.9–9.7)	7.7 (1.4–20)
CB-153	19 (7.2–38)	11 (2.2–29)	17 (5.5–37)	43 (15–110)	31 (3.8–59)	20 (8.6–36)	32 (3.8–110)
Σ PCB (15 congeners)	84 (36–150)	56 (26–130)	61 (20–128)	150* (69–360)	110 (14–210)	79 (39–140)	110** (14–360)
BDE-28	0.04 (nd–0.23)	0.43 (0.11–2.8)	0.14 (0.01–0.52)	0.08 (0.05–0.25)	0.04 (nd–0.12)	0.08 (0.02–0.22)	0.07 (nd–0.25)
BDE-47	0.19 (0.07–0.40)	0.89 (0.34–4.0)	2.0 (0.33–20)	0.76 (0.16–2.0)	0.58 (0.21–1.1)	0.57 (0.18–1.1)	0.64 (0.16–2.0)
BDE-99	0.02 (nd–0.07)	0.07 (0.01–0.36)	0.33 (0.06–1.3)	0.14 (nd–0.38)	0.11 (nd–0.30)	0.11 (0.03–0.22)	0.12 (nd–0.38)
BDE-100	0.02 (nd–0.17)	0.08 (0.02–0.33)	0.28 (0.01–2.2)	0.10 (nd–0.49)	0.10 (nd–0.22)	0.12 (0.02–0.48)	0.11 (nd–0.49)
BDE-153	0.14 (nd–0.49)	0.38 (0.12–0.92)	0.86 (0.14–3.49)	0.45 (0.08–1.5)	0.42 (0.15–0.73)	0.29 (0.10–0.77)	0.39 (0.08–1.54)
BDE-154	0.005 (nd–0.02)	0.05 (nd–0.11)	0.13 (0.01–0.36)	0.19 (nd–0.70)	0.19 (nd–1.2)	0.10 (0.02–0.25)	0.16 (nd–1.2)
Σ PBDE (6 congeners)	0.42 (0.15–0.83)	1.9 (0.88–7.7)	3.7* (0.82–24)	1.7 (0.36–4.7)	1.4 (0.55–2.8)	1.3 (0.50–2.0)	1.5 (0.36–4.7)
Ratio							
p,p'-DDE/*p,p'*-DDT	30	53**	18	39	28	27	32
o,p'-DDT/*p,p'*-DDT	0.095	0.28**	0.19	0.18	0.091	0.15	0.14
CB-153/BDE-153	140	29	20	96	74	70	82
BDE-47/BDE-153	1.4	2.3	2.3	1.7	1.4	2.0	1.6

The asterisk represents the significant difference from the other countries (*$p<0.05$, **$p<0.01$).
The concentration below the detection limit was treated as zero for the arithmetic mean.

in Chinese milk may be attributed to contamination by dicofol products. Even after the ban of technical DDTs in 1983, DDTs are still being produced in China for use in malaria control and are also present in dicofol, which contains 3–7% DDT as impurities (Qiu et al., 2004, 2005). When compared, the ratios of *o,p'*-DDT/*p,p'*-DDT in human milk were on average 0.28 in Chinese women, whereas they were less than 0.1 in Vietnamese women (Table 3). The significant difference in ratios may be explained by the fact that Chinese women were exposed to dicofol that contains higher levels of *o,p'*-DDT (Qiu et al., 2004; Li et al., 2006), whereas Vietnamese women were exposed to higher levels of *p,p'*-DDT by technical DDT and/or its historical accumulation. The source of DDT contamination in Japanese and Korean populations may be partly due to dicofol, since the ratios of *o,p'*-DDT/*p,p'*-DDT are higher than the ratios found in Hanoi. Furthermore, in areas with intensive use of dicofol containing high DDT concentrations, it has been suggested that *p,p'*-DDE in the air or other media originated not only from the degradation of *p,p'*-DDT but also from the degradation of α-chloro-DDT (*p,p'*-Cl-DDT), an intermediate (impurity) in dicofol manufacture and during GC analysis (Qiu et al., 2004). We suspect that these findings may cause an overestimation of the *p,p'*-DDE concentrations in breast milk, but we could not conclude the possible source of *p,p'*-DDE contamination in human milk without further investigation.

Table 4
Comparison of mean concentrations (ng/g lipid) of OCPs and PCBs in breast milk from different countries or regions.

Country	Region	Year	n[a]	ΣDDT	ΣCHL	HCB	ΣHCH	ΣPCB	Reference
Japan	Sendai (Miyagi)	2007	20 (14)	260	46	18	190	150	This study
	Kyoto	2007–2008	20 (11)	160	31	13	77	110	This study
	Takayama (Gifu)	2007	20 (6)	97	17	8.1	49	79	This study
	Fukuoka	2001–2004	(38)	340	80	14	110	240	Kunisue et al. (2006)
	Osaka	1998	49	290	85	14	210	200	Konishi et al. (2001)
Korea	Seoul	2007	29 (20)	180	14	13	110	61	This study
China	Beijing	2007	25 (25)	1300	3.8	86	570	56	This study
	Beijing	1998	60	2000[b]	–	–	1200[b]	–	Yu et al. (2006)
	Shenyang	2002	20	870	6.7	56	550	28	Kunisue et al. (2004)
	Dalian	2002	20	2100	16	81	1400	42	Kunisue et al. (2004)
	Guangzhou	2000	54	3600	–	–	1100	33	Wong et al. (2002)
	Hong Kong	1999	132	2900	–	–	950	42	Wong et al. (2002)
Vietnam	Hanoi	2007	20 (20)	1200	0.75	7.4	140	84	This study
	Hanoi	2000	42	2100	2.0	3.9	58	74	Minh et al. (2004)
	Hochiminh	2001	44	2300	6.9	2.5	14	79	Minh et al. (2004)
Taiwan	Taichung	2000–2001	36 (30)	330	10	–	3.4	–	Chao et al. (2006)
Indonesia	Jakarta	2001	16	640	2.0	2.2	14	33	Sudaryanto et al. (2006)
	Purwakarta	2002	18	1300	7.7	1.8	30	24	Sudaryanto et al. (2006)
Australia	Melbourne	2002–2003	39	480	14	12	190	–	Mueller et al. (2008)
Russia	Buryatia	2003–2004	10	660	19	100	810	240	Tsydenova et al. (2007)
	Arkhangelsk	1996–1997	(40)	1400	36	77	410	370	Polder et al. (2003)
USA	Massachusetts	2004	38	64	32	2.3	19	–	Johnson-Restrepo et al. (2007)
UK	Lancaster/London	2001–2003	54	150[b]	–	17	40	150	Kalantzi et al. (2004)

[a] The figures in parentheses represent the number of primiparae.
[b] Median.

3.1.2. CHLs

CHLs were relatively abundant (31 ng/g lipid) in Japanese breast milk compared with mothers' milk from the other countries, where the levels were 14 ng/g lipid in Seoul, 3.8 ng/g lipid in Beijing and 0.75 ng/g lipid in Hanoi (Table 3). CHL composition was dominated by trans-nonachlor, followed by oxychlordane or cis-nonachlor in most cases. High percentages of oxychlordane (36% of ΣCHL) were found in the milk from Korea. In Japan, the use of CHLs rapidly increased during the 1980s, and then its use was prohibited in 1986. Based on the results from a survey during 1986–1998, CHL levels in breast milk slightly decreased from 110 to 85 ng/g lipid in the Osaka area (Konishi et al., 2001) and to 80 ng/g lipid during 2001–2004 in the Fukuoka area (Kunisue et al., 2006). The present study indicated a continuous reduction of CHLs in Japan. The levels are comparable to those from Russia (Polder et al., 2003; Tsydenova et al., 2007) and North America (Johnson-Restrepo et al., 2007), but are still higher than those from the other Asian countries (Table 4). A potential source of CHLs in breast milk might be the diet. However, another source may be the house (closed system), since CHLs have been used as an insecticide which is sprayed under the floor of a house for termite pest control (Taguchi and Yakushiji, 1988; Konishi et al., 2001).

3.1.3. HCHs

HCHs in breast milk were dominated by β-HCH. We could not detect any other isomers of HCHs in all samples. HCH levels were the highest in Beijing (570 ng/g lipid), followed by Sendai (190 ng/g lipid) and the lowest in Takayama (49 ng/g lipid) (Table 3). In the 1998 and 2000 surveys (Minh et al., 2004; Yu et al., 2006), HCH levels were 1200 ng/g lipid in Beijing and 58 ng/g lipid in Hanoi. The current study indicated that the levels in Hanoi increased twice, whereas the levels in Beijing decreased by half during the last decade. The contamination levels of HCHs in China are in the same range as those in Russia, and still higher than those found in the developed nations (Table 4). These results indicate that technical HCH may be still illegally used for public health purposes. In Japan, regional variation in HCH levels was seen between Sendai and Takayama by a factor of 4. The present data were higher or comparable to the data from the 2001 survey (Konishi et al., 2001; Kunisue et al., 2006), indicating that HCH levels have not decreased since 2001. In particular, the human exposure to HCHs in Sendai, where the maximum was 1200 ng/g lipid in milk, may be explained by continuous intake of contaminated foods (Polder et al., 2003). The exposure of infants to β-HCH as a result of breast-feeding has been recognized as a matter of concern because of its possible health implications (Pohl and Tylenda, 2000). Dietary products should be further monitored for β-HCH in order to elucidate future pollution trends in Japan and China.

3.1.4. HCB

The mean concentrations of HCB in human breast milk from Beijing (86 ng/g lipid) were about one order of magnitude higher than those from the other regions investigated (Table 3). The 1998–2002 survey in China reported that HCB levels were 81 and 56 ng/g lipid in mothers' milk from Dalian and Shenyang, respectively (Kunisue et al., 2004). On this basis, the present data imply that HCB has not decreased since 2002. The reason for the fact that there has not been a reduction may be partially explained by the fact that HCB was used annually for agricultural purposes until 1991 in China (Nakata et al., 2002). In Russia, HCB is still being used for production of pyrotechnic and ordinance materials for the military (Barber et al., 2005). The present concentrations of HCB in China are comparable to those from Russia (mean = 100 ng/g lipid) in 2003–2004 (Tsydenova et al., 2007), and seem to be among the highest in East-Asian countries (Table 4). Lower levels of HCB were observed in breast milk from Vietnam, Korea and Japan, reflecting that there is no usage or a lack of a source of HCB in these countries. The contamination trends of OCPs including HCB in this study are similar to the results from studies which monitor the levels of OCPs in the environment and mussels in Asian countries (Ramu et al., 2007).

3.1.5. PCBs

The mean concentrations of PCBs in human milk were higher in Japan (110 ng/g lipid, $n = 60$) than in the other three countries (56–84 ng/g lipid) (Table 3). Major components were CB-153 (20–28%), followed by CB-138 (18–19%) and CB-180 (6–13%) in all samples investigated. No pattern difference was observed between Japan and Korea, while the lower chlorinated PCB congeners (e.g. CB-74, CB-99 and CB-118) were relatively abundant in Chinese and Vietnamese milk. This is most likely due to the dietary exposure to less chlorinated PCB homologues via seafood from a specific source in China (Mai et al., 2005; Yang et al., 2009).

The present PCB levels found in Japan were in the same range as recent research that reported a concentration of 200 ng/g lipid in Osaka (Konishi et al., 2001), and 240 ng/g lipid in Fukuoka (Kunisue et al., 2006) (Table 4). A regional difference was found between Sendai (150 ng/g lipid) and Takayama (79 ng/g lipid) (Table 3), indicating that the geographical factor (coastal, urban or rural) is a major determinant of PCB levels in milk. The current PCB levels in Vietnamese, Chinese and Korean milk were higher than those from the other developing countries (e.g. Indonesia), but were lower than those from the UK or Russia (Table 4). We assume that PCB concentrations are strongly related to the amount of industrial activities taking place in the surrounding environment as well as dietary habits of consuming seafood.

3.1.6. PBDEs

The congener profile was dominated by BDE-47 (43–54%), followed by BDE-153 (23–33%) in Japan, Korea and Vietnam (Table 3). In China, BDE-28 levels were relatively high (accounted for 23% of total PBDEs), where the levels were similar to or higher than BDE-153. This implies that some specific PBDE commercial mixtures containing lower BDEs might be employed in China. In fact, several studies dealing with other

Table 5
Comparison of mean concentrations (ng/g lipid) of major PBDE congeners in breast milk from different countries or regions.

Country	Region	Year	n[a]	ΣPBDE	BDE-47	BDE-153	BDE-209	Reference
Japan	Sendai (Miyagi)	2007	20 (14)	1.7	0.76	0.45	na	This study
	Kyoto	2007–2008	20 (11)	1.4	0.58	0.42	na	This study
	Takayama (Gifu)	2007	20 (6)	1.3	0.57	0.29	na	This study
	Kashiwa (Chiba)	2006	8	4.9	2.9	0.46	nd	Kawashiro et al. (2008)
	4 regions	2005	89	1.7	0.49	0.4	0.14	Inoue et al. (2006)
	13 regions	2004	105	1.3[b]	0.68	0.27	na	Eslami et al. (2006)
	Osaka	2000	27	2.5	0.53	0.34	na	Akutsu et al. (2003)
Korea	Seoul	2007	29	3.7	2.0	0.86	na	This study
	ni	2004	9	2.6	0.81	0.79	na	Sudaryanto et al. (2005)
China	Beijing	2007	25	1.9	0.89	0.38	na	This study
	Beijing	2005	23	1.2	0.51	0.33	na	Li et al. (2008)
	Nanjing (urban)	2004	9	7.7	0.66	1.0	1.3	Sudaryanto et al. (2008b)
	Zoushan (rural)	2004	10	4.7	0.33	0.52	0.95	Sudaryanto et al. (2008b)
	South China	2005	27	3.5[b]	1.3	0.8	na	Bi et al. (2006)
Vietnam	Hanoi	2007	20	0.42	0.19	0.14	na	This study
	ni	2000	10	0.91	0.31	0.17	na	Sudaryanto et al. (2005)
Taiwan	Taichung	2000–2001	20 (17)	3.9[b]	1.5	0.87	0.27	Wang et al. (2008)
Indonesia	Java Is.	2001–2003	30	2.2	0.39	0.32	0.27	Sudaryanto et al. (2008a)
Australia	12 regions	2002–2003	157	11	5.6	1.1	na	Toms et al. (2007)
Russia	Buryatia	2003–2004	10	0.96	0.14	0.32	na	Tsydenova et al. (2007)
USA	Pacific Northwest	2003	40 (40)	96	50	16	0.8	She et al. (2007)
	Massachusetts	2004	38 (30)	75	41	5.2	<204	Johnson-Restrepo et al. (2007)
UK	Lancaster/London	2001–2003	54	6.6	3	1.4	na	Kalantzi et al. (2004)
Sweden	Stockholm	2000–2001	15	2.1	1.2	0.32	na	Guvenius et al. (2003)
	Uppsala	1998–1999	93	4.0	2.4	0.6	na	Lind et al. (2003)
Faroe Is.	Tórshavn	1999	9	7.2	1.9	2.4	1.0	Fängström et al. (2005)

ni = not indicated; na = not analyzed; nd = not detected.
[a] The figures in parentheses represent the number of primiparae.
[b] Geometric mean.

environmental matrices also found elevated proportions of tri- and tetraBDEs in sediments (Chen et al., 2006) and fishes (Xian et al., 2008) from the Yangtze River in China.

The mean concentration of PBDEs was the highest in mothers from Korea (3.7 ng/g lipid), followed by China (1.9 ng/g lipid), Japan (1.5 ng/g lipid), and the lowest in Vietnam (0.42 ng/g lipid) (Table 3). The results were compared with those observed for other countries (Table 5). In Korea, the ranges of PBDE levels were wide (0.82–24 ng/g lipid), implying that humans are still undergoing high exposure to PBDEs. Although there is little information about usage/production of PBDEs in Korea, the concentrations of PBDEs in human blood from Korea have been reported to be much higher (Kim et al., 2005) as compared with those from Japan (Inoue et al., 2006) and China (Bi et al., 2006). Additionally, apparently higher concentrations of PBDEs have been found in mussels from the coastal waters of Korea (Ramu et al., 2007). In Japanese milk samples, the present study showed no increasing trends in PBDE levels, as compared with a recent large-scale report (Eslami et al., 2006). The potential exposure pathways are thought to be via dietary intake or housedust ingestion/inhalation in coastal/urban and inland/rural areas. Although regional differences in PBDE levels have been observed in a previous report (Eslami et al., 2006), no such difference was observed in this study. In Chinese breast milk, present levels (1.9 ng/g lipid) were higher than those in the 2005 survey from Beijing (1.2 ng/g lipid) (Li et al., 2008), indicating an increasing trend of PBDEs. The regional difference in PBDE levels in China has also been observed between Nanjing (urban) and Zoushan (rural) in 2004 (Sudaryanto et al., 2008b), and in South China in 2005 (Bi et al., 2006). This study also showed that PBDE levels in Vietnam have decreased by half since the 2000 survey (Sudaryanto et al., 2005), and remain lower than those from Russia, Indonesia, Taiwan and Australia (Table 5). In summary, the current levels of PBDEs in the four regions studied appear to still be 2–3 fold lower than in Europe (Kalantzi et al., 2004; Fängström et al., 2005) and approximately one order of magnitude lower than those in North America (She et al., 2007).

3.2. Parity-dependent concentration

Concentrations of contaminants in human breast milk frequently vary with several factors such as the number of children, age of the mother and dietary preferences. In this study, we considered the difference in POP concentrations between two groups: primiparae and multiparae from Japan and Korea (Table 6). The mean concentrations of OCPs and PCBs in primiparae ($n=31$) were significantly higher ($p<0.001$) than those in multiparae ($n=29$) in Takayama and all Japanese women combined. For mothers in Korea, CHLs, the levels of β-HCH and HCB were significantly higher in primiparae. A higher concentration of OCPs in primiparae supports the previous investigations from Japan (Kunisue et al., 2006), Vietnam (Minh et al., 2004) and China (Kunisue et al., 2004), and the hypothesis that lower levels of OCPs in multiparae may be due to elimination of contaminants via previous periods of lactation. In contrast, no parity-dependency for PBDEs was observed in Japan and Korea, which may be explained by the specific exposure pathway of PBDE congeners.

Table 6
Comparison of POP concentrations in human breast milk between primipara and multipara from Japan and Korea.

	Concentration (mean ± SD (ranges), ng/g lipid)					
	ΣDDT	ΣCHL	β-HCH	HCB	ΣPCB	ΣPBDE
Sendai						
Primipara	300 ± 250	52 ± 36	220 ± 320	19 ± 4.9	160 ± 85	1.9 ± 1.2
($n=14$)	(102–970)	(23–140)	(9.0–1200)	(11–27)	(71–360)	(0.36–4.7)
Multipara	160 ± 160	36 ± 33	120 ± 200	13 ± 9.2	120 ± 100	1.4 ± 0.70
($n=6$)	(65–470)	(8.6–100)	(9.0–520)	(6.4–31)	(69–330)	(0.63–2.4)
p-value	0.032*	0.099	0.127	0.048*	0.117	0.409
Kyoto						
Primipara	190 ± 150	38 ± 11	96 ± 78	15 ± 4.9	120 ± 47	1.5 ± 0.41
($n=11$)	(73–550)	(24–58)	(36–270)	(7.8–25)	(63–210)	(0.78–2.1)
Multipara	120 ± 44	24 ± 8.0	55 ± 24	10 ± 3.7	92 ± 50	1.4 ± 0.65
($n=9$)	(49–170)	(11–36)	(31–94)	(4.5–17)	(14–160)	(0.55–2.8)
p-value	0.470	0.006*	0.239	0.057	0.342	0.518
Takayama						
Primipara	140 ± 44	23 ± 3.1	97 ± 140	11 ± 2.3	110 ± 19	1.3 ± 0.56
($n=6$)	(73–180)	(19–27)	(21–370)	(8.2–14)	(90–140)	(0.60–1.9)
Multipara	80 ± 39	15 ± 5.5	29 ± 19	6.8 ± 2.3	67 ± 17	1.3 ± 0.45
($n=14$)	(23–180)	(6.3–23)	(10–70)	(2.7–11)	(39–92)	(0.50–2.0)
p-value	0.026*	0.006*	0.047*	0.003*	<0.001*	0.967
Japan (all regions)						
Primipara	230 ± 200	41 ± 27	150 ± 230	16 ± 5.4	140 ± 68	1.6 ± 0.90
($n=31$)	(73–970)	(19–140)	(9.0–1200)	(7.8–27)	(63–360)	(0.36–4.7)
Multipara	110 ± 82	22 ± 17	57 ± 93	9.3 ± 5.4	87 ± 56	1.3 ± 0.56
($n=29$)	(23–470)	(6.3–100)	(9.0–520)	(2.7–31)	(14–330)	(0.5–2.8)
p-value	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	0.214
Seoul						
Primipara	200 ± 120	15 ± 5.9	140 ± 240	14 ± 3.7	66 ± 31	3.0 ± 2.1
($n=20$)	(49–580)	(6.4–31)	(28–830)	(8.1–21)	(23–130)	(0.82–9.5)
Multipara	150 ± 60	11 ± 2.3	39 ± 20	11 ± 1.7	50 ± 23	5.4 ± 7.4
($n=9$)	(91–270)	(8.1–14)	(17–72)	(8.2–13)	(20–96)	(1.1–24)
p-value	0.509	0.030*	0.032*	0.017*	0.203	0.540

SD = standard deviation.
* Statistically significant.

Table 7
Spearman's rank correlation coefficients between concentrations of major POPs in Japanese breast milk ($n=60$).

	Lipid	p,p'-DDE	t-Nonachlor	HCB	β-HCH	CB-153	BDE-47
p,p'-DDE	−0.389*						
t-Nonachlor	−0.333*	0.681**					
HCB	−0.208	0.712**	0.803**				
β-HCH	−0.474**	0.589**	0.558**	0.553**			
CB-153	−0.279*	0.690**	0.768**	0.671**	0.430**		
BDE-47	−0.272*	0.298*	0.217	0.128	0.267*	0.308*	
BDE-153	−0.462**	0.507**	0.589**	0.366**	0.274*	0.636**	0.244

* $p<0.05$.
** $p<0.01$.

3.3. Relationship between OCPs and PBDEs

Table 7 shows the relationship between two variables (lipid content, concentrations of major contaminants, p,p'-DDE, trans-nonachlor, β-HCH and HCB, CB-153, BDE-47 and BDE-153) in Japanese mothers' milk ($n=60$). All compounds except HCBs were negatively correlated ($p<0.05$) to the lipid content (%). High Spearman's rank correlation coefficients were found among three OCPs and between CB-153 and BDE-153, but not between BDE-47 and BDE-153. This finding indicates that humans are exposed to these OCPs, PCBs and BDE-153 via a similar dietary pathway, but from a different route than BDE-47.

3.4. Age-dependent concentration

We examined the relationship between POP concentrations in breast milk and the age of mothers in six regions. As shown in Table 8, CHL levels in breast milk from Seoul were positively correlated to the age of mothers ($p<0.05$), but no age-dependency was observed in the other regions, even in the case of primiparous mothers in Korea and Japan. Age-dependency for OCPs has been observed in Japanese breast milk (Kunisue et al., 2004) and adipose tissues (Kunisue et al., 2007). The age-related increase of CHLs may be explained by a continuous human exposure to technical CHL, which was previously used for timber houses as a termiticide. On the other hand, PCB and PBDE levels were positively correlated to the age of mothers in Sendai. Among PBDE congeners, BDE-153 more strongly contributed to the age-dependency than BDE-47, suggesting that BDE-153 has different exposure kinetics from BDE-47. A potential source of BDE-47 is considered to be inhalation or ingestion of indoor air dust from pentaPBDE treated consumer products, whereas BDE-153 is derived from the wide industrial usage of commercial octa-BDE products in Japan (Watanabe and Sakai, 2003). Interestingly, BDE-153 was positively correlated ($r=0.444$, $p<0.05$) to the age of women in Sendai, but negatively to the age of mothers from Kyoto ($r=-0.559$, $p<0.05$) (Fig. 2). No such correlation was observed for BDE-47. The difference in age-dependency for BDE-153 between Sendai and Kyoto may be attributed to other factors such as their occupations, since many of the participants from Kyoto (17/20) were housewives, whereas those from Sendai (17/20) were occupational workers (Table 2). Jakobsson et al. (2002) found that the levels of BDE-153 in human serum were positively correlated with the duration of computer work among technicians, whereas there

Table 8
Spearman's rank correlation coefficients between the ages of mothers and POP concentrations in breast milk.

POPs	Hanoi	Beijing	Seoul	Sendai	Kyoto	Takayama
	($n=20$)	($n=25$)	($n=29$)	($n=20$)	($n=20$)	($n=20$)
ΣDDT	0.048	0.109	0.298	0.233	−0.127	0.014
ΣCHL	−0.019	−0.133	0.422*	0.408	−0.217	−0.274
HCB	−0.020	−0.176	0.145	0.278	0.139	−0.392
β-HCH	−0.364	0.110	0.084	−0.271	−0.207	0.254
CB-153	−0.320	−0.041	0.248	0.554*	0.342	−0.310
ΣPCB	−0.295	−0.170	0.259	0.577**	0.270	−0.303
BDE-47	0.014	−0.091	0.000	0.264	−0.217	0.322
BDE-153	0.225	0.087	0.068	0.444*	−0.559*	−0.035
ΣPBDE	0.288	−0.045	0.009	0.432	−0.464*	0.194

* $p<0.05$.
** $p<0.01$.

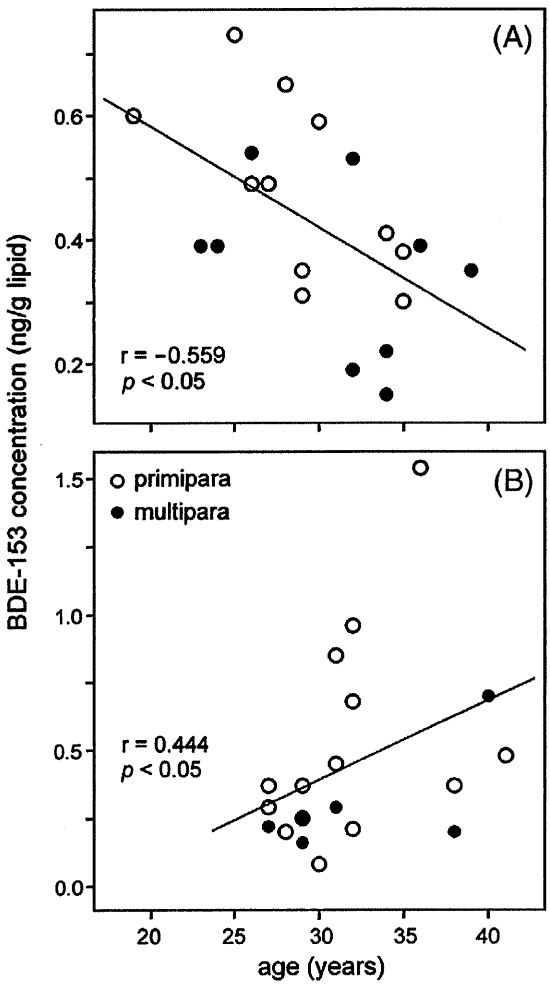

Fig. 2. Relationships between the age of mothers and concentrations of BDE-153 in the two groups from Kyoto (A) and Sendai (B) areas of Japan.

were no correlations between BDE-47 and age in any of the occupational groups. In Japan, the high proportion of BDE-153 was observed in adipose tissue (Kunisue et al., 2007; Choi et al., 2003). Therefore, detection of BDE-153 in human milk from Japan may be an indicator of recent exposure to commercial octa-BDE as well as mobilization from adipose tissue.

In this study, we did not quantify BDE-209, which was derived from technical deca-BDE products currently in use, although BDE-183, one of the predominant isomers derived from octa-BDE products, was not detected in all samples (detection limit = 2.5 ng/g lipid, data not shown). BDE-209 has been detected in human breast milk as a minor component, while it was a major component in the blood (Inoue et al., 2006; Kawashiro et al., 2008), suggesting that the blood-to-milk transfer of higher brominated congeners such as BDE-209 is low. This finding results in the maternal transfer of lower brominated BDEs to the child. Thus the different PBDE congener patterns in human breast milk (BDE-47), adipose tissues (BDE-153) and blood (BDE-209) may imply that there are congener-specific kinetics in the human body.

4. Conclusion

The present study revealed that the contamination trends of POPs in human breast milk from Asian countries were as follows: (1) DDT levels are still high in mothers from China and Vietnam, (2) β-HCH and HCB are predominant in Chinese mothers, (3) CHL and PCB levels are still higher in Japan, and (4) PBDEs levels are relatively higher in Korea. This trend is mostly in accordance with the results from the Asian Mussel Watch Program of POPs (Ramu et al., 2007). Although regional factors were one of the major determinants of OCPs and PCBs, their concentrations in primiparae were significantly higher than those in multiparae. This finding suggests that the mothers may transfer higher amounts of OCPs and PCBs to the first born infant who then might be at a higher risk of exposure of contaminants. For PBDEs, no parity/regional differences were observed, and the levels were lower than those from Europe and USA. However, large quantities of PBDEs are still used in many products and it can be anticipated that PBDE levels will further increase in Asia. It is therefore necessary to continuously monitor the breast milk to elucidate whether any new input of these contaminants is occurring.

Finally, the present study demonstrated prototypical breast milk POPs signatures unique to individual countries. Such POPs signatures represent profiles of OCPs, to which the population is exposed through various routes. OCPs are now used legally or illegally in Asian countries. Thus a shift in breast milk POPs signatures may be a good indicator for detecting the spread of POPs contaminations from one country to another. A breast POPs signature pattern in Sendai is especially interesting since its similarity to that in Beijing.

Acknowledgments

Human milk samples were provided by The Kyoto University Human Specimen Bank. The authors thank Ms. Seika Sai for assistance in GC/MS analysis. This work was supported by the Japan Science and Technology Agency (1300001) for AK and a Grant-in-Aid for Scientific Research (19310042 and 20404006) for KH from the Japan Society for the Promotion of Science.

References

Akutsu K, Kitagawa M, Nakazawa H, Makino T, Iwasaki K, Oda H, et al. Time-trend (1973–2000) of polybrominated diphenyl ethers in Japanese mother's milk. Chemosphere 2003;53:645–54.

Barber JL, Sweetman AJ, van Wijk D, Jones KC. Hexachlorobenzene in the global environment: emissions, levels, distribution, trends and processes. Sci Total Environ 2005;349: 1-44.

Bi X, Qu W, Sheng G, Zhang W, Mai B, Chen D, et al. Polybrominated diphenyl ethers in South China maternal and fetal blood and breast milk. Environ Pollut 2006;144: 1024–30.

Chao HR, Wang SL, Lin TC, Chung XH. Levels of organochlorine pesticides in human milk from central Taiwan. Chemosphere 2006;62:1774–85.

Chen SJ, Gao XJ, Mai BX, Chen ZM, Luo XJ, Sheng GY, et al. Polybrominated diphenyl ethers in surface sediments of the Yangtze River Delta: levels, distribution and potential hydrodynamic influence. Environ Pollut 2006;144:951–7.

Choi JW, Fujimaki S, Kitamura K, Hashimoto S, Ito H, Suzuki N, et al. Polybrominated dibenzo-*p*-dioxins, dibenzofurans, and diphenyl ethers in Japanese human adipose tissue. Environ Sci Technol 2003;37:817–21.

Costa LG, Giordano G. Developmental neurotoxicity of poybrominated diphenyl ether (PBDE) flame retardants. Neurotoxicol 2007;28:1047–67.

Eslami B, Koizumi A, Ohta S, Inoue K, Aozasa O, Harada K, et al. Large-scale evaluation of the current level of polybrominated diphenyl ethers (PBDEs) in breast milk from 13 regions of Japan. Chemosphere 2006;63:554–61.

Fängström B, Strid A, Grandjean P, Weihe P, Bergman Å. A retrospective study of PBDEs and PCBs in human milk from the Faroe Islands. Environ Health 2005;4:12.

Guvenius DM, Aronsson A, Ekman-Ordeberg G, Bergman Å, Norén K. Human prenatal and postnatal exposure to polybrominated diphenyl ethers, polychlorinated biphenyls, polychlorobiphenylols, and pentachlorophenol. Environ Health Perspect 2003;111:1235–41.

Inoue K, Harada K, Takenaka K, Uehara S, Kono M, Shimizu T, et al. Levels and concentration ratios of polychlorinated biphenyls and polybrominated diphenyl ethers in serum and breast milk in Japanese mothers. Environ Health Perspect 2006;114:1179–85.

Jakobsson K, Thuresson K, Rylander L, Sjödin A, Hagmar L, Bergman Å. Exposure to polybrominated diphenyl ethers and tetrabromobisphenol A among computer technicians. Chemosphere 2002;46:709–16.

Johnson-Restrepo B, Addink R, Wong C, Arcaro K, Kannan K. Polybrominated diphenyl ethers and organochlorine pesticides in human breast milk from Massachusetts, USA. J Environ Monit 2007;9:1205–12.

Kalantzi OI, Martin FL, Thomas GO, Alcock RE, Tang HR, Drury SC, et al. Different levels of polybrominated diphenyl ethers (PBDEs) and chlorinated compounds in breast milk from two U.K. regions. Environ Health Perspect 2004;112:1085–91.

Kawashiro Y, Fukata H, Omori-Inoue M, Kubonoya K, Jotaki T, Takigami H, et al. Perinatal exposure to brominated flame retardants and polychlorinated biphenyls in Japan. Endocr J 2008;55:1071–84.

Kim BH, Ikonomou MG, Lee SJ, Kim HS, Chang YS. Concentrations of polybrominated diphenyl ethers, polychlorinated dibenzo-*p*-dioxins and dibenzofurans, and polychlorinated biphenyls in human blood samples from Korea. Sci Total Environ 2005;336: 45–56.

Koizumi A, Yoshinaga T, Harada K, Inoue K, Morikawa A, Muroi J, et al. Assessment of human exposure to polychlorinated and polybrominated diphenyl ethers in Japan

using archived samples from the early 1980s and mid-1990s. Environ Res 2005;99:31–9.
Konishi Y, Kuwabara K, Hori S. Continuous surveillance of organochlorine compounds in human breast milk from 1972 to 1998 in Osaka, Japan. Arch Environ Contam Toxicol 2001;40:571–8.
Kunisue T, Someya M, Kayama F, Jin Y, Tanabe S. Persistent organochlorines in human breast milk collected from primiparae in Dalian and Shenyang, China. Environ Pollut 2004;131:381–92.
Kunisue T, Muraoka M, Ohtake M, Sudaryanto A, Minh NH, Ueno D, et al. Contamination status of persistent organochlorines in human breast milk from Japan: recent levels and temporal trend. Chemosphere 2006;64:1601–8.
Kunisue T, Takayanagi N, Isobe T, Takahashi S, Nose M, Yamada T, et al. Polybrominated diphenyl ethers and persistent organochlorines in Japanese human adipose tissues. Environ Int 2007;33:1048–56.
Li X, Zhu Y, Liu X, Fu Z, Xu X, Cheng H. Distribution of HCHs and DDTs in soils from Beijing City, China. Arch Environ Contam Toxicol 2006;51:329–36.
Li J, Yu H, Zhao Y, Zhang G, Wu Y. Levels of polybrominated diphenyl ethers (PBDEs) in breast milk from Beijing, China. Chemosphere 2008;73:182–6.
Lind Y, Darnerud PO, Atuma S, Aune M, Becker W, Bjerselius R, et al. Polybrominated diphenyl ethers in breast milk from Uppsala County, Sweden. Environ Res 2003;93: 186–94.
Mai B, Zeng EY, Luo X, Yang Q, Zhang G, Li X, et al. Abundances, depositional fluxes, and homologue patterns of polychlorinated biphenyls in dated sediment cores from the Pearl River Delta, China. Environ Sci Technol 2005;39:49–56.
Meerts IA, van Zanden JJ, Luijks EA, van Leeuwen-Bol I, Marsh G, Jakobsson E, et al. Potent competitive interactions of some brominated flame retardants and related compounds with human transthyretin in vitro. Toxicol Sci 2000;56:95-104.
Minh NH, Someya M, Minh TB, Kunisue T, Iwata H, Watanabe M, et al. Persistent organochlorine residues in human breast milk from Hanoi and Hochiminh City, Vietnam: contamination, accumulation kinetics and risk assessment for infants. Environ Pollut 2004;129:431–41.
Mueller JF, Harden F, Toms LM, Symons R, Fürst P. Persistent organochlorine pesticides in human milk samples from Australia. Chemosphere 2008;70:712–20.
Nakata H, Kawazoe M, Arizono K, Abe S, Kitano T, Shimada H, et al. Organochlorine pesticides and polychlorinated biphenyl residues in food stuffs and human tissues from China: status of contamination, historical trend, and human dietary exposure. Arch Environ Contam Toxicol 2002;43:473–80.
Nakata H, Nasu T, Abe S, Kitano T, Fan Q, Li W, et al. Organochlorine contaminants in human adipose tissues from China: mass balance approach for estimating historical Chinese exposure to DDTs. Environ Sci Technol 2005;39:4714–20.
Ohta S, Ishizaka D, Nishimura H, Nakao T, Aozasa O, Shimidzu Y, et al. Comparison of polybrominated diphenyl ethers in fish, vegetables, and meats and levels in human milk of nursing women in Japan. Chemosphere 2002;46:689–96.
Pohl HR, Tylenda CA. Breast-feeding exposure of infants to selected pesticides: a public health viewpoint. Toxicol Ind Health 2000;16:65–77.
Polder A, Odland JO, Tkachev A, Føreid S, Savinova TN, Skaare JU. Geographic variation of chlorinated pesticides, toxaphenes and PCBs in human milk from sub-arctic and arctic locations in Russia. Sci Total Environ 2003;306:179–95.
Qiu X, Zhu T, Li J, Pan H, Li Q, Miao G, et al. Organochlorine pesticides in the air around the Taihu Lake, China. Environ Sci Technol 2004;38:1368–74.
Qiu X, Zhu T, Yao B, Hu J, Hu S. Contribution of dicofol to the current DDT pollution in China. Environ Sci Technol 2005;39:4385–90.
Ramu K, Kajiwara N, Sudaryanto A, Isobe T, Takahashi S, Subramanian A, et al. Asian mussel watch program: contamination status of polybrominated diphenyl ethers and organochlorines in coastal waters of Asian countries. Environ Sci Technol 2007;41: 4580–6.
She J, Holden A, Sharp M, Tanner M, Williams-Derry C, Hooper K. Polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) in breast milk from the Pacific Northwest. Chemosphere 2007;67:307–17.
Sjödin A, Carlsson H, Thuresson K, Sjölin S, Bergman Å, Östman C. Flame retardants in indoor air at an electronics recycling plant and at other work environments. Environ Sci Technol 2001;35:448–54.
Sjödin A, Päpke O, McGahee E, Focant JF, Jones RS, Pless-Mulloli T, et al. Concentration of polybrominated diphenyl ethers (PBDEs) in household dust from various countries. Chemosphere 2008;73:131–6.
Sudaryanto A, Kajiwara N, Takahashi S, Muawanah, Tanabe S. Geographical distribution and accumulation features of PBDEs in human breast milk from Indonesia. Environ Pollut 2008a;151:130–8.
Sudaryanto A, Kajiwara N, Tsydenova OV, Isobe T, Yu H, Takahashi S, et al. Levels and congener specific profiles of PBDEs in human breast milk from China: implication on exposure sources and pathways. Chemosphere 2008b;73:1661–8.
Sudaryanto A, Kajiwara N, Tsydenova O, Iwata H, Adibroto TA, Yu H, et al. Global contamination of PBDEs in human milk from Asia. Organohalog Compd 2005;67: 1315–8.
Sudaryanto A, Kunisue T, Kajiwara N, Iwata H, Adibroto TA, Hartono P, et al. Specific accumulation of organochlorines in human breast milk from Indonesia: levels, distribution, accumulation kinetics and infant health risk. Environ Pollut 2006;139:107–17.
Taguchi S, Yakushiji T. Influence of termite treatment in the home on the chlordane concentration in human milk. Arch Environ Contam Toxicol 1988;17:65–71.
Toms LML, Harden FA, Symons RK, Burniston D, Fürst P, Müller JF. Polybrominated diphenyl ethers (PBDEs) in human milk from Australia. Chemosphere 2007;68: 797–803.
Tsydenova OV, Sudaryanto A, Kajiwara N, Kunisue T, Batoev VB, Tanabe S. Organohalogen compounds in human breast milk from Republic of Buryatia, Russia. Environ Pollut 2007;146:225–32.
Wada Y, Koizumi A, Yoshinaga T, Harada K, Inoue K, Morikawa A, et al. Secular trends and geographical variations in the dietary intake of polybrominated diphenyl ethers (PBDEs) using archived samples from the early 1980s and mid 1990s in Japan. J Occup Health 2005;47:236–41.
Wang YF, Wang SL, Chen FA, Chao HA, Tsou TC, Shy CG, et al. Associations of polybrominated diphenyl ethers (PBDEs) in breast milk and dietary habits and demographic factors in Taiwan. Food Chem Toxicol 2008;46:1925–32.
Watanabe I, Sakai S. Environmental release and behavior of brominated flame retardants. Environ Int 2003;29:665–82.
Wong CKC, Leung KM, Poon BHT, Lan CY, Wong MH. Organochlorine hydrocarbons in human breast milk collected in Hong Kong and Guangzhou. Arch Environ Contam Toxicol 2002;43:364–72.
Wong MH, Leung AOW, Chan JKY, Choi MPK. A review on the usage of POP pesticides in China, with emphasis on DDT loadings in human milk. Chemosphere 2005;60: 740–52.
Wu N, Herrmann T, Peapke O, Tickner J, Hale R, Harvey E, et al. Human exposure to PBDEs: associations of PBDE body burdens with food consumption and house dust concentrations. Environ Sci Technol 2007;41:1584–9.
Xian Q, Ramu K, Isobe T, Sudaryanto A, Liu X, Gao Z, et al. Levels and body distribution of polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDs) in freshwater fishes from the Yangtze River, China. Chemosphere 2008;71:268–76.
Yang Z, Shen Z, Gao F, Tang Z, Nju J. Occurrence and possible sources of polychlorinated biphenyls in surface sediments from the Wuhan reach of the Yangtze River, China. Chemosphere 2009;74:1522–30.
Yu HF, Zhao XD, Zhao JH, Zhu ZQ, Zhao Z. Continuous surveillance of organochlorine pesticides in human milk from 1983 to 1998 in Beijing, China. Int J Environ Health Res 2006;16:21–6.