from Kanto Chemical Co. Ltd. (Tokyo, Japan). Silica-gel (Wako gel S-1) was used for purification (Wako Pure Industries Ltd., Osaka, Japan) and heated at 130 °C for 3 h prior to use. The chemical structures of the target analytes of natural origin are shown in Fig. 2.

2.3. Clean-up procedure

The methodology used to analyze brominated contaminants in the breast milk samples was based on lipid extraction, gel permeation chromatography (GPC) and silica-gel column cleanup, and gas chromatography–negative chemical ionization–mass spectrometry (GC–NCI–MS). Briefly, 5 mL of each breast milk was spiked with 4′-MeO-BDE121 (0.2 ng) and extracted with n-hexane, after adding potassium oxalate solution, ethanol and ethylether (1:1:1, v/v/v). The extract was washed with water and dried over sodium sulfate. After solvent evaporation, lipid was determined gravimetrically.

An aliquot of lipid (50–300 mg) was dissolved in 1.5 mL of dichloromethane (DCM)/n-hexane (1:1, v/v), and subjected to GPC with a Bio-Beads S-X3 column (35 g of gel material; Bio-Rad Laboratories, Hercules, CA, USA) with DCM/hexane as the eluting solvent at a flow rate of 4 mL/min. The first 90-mL fraction of the eluate containing lipid was discarded, and the subsequent 80-mL fraction was collected. To remove the remaining trace amount of lipid, the residue was loaded onto a silica-gel column (0.2 g of Wako gel S-1). The fraction was eluted with 15 mL of 12% DCM/n-hexane, and concentrated to 200 μL for GC/MS analysis.

2.4. Instruments and quantification

Thirteen analytes were measured by GC–NCI–MS using an Agilent HP5973MSD 5973i (Agilent Technologies, Palo Alto, CA, USA) coupled with a 6890N gas chromatograph. The GC/MS conditions and target ions for determination of POPs are summarized in Table 2. Quantification of the compounds was based on the signals in the mass chromatograms and on comparisons with the internal standard (4′-MeO-BDE121). 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.

2.5. Quality control and quality assurance

Procedural blanks were analyzed simultaneously with every batch of ten samples to check for interference or contamination from solvents and glassware. For recovery tests, a matrix (cow milk) spiking test was conducted with two spiked levels (2.0 and 10.0 ng/g) of 13 analytes and an internal standard. Based on GC/MS-selected ion monitoring (SIM), their recoveries were 84–91% with relative standard deviations (RSDs) of <10% ($n = 5$). The limits of quantification (LOQs) were defined as five times the noise value and ranged from 0.01 to 0.2 ng/g lipid (Table 3). When the level of the target chemical was less than the LOQ, we allocated one-half of the LOQ as the value for the calculation. The calibrations (0.1–5.0 ng/mL of each analyte) were linear and characterized by good correlation coefficients (>0.99) for all compounds studied. The quality of the method under validation was verified by analysis of a Standard Reference Material (cod liver oil, SRM1588b, NIST) (Stapleton et al., 2007). The data from our laboratory were in good agreement with the certified values (<11% of RSD, $n = 5$) for PBDEs.

Table 2
GC/MS conditions for analysis of brominated compounds in human breast milk.

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)
Ionization mode	ECNI (electron capture negative ionization)
Reagent gas	Methane
Target ions, (confirmed ions), m/z	79 (81) for brominated contaminants, 386 (388) for MBP-Cl_7

2.6. Statistical analysis

The obtained data were analyzed statistically using SPSS software version 18.0 for Windows 2007 (SPSS Inc., Chicago, IL, USA). One-way analysis of variance was used to examine differences in the target chemical concentrations between regions. Pearson's correlation coefficient was used to examine the strength of the associations between the mothers' ages and the organobromine concentrations. Probability values of less than 0.05 were considered to indicate statistical significance.

3. Results

We detected six PBDE congeners, HeBB and TeBB in breast milk samples from Hokkaido and Okinawa. The major components of the PBDEs were BDE-47 and BDE-153, which were detected at higher frequencies in Okinawa. The congener levels are shown in Table 3. The levels of ΣPBDE ranged from <0.2 to 69 ng/g lipid (median, 1.5 ng/g lipid) and were higher in mothers from Okinawa, although one sample from Hokkaido was considerably highly contaminated with PBDEs (i.e. 46 ng/g lipid for BDE-47 and 4.0 ng/g lipid for BDE-153). HeBB and TeBB were found at ranges of <0.05–2.5 (mean, 0.53) ng/g lipid and 0.76 to 6.6 (mean, 2.6) ng/g lipid, respectively. The HeBB levels were significantly higher in breast milk from Hokkaido ($p < 0.01$), whereas no regional

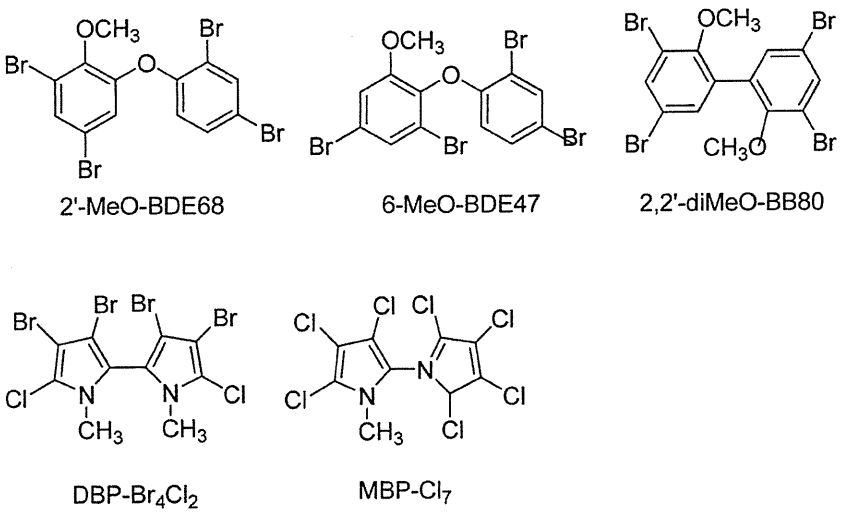

Fig. 2. Structures of naturally produced brominated contaminants. 2′-MeO-BDE68: 4,6-dibromo-2-(2′,4′-dibromo)phenoxyanisole; 6-MeO-BDE-47: 3,5-dibromo-2-(2′,4′-dibromo) phenoxyanisole; 2,2′-diMeO-BB80: 2,2′-dimethoxy-3,3′5,5′-tetrabromobiphenyl; DBP-Br_4Cl_2: 1,1′-dimethyl-2,2′-bipyrrole; MBP-Cl_7: 2,3,3′,4,4′,5,5′-heptachloro-1′-methyl-1,2′-bipyrrole.

Table 3
Concentrations of polybrominated diphenyl ethers and related compounds in breast milk collected from Okinawa and Hokkaido.

	Okinawa $n = 20$				Hokkaido $n = 20$				Overall		LOQ (ng/g lipid)
	Freq (n > LOQ)	Mean	Median	Range	Freq (n > LOQ)	Mean	Median	Range	Mean	Median	
Concentration (ng/g lipid)											
BFRs											
BDE-28	16	0.12	0.12	<0.06–0.38	6	0.16	0.030	<0.06–1.9	0.14	0.040	0.06
BDE-47	20	0.97	0.87	0.10–2.2	16	2.7	0.40	<0.08–46	1.9	0.56	0.08
BDE-99	14	0.20	0.16	<0.1–0.48	4	0.62	0.050	<0.1–10	0.41	0.050	0.1
BDE-100	11	0.16	0.080	<0.1–0.56	4	0.41	0.050	<0.1–6.7	0.29	0.050	0.1
BDE-153	20	0.60	0.56	<0.2–1.6	10	0.54	0.19	<0.2–4.0	0.57	0.48	0.2
BDE-154	14	0.19	0.16	<0.2–0.41	3	0.13	0.10	<0.2–0.57	0.16	0.10	0.2
ΣPBDE	20	2.1	2.1	0.55–5.1	16	4.3	1.0	<0.2–69	3.4	1.5	–
TeBB	20	2.4	2.0	0.83–6.0	20	2.6	2.6	0.76–6.6	2.5	2.1	0.01
HeBB	19	0.19	0.20	<0.05–0.46	20	0.86**	0.71	0.20–2.5	0.53	0.32	0.05
Natural products											
2′-MeO-BDE68	18	0.39*	0.28	<0.06–1.6	12	0.17	0.070	<0.06–0.69	0.28	0.14	0.06
6-MeO-BDE-47	8	0.050*	0.030	<0.05–0.13	0	<0.05	<0.05	<0.05	0.040	0.030	0.05
2,2′-diMeO-BB80	17	0.20**	0.22	<0.04–0.45	7	0.040	0.020	<0.04–0.12	0.12	0.070	0.04
MBP-Cl_7	19	0.19	0.11	<0.01–0.94	17	0.090	0.070	<0.01–0.43	0.14	0.080	0.01
DBP-Br_4Cl_2	17	0.23	0.20	<0.04–0.062	18	0.45	0.28	<0.04–2.7	0.34	0.25	0.04
Ratio											
BDE-47/BDE-153		1.6	1.6			5.0	2.1		3.3	1.2	
TeBB/HeBB		12	9.8			3.1	3.7		4.7	6.6	
2′-MeO-BDE68/BDE-47		0.40	0.32			0.06	0.18		0.15	0.25	

All data were calculated by assuming that values below the LOQ were equal to one-half of the LOQ. *$p < 0.05$, **$p < 0.01$.

difference was found in the TeBB levels. Regarding other brominated contaminants, we detected three methoxylated analogs of tetra-BDEs and two halogenated bipyrroles (Fig. 2). The levels of 2′-MeO-BDE68 and 2,2′-diMeO-BB80 were significantly higher in mothers from Okinawa (0.39 and 0.20 ng/g lipid, respectively, $p < 0.01$ for each) than in mothers from Hokkaido. The levels of MBP-Cl_7 and DBP-Br_4Cl_2 ranged from <0.01 to 0.94 ng/g lipid and <0.01–2.7 ng/g lipid, respectively. No regional differences in the levels of these two bipyrroles were observed between the two areas.

The correlations between the concentrations of individual contaminants in Okinawa ($n = 20$) and Hokkaido ($n = 20$) are shown in Table 4. BDE-47 was correlated with BDE-153 in Hokkaido ($r = 0.927$, $p < 0.01$), but not in Okinawa. In accordance, HeBB was correlated with TeBB in Hokkaido ($r = 0.628$, $p < 0.01$), but not in Okinawa. 2′-MeO-BDE68 was positively correlated with 2,2′-diMeO-BB80 in Okinawa ($r = 0.522$, $p < 0.05$), but not in Hokkaido. DBP-Br_4Cl_2 was not correlated with MBP-Cl_7 in both areas, but well correlated with 2′-MeO-BDE68 ($r = 0.478$, $p < 0.05$) and 2,2′-diMeO-BB80 ($r = 0.767$, $p < 0.01$) in Okinawa. No age dependency was found for any of the congeners investigated in both areas.

4. Discussion

4.1. PBDEs

The contamination trends of PBDEs in this study were of similar magnitude to recent results in Japan (Haraguchi et al., 2009c; Kawashiro et al., 2008) and Europe (Thomsen et al., 2010). The present study showed regional differences in the concentrations of PBDEs in breast milk. These trends were also observed in a recent large-scale survey of PBDEs in Japanese breast milk (Eslami et al., 2006). The variation of PBDE levels in Japanese people may be caused by factors related to food culture. However, one milk sample from Hokkaido contained considerably high levels of PBDEs (69 ng/g lipid), despite the other samples from the same area showing lower levels (median, 1.0 ng/g lipid) of PBDEs. It is assumed that the high concentration of PBDEs may be attributed to occupational exposure via house dust or electric waste consumption (Fromme et al., 2009; Thomsen et al., 2010), rather than food sources and habitual dietary intake. A previous survey using tuna fish as biomarker in the Asia-Pacific region revealed that the highest concentrations of PBDEs were detected in fish from off-Taiwan coastal water, near the Okinawa area (Ueno et al., 2004). The levels of congeners were higher in the order of BDE-47 > BDE-153 > BDE-100 in most samples, although BDE-47 was not correlated with BDE-153 in Okinawa, indicating their different sources. The relative contribution of lower brominated PBDEs (i.e. ratio of BDE-47 to BDE-153) was higher in Hokkaido (5.0) than in Okinawa

Table 4
Peason's correlation coefficients between the levels of the major brominated contaminants in breast milk from Okinawa ($n = 20$) and Hokkaido ($n = 20$).

	BDE-47	BDE-153	TeBB	HeBB	2′-MeO-BDE68	2,2′-diMeO-BB80	MBP-Cl_7
Okinawa							
BDE-153	0.348						
TeBB	−0.202	0.107					
HeBB	0.364	0.775**	0.053				
2′-MeO-BDE68	0.070	−0.189	−0.199	−0.078			
2,2′-diMeO-BB80	0.299	−0.188	−0.104	0.074	0.522*		
MBP-Cl_7	0.432	0.540*	−0.168	0.490*	0.029	0.021	
DBP-Br_4Cl_2	0.284	−0.059*	−0.137	0.158	0.478*	0.767**	0.279
Hokkaido							
BDE-153	0.927**						
TeBB	−0.214	−0.088					
HeBB	−0.117	−0.031	0.628**				
2′-MeO-BDE68	0.054	0.197	−0.077	0.069			
2,2′-diMeO-BB80	0.004	0.071	0.049	−0.273	0.221		
MBP-Cl_7	0.268	0.298	0.054	0.069	0.183	−0.090	
DBP-Br_4Cl_2	−0.064	−0.108	0.301	−0.024	0.408	0.480*	0.129

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

(1.6) (Table 3). The results may be related to the finding that the percentage contributions of lower brominated congeners (BDE-28 and BDE-47) increased with increasing latitude and the highest ratio of lower PBDEs was found in seafood from the northern colder region in the North Pacific (Ueno et al., 2004).

4.2. HeBB and its metabolite

Although HeBB has been used as one of the BFRs at low volumes in Japan (350 tons per year between 1994 and 2001) (Watanabe and Sakai, 2003), recent contamination trends of HeBB have not been available. This study revealed that, as well as HeBB, debrominated TeBB was present at higher levels than HeBB in most samples, indicating that these compounds are widely distributed as persistent brominated contaminants in the Japanese environment. The HeBB levels were significantly higher in mothers from Hokkaido than in mothers from Okinawa, while no regional difference was observed for the TeBB levels (Table 3). The HeBB levels were not significantly correlated with the TeBB and BDE-47 levels, but were positively correlated with the BDE-153 levels (Table 4), indicating that HeBB may be exposed via the same route as BDE-153. Miyazaki et al. (1986) first detected TeBB in human milk, but not HeBB. Although we have no information that TeBB is contained as a byproduct in agricultural and/or industrial chemicals, the source of TeBB may be partly different from that of HeBB. In a 1988 survey, similar levels of HeBB and TeBB were determined in human adipose tissues (range, 2.1–4.1 ng wet weight) (Yamaguchi et al., 1988) and rat experiments showed that TeBB may be a metabolite (debrominated product) of HeBB. The HeBB levels were positively correlated with the TeBB levels in Hokkaido, but not in Okinawa, suggesting that there may be other factors affecting the variation of HeBB levels.

4.3. MeO-PBDE analogs

Regarding PBDE-related products detected in this study, three methoxylated PBDE analogs, 2′-MeO-BDE68, 6-MeO-BDE-47 and 2,2′-diMeO-BB80, are considered to be of natural origin. The levels of both 2′-MeO-BDE68 and 2,2′-diMeO-BB80 were slightly lower than those of BDE-47. The ratios of 2′-MeO-BDE68 to BDE-47 were higher in samples from Okinawa (0.40) than in samples from Hokkaido (0.06) (Table 3), and the levels of 2′-MeO-BDE68 were not correlated to those of BFRs (Table 4), indicating a specific source via a different exposure pathway. Recent studies have shown that whale blubber, shark liver and seafood (grouper, bluefin tuna etc.) from Okinawa coastal water have accumulated these MeO-PBDE analogs (Haraguchi et al., 2009b; Hisamichi et al., 2007; Marsh et al., 2005). Therefore, the source of MeO-PBDEs in breast milk may be seafood contaminated with naturally produced brominated analogs. The regional difference may be attributed to the extent of occurrence of MeO-PBDEs in nature. For example, these compounds could be produced by specific seaweeds inhabiting the tropical seashore (Haraguchi et al., 2010). MeO-PBDEs and the corresponding OH-PBDEs have also been found in human milk from Italy (Lacorte and Ikonomou, 2009) and Nicaragua (Athanasiadou et al., 2008), although their profiles in breast milk were different from our results. The toxicity of MeO-PBDEs is still unknown but the corresponding OH-PBDEs are known to have endocrine-disrupting properties that allow transfer from mothers to infants via the placenta or breastfeeding (Kawashiro et al., 2008). Wan et al. (2009) reported that OH-PBDEs formed in the livers of marine mammals and fish are demethylation products of MeO-PBDEs rather than hydroxylated metabolites of PBDEs. It is therefore possible that MeO-PBDEs are converted to more toxic OH-PBDEs in the human body. The levels of 2,2′-diMeO-BB80 were positively correlated with those of 2′-MeO-BDE68, indicating that both compounds had the same exposure route. The 2,2′-diMeO-BB80 detected in human milk has also accumulated in whales and sharks (Haraguchi et al., 2009a, 2009b; Marsh et al., 2005). The source may be derived from 2,2′-diOH-BB80 that can be isolated from a marine bacterium (Isnansetyo and Kamei, 2003).

4.4. Halogenated bipyrroles

The present study further showed that two types of halogenated bipyrroles, DBP-Br_4Cl_2 (2,2′-bipyrrole) and MBP-Cl_7 (1′,2-bipyrrole), were distributed at similar levels to 2′-MeO-BDE68 in Japanese breast milk. The greater abundance of DBP-Br_4Cl_2 in mothers from Hokkaido suggests that the source may be biota (foodweb) in the northern latitude of the North Pacific area. In fact, killer whales stranded in Hokkaido had accumulated DBP-Br_4Cl_2 at much higher levels (Haraguchi et al., 2009a). However, DBP-Br_4Cl_2 was also found in the liver of tiger sharks in Okinawa coastal water (Haraguchi et al., 2009b), whale products in the Japanese market (Haraguchi et al., 2006) and Canadian seafood (Tittlemier, 2004), indicating the widespread distribution of DBP-Br_4Cl_2 in the Pacific. In Okinawa breast milk, the levels of DBP-Br_4Cl_2 were significantly correlated with those of the other natural contaminants, such as 2′-MeO-BDE68 and 2,2′-diMeO-BB80 (Table 3), but were not correlated with the levels of MBP-Cl_7. These findings suggest that these bipyrroles may be derived from different biogenic sources. In fact, MBP-Cl_7 has been detected in mammals from Oceania (Vetter et al., 2001), while DBP-Br_4Cl_2 has not. Nevertheless, both bipyrroles appear to have similar physicochemical properties to BDE-47 and 2′-MeO-BDE68 in their potential for global distribution (Hackenberg et al., 2003; Tittlemier et al., 2004). Although the toxicological significance of these bipyrroles is unknown, some reports have shown hepatic enzyme induction by DBP-Br_4Cl_2 (Tittlemier et al., 2003) and moderate biological activity of MBP-Cl_7 (Vetter et al., 2004).

4.5. Daily intake estimates for infants

The estimation of daily intake (EDI) for the brominated contaminants for infants was assessed based on average breast milk consumption by infants (Van Oostdam et al., 1999) (Supplemental Table 1). In this study, the EDIs of PBDEs were less than one-thousand of the No Observed Adverse Effect Level (NOAEL) of Penta-BDEs (NOAEL:0.4 mg/kg body weight/day) (Viberg et al., 2004), indicating that the health risks for PBDEs intake from breast milk are limited. However, infants have different susceptibilities to adults with regard to their dynamic growth and developmental processes (Sly and Flack, 2008). In addition, the toxicokinetics and toxicities of HeBB, naturally occurring MeO-PBDEs and halogenated bipyrroles are still unclear. These uncertainties necessitate more comprehensive toxicological studies on those compounds.

5. Conclusions

The present study showed that Japanese breast milk samples were contaminated with anthropogenic (PBDEs and HeBB) and natural origin (MeO-PBDEs and bipyrroles) compounds The levels of PBDEs (BDE-47 and BDE-153) tended to be higher in mothers from Okinawa, while the levels of HeBB were significantly higher in mothers from Hokkaido. These findings indicate that PBDEs and HeBB have different exposure pathways. Two MeO-PBDEs (2′-MeO-BDE68 and 2,2′-diMeO-BB80) showed higher concentrations in mothers from Okinawa, whereas two bipyrroles (DBP-Br_4Cl_2 and MBP-Cl_7) may be derived from different biota in the Japanese coastal waters. To clarify the exposure pathways and health effects of these brominated contaminants, the spatial trends of these contaminants need to be further investigated.

Acknowledgments

This study was mainly supported by Special Coordination Funds for Promoting Science and Technology (No. 1300001) sponsored by the Japan Science and Technology Agency, and a Grant-in-Aid for Health Sciences Research from the Ministry of Health, Labour and Welfare of Japan (H21-Food-003). The funding bodies had no roles in the study design, data collection and analysis, or in decisions concerning the preparation and publication of the manuscript. The authors have no financial conflicts of interest.

Appendix. Supplementary data

Supplementary data related to this article can be found online at doi:10.1016/j.envpol.2011.11.022.

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Chemosphere 86 (2012) 315–321

Contents lists available at SciVerse ScienceDirect

Chemosphere

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

Levels and profiles of long-chain perfluorinated carboxylic acids in human breast milk and infant formulas in East Asia

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ARTICLE INFO

Article history:
Received 16 April 2011
Received in revised form 16 September 2011
Accepted 20 October 2011
Available online 21 November 2011

Keywords:
Human breast milk
Perfluorinated carboxylic acids
Japan
Korea
China
Asia

ABSTRACT

In this study, 90 human breast milk samples collected from Japan, Korea, and China were analyzed for perfluorooctanoic acid (PFOA) (C8), perfluorononanoic acid (PFNA) (C9), perfluorodecanoic acid (PFDA) (C10), perfluoroundecanoic acid (PFUnDA) (C11), perfluorododecanoic acid (PFDoDA) (C12), and perfluorotridecanoic acid (PFTrDA) (C13). In addition, infant formulas ($n = 9$) obtained from retail stores in China and Japan were analyzed. PFOA was the predominant compound and was detected in more than 60% of samples in all three countries. The PFOA, PFNA, PFDA, and PFUnDA levels in Japan were significantly higher than those in Korea and China ($p < 0.05$). The PFTrDA level was highest in Korea ($p < 0.05$). The median PFOA concentrations were 89 pg mL^{-1} (48% of total perfluorinated carboxylic acids (PFCAs) (C8–C13)) in Japan, 62 pg mL^{-1} (54%) in Korea, and 51 pg mL^{-1} (61%) in China. The remaining $\sum$PFCAs (C9–C13) were 95 pg mL^{-1} in Japan, 52 pg mL^{-1} in Korea, and 33 pg mL^{-1} in China. Among the long-chain PFCAs, odd-numbered PFCAs were more frequently detected than even-numbered PFCAs, except for PFDA in Japan. There were no evident correlations between the mother's demographic factors and the PFCA concentrations. PFOA, PFNA, and PFDA were frequently detected in both Japan and China, but there were no significant differences between the two countries. The total PFCA concentrations in the infant formulas were lower than those in the breast milk samples in Japan ($p < 0.05$), but not in China ($p > 0.05$). In conclusion, various PFCAs were detected in human breast milk samples from East Asian countries.

1. Introduction

Perfluorinated compounds (PFCs) comprise a large group of man-made fluorinated organic chemicals. They have been produced since the 1950s and are used for various industrial and consumer-related applications, such as food packaging materials, protective coatings for textiles, carpets, papers, and surfactants (Key et al., 1997). During the last decade, PFCs such as perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) have been found at considerable levels in various biota samples including the liver and tissues, and especially human blood and serum, worldwide (Fromme et al., 2009).

The toxic effects of PFOS and PFOA have been investigated in animal studies. Prenatal as well as postnatal toxic effects of PFOA and PFOS were observed in rats and mice, including increased liver weights, growth lags, and delayed development. The reproductive and developmental toxicities of these chemicals toward humans are of particular concern (Lau et al., 2004). Several epidemiological investigations have raised concerns regarding the developmental effects of PFOS and PFOA on children, such as low birth weights (Steenland et al., 2010).

In the Stockholm Convention on Persistent Organic Pollutants, PFOS is listed in Annex B (Wang et al., 2009). Fluoropolymer manufacturers have also committed themselves to voluntarily reducing PFOA emissions under a stewardship program by the US EPA (EPA, 2006). The temporal trends in serum levels have revealed decreases in the serum levels of both PFOA and PFOS in the United States, Norway, and Japan since 2000 (Olsen et al., 2007; Harada and Koizumi, 2009; Haug et al., 2009; Harada et al., 2010).

In contrast to PFOS and PFOA, little information is available for perfluorinated carboxylic acids (PFCAs) with longer chains than PFOA. The emissions of perfluorononanoic acid (PFNA) and perfluoroundecanoic acid (PFUnDA) were 25 and 7 metric tons, respectively, in 2000 (Prevedouros et al., 2006). A modeling study indicated that these PFCAs could also have been emitted from precursor compounds, such as fluorotelomer alcohols (FTOHs), for

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doi:10.1016/j.chemosphere.2011.10.035

decades (Van Zelm et al., 2008). Recent evidence suggests that the toxicological effects of PFCAs are strongly correlated with their chain lengths and functional groups (Upham et al., 1998; Matsubara et al., 2006; Wolf et al., 2008; Liao et al., 2009). Therefore, the effects of exposure to long-chain PFCAs need to be clarified, especially in infants.

Human breast milk and infant formulas are considered to be the main PFC exposure sources for infants during the lactation period. Indeed, contamination of PFCs in human breast milk has been reported in various studies from Asia (So et al., 2006; Tao et al., 2008b; Nakata et al., 2009; Liu et al., 2010, 2011; Kim et al., 2011), the United States (Kuklenyik et al., 2004; Tao et al., 2008a; von Ehrenstein et al., 2009), and Europe (Kärrman et al., 2007; Bernsmann and Furst, 2008). However, the available data for PFCAs with longer chains than PFNA in human breast milk are limited, because of the low recoveries of long-chain PFCAs from human breast milk samples (Kärrman et al., 2007).

The aim of the present study was to investigate the current levels of long-chain PFCAs in human breast milk in East Asian countries, which were reported to show increasing trends for long-chain PFCAs in serum (Kärrman et al., 2009; Harada et al., 2011). Human breast milk samples collected from Japan, Korea, and China were analyzed for PFOA, PFNA, perfluorodecanoic acid (PFDA), PFUnDA, perfluorododecanoic acid (PFDoDA), and perfluorotridecanoic acid (PFTrDA) using an ion-pair extraction method (Hansen et al., 2001) with modifications. In addition, infant formulas from representative manufacturers in the Japanese and Chinese markets were analyzed for comparison with the PFCA concentrations in the breast milk samples from the same regions.

2. Methods and materials

2.1. Study population and sample information

To evaluate the geographical differences in the PFCA levels in human breast milk, we selected 30 samples each from Japan, Korea, and China that were stored in the Human Specimen Bank of Kyoto University (Koizumi et al., 2005, 2009). For infant formulas, we obtained five products from five different companies in the Japanese market and four products from four different companies in the Chinese market. The main ingredients of these infant formulas were cow milk, cow milk-related products (milk whey protein, lactose, and casein), and edible oils (palm olein and soybean oil). A summary of the sample information is provided in Table 1.

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

2.2. Standards and reagents

Analytical standards for the PFCAs, $^{13}C_4$-labeled PFOA and $^{13}C_5$-labeled PFNA, were obtained from Wellington Laboratories (PFC-MXA, MPFOA, and MPFNA; Guelph, Ontario, Canada).

Methanol, acetone, dichloromethane (DCM), and hexane (purity: >99%, pesticide analysis grade) were obtained from Kanto Chemicals (Tokyo, Japan). Ethyl acetate (pesticide analysis grade), methyl *t*-butyl ether (MTBE, pesticide analysis grade), tetrabutylammonium hydrogen sulfate (TBA), sodium carbonate, sodium bicarbonate, and benzyl bromide were purchased from Wako Pure Chemicals (Osaka, Japan). Ultrapure water (Milli-Q™ Reference; Millipore, Billerica, MA) was used for all solutions. MTBE, DCM, and hexane were prefiltered through silica gel (Presep-C silica gel; Wako Pure Chemicals). Methanol, ethyl acetate, and acetone were distilled before use. Milli-Q water was filtered through an Oasis WAX column (Waters, Milford, MA).

2.3. Sample preparation and extraction

Frozen human breast milk samples were thawed and returned to room temperature before extraction. A liquid–liquid and solid–phase extraction method was used to extract the PFCAs in the samples. Aliquots of breast milk (2 mL) together with an internal standard ($^{13}C_4$-PFOA, 1 ng) were placed in 15-mL polypropylene sample tubes. Next, 2 mL of 0.5 M TBA/0.25 M sodium carbonate buffer (pH adjusted to 10 using NaOH) and 2 mL of methanol were added to the samples and vortexed for 15 s. After addition of 3 mL of MTBE, the samples were mixed again and centrifuged at 10000 rpm for 5 min. The supernatants were separated into new glass tubes. Another 3 mL of MTBE was added and the extraction was performed again. The combined sample extracts were dried under a gentle stream of nitrogen. Subsequently, each extract was dissolved in 4 mL of 1:1 MTBE/DCM and loaded onto a Presep-C silica gel column preconditioned with 45 mL of methanol and 4 mL of 1:1 MTBE/DCM on a vacuum manifold. The silica gel column was washed with 10 mL of hexane and 30 mL of ethyl acetate that had been prefiltered through another Presep-C silica gel column. The target fraction was eluted using 12 mL of acetone that had been prefiltered through an alumina column (Sep-Pak plus alumina N; Waters). The eluate was dried under a gentle stream of dry nitrogen. The residue was then redissolved in 100 μL of 0.1 M benzyl bromide/acetone solution and derivatized at 60 °C for 1 h. No further clean-up was conducted.

The infant formulas were dissolved in Milli-Q water according to the guidelines on the packages. Cow milk (4 mL), Milli-Q water (2 mL, procedural blank), and infant formulas (2 mL) were treated by the same procedure used for the human breast milk samples.

2.4. Instrumental analysis

The extracts were analyzed by gas chromatography–mass spectrometry (Agilent 6890GC/5973MSD; Agilent Technologies Japan Ltd., Tokyo, Japan) in the electron impact ionization mode. The PFCAs were separated on a J&W DB-5MS column with a helium carrier gas (1.5 mL min^{-1}). The splitless injection volume was 2 μL. The oven temperature was 70 °C for 2 min initially, and then ramped up to 280 °C at 20 °C min^{-1}. The monitored ions are listed in Table 2. Standard stock solutions (2 μg mL^{-1}) were diluted to seven working standard solutions (4, 2, 1, 0.8, 0.4, 0.2, and 0.1 ng mL^{-1}) by serial dilutions in acetone. All the standard solutions were stored in a refrigerator at 4 ± 2 °C for a maximum period of 3 months from the date of preparation.

The instrumental detection limits (IDLs) were defined as the mass of analyte producing a peak with a signal-to-noise ratio of 3, and ranged from 0.5 pg (PFUnDA, PFDoDA, and PFTrDA) to 0.2 pg (other PFCAs).

2.5. Quality assurance

We used Milli-Q water as the procedural blank control. The average blank values ($n = 6$) were 20.5 pg mL^{-1} (PFOA), 5.2 pg mL^{-1} (PFNA), and 7.1 pg mL^{-1} (PFDA). In the case of blank levels, the mean blank signal was subtracted from the calculated sample concentration only if the calculated sample concentration was three times higher than the blank concentration. If no signal was detected in the blank samples, the method detection limits (MDLs) were based on the IDLs and 2-mL milk samples. Using this method, we established that the MDLs ranged from 40 to 10 pg mL^{-1} (Table 2).

Table 1
Study areas and sample information.

Sampling site	n	Year	Age (year)[a]	(range)	Parity (n)	Smoking[b,c]	Drinking[c]	Lactation period (week)
A. Human milk								
Japan Kyoto	30	2010	27.8 ± 3.4	(21–33)	1(30)	Ex (7), non (23)	Ex (18), non (12)	3.0 ± 0.5
Korea Seoul	30	2010	30.9 ± 2.3	(26–36)	1(22), 2(8)	Ex (3), non (27)	Curr (3), ex (2), non (25)	1.6 + 1.1
China Beijing	30	2008, 2009	27.0 ± 1.7	(23–30)	1(30)	Non (30)	Curr (2), ex (27), non (1)	NA
B. Infant formula								
			Targeted infant age (month)					
Japan Kyoto	5	2010	0–12					
China Beijing	4	2010	0–12					

[a] Data are presented as the mean ± standard deviation.
[b] Including second-hand tobacco smoke.
[c] Curr: current; ex: experienced; non: never.

Table 2
Recoveries and detection limits for the PFCA analyses in human serum samples.

Compound	Quantification (confirmation)	Instrument detection limit[a] (pg)	Blank (pg mL^{-1}) range (mean)	Detection limit[b] (pg mL^{-1})	Recovery and (reproducibility) mean percentage (SD) (n = 9)	Standard reference material 1954[c]		
						This study (pg g^{-1}) U	Toronto[d] (pg g^{-1})	Env. Canada[d] (pg g^{-1}
PFOA	504 (485)	0.2	12.0–32.1(20.5)	40	104 (14)	117	149	116
$^{13}C_4$ PFOA	508 (489)	–	–	–	99 (12)	–	–	–
PFNA	554 (535)	0.2	<5–14.7(5.2)	10	84 (44)	24	22	<16
$^{13}C_5$ PFNA	559 (540)	–	–	–	–	–	–	–
PFDA	604 (585)	0.2	<5–25.8(7.1)	15	109 (32)	16	14	<6
PFUnDA	654 (635)	0.5	<10	10	95 (45)	12	7	<14
PFDoDA	704 (685)	0.5	<10	10	92 (25)	<10	3	<8
PFTrDA	754 (735)	0.5	<10	10	97 (27)	<10		

[a] Injection of 2 μL.
[b] Milk sample of 2 mL (the mean blank signal was subtracted from the calculated sample concentration only if the calculated sample concentration was three times higher than the blank concentration).
[c] Milk standard reference material from the National Institute of Standards and Technology, 1954.
[d] Analyzed by the University of Toronto and Environment Canada (Keller et al., 2010).

$^{13}C_4$-PFOA was used as an internal standard for the PFCAs. $^{13}C_5$-PFNA was used to monitor the recovery of the internal standard. The recoveries of the PFCAs were examined by spiking 500 pg of each standard compound into cow milk. The mean recoveries of PFOA, PFNA, PFDA, PFUnDA, PFDoDA, and PFTrDA were 104%, 84%, 109%, 95%, 92%, and 97%, respectively. Typical chromatograms of PFCAs obtained in this study are shown in Supplemental Fig. 1.

For quality assurance and quality control of our analytical methods and procedures in the analysis of PFCAs in the breast milk samples, we measured PFCAs in standard reference materials from the National Institute of Standards and Technology (Table 2). The PFCA values were comparable to those reported previously (Keller et al., 2010).

2.6. Statistical analysis

We calculated the percentages of detection of the PFCAs in each country, and determined the range, median, mean, standard deviation, geometric mean, and 90th percentile concentration. Concentrations below the MDL were replaced by half of the MDL for statistical analyses. Nonparametric statistical tests were applied to assess the statistical significance of differences between values. The Steel–Dwass test was used to compare differences in the PFCA concentrations among different countries after the Kruskal–Wallis test. Spearman's rank correlation analysis was used to examine the relationships between the PFCA levels and the mother's age and child's birth weight. The Mann–Whitney test was used to examine the relationships between the PFCA levels and alcohol drinking and cigarette smoking. The level of statistical significance was set at $p < 0.05$. A factor analysis was used to elucidate the number of potential factors of sources. The analyses were conducted via a correlation matrix. Eigenvectors were employed for the analysis when the eigenvalues were greater than 1. Normalized varimax rotation was applied to these eigenvectors. The statistical analyses were carried out using the software JMP® 4 (SAS Institute Inc., Cary, NC) or R Ver. 2.12.1. (Ihaka and Gentleman, 1996) for the Steel–Dwass test.

3. Results

3.1. PFCA concentrations in breast milk in Japan, Korea, and China

The demographic characteristics of the participants are shown in Table 1. The participants in Korea were, on average, about 3 years older than those in Japan and China. The descriptive statistical data are summarized in Table 3. PFOA was the predominant compound and was detected in more than 60% of samples in all three Asian countries. The median concentration of PFOA ranged from 51 pg mL^{-1} in China to 89 pg mL^{-1} in Japan. The PFOA levels in Japan were significantly higher than those in Korea and China ($p < 0.05$, Steel–Dwass test).

PFNA and PFUnDA were detected at comparable rates to PFOA in the three countries. The levels of PFNA and PFUnDA were higher in Japan than in Korea and China ($p < 0.05$, Steel–Dwass test). PFDA was frequently detected in Japan (67%), but rarely detected in Korea (13%) and China (13%). In Korea, half of the milk samples contained detectable levels of PFTrDA, which was the highest among the three countries ($p < 0.05$, Steel–Dwass test). PFDoDA was detected in few samples in the three Asian countries and there

Table 3
Concentrations of PFCAs in breast milk samples.

Sampling site		Concentration (pg mL^{-1})						
		PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	∑PFCAs
Japan	n > MDL(%)	28(93.3)	27(90.0)	20(66.7)	28(93.3)	5(16.7)	10(33.3)	30(100.0)
Kyoto	Median	89(<40–194)A*	31(<10–72)A*	17(<15–65)A*	35(<10–100)A*	<10(<10–29) n.s.	<10(<10–91)AB*	184(50.3–413.5)A*
	Mean	93.5 ± 43.7	32.1 ± 17.2	21.3 ± 15.0	36.6 ± 21.8	<10	15.2 ± 20.6	194.5 ± 83.6
	GM(GSD)	82.7(1.7)	26.5(2.0)	16.9(2.0)	30.4(2.0)	<10	<10	176.7(1.6)
	P90	173	62	44	65	22	36	315
Korea	n > MDL(%)	24(80.0)	20(66.7)	4(13.3)	22(73.3)	4(13.3)	15(50.0)	28(93.3)
Seoul	Median	62(<40–173)B*	15(<10–41)B*	<15(<15–19)B*	19(<10–51)B*	<10(<10–41) n.s.	10(<10–43)A*	114(<10–283.9)B*
	Mean	64.5 ± 33.7	14.7 ± 9.3	<15	19.6 ± 13.1	<10	16.8 ± 13.5	118.8 ± 50.9
	GM(GSD)	55.5(1.8)	11.9(2.0)	<15	15.3(2.2)	<10	11.7(2.4)	109.7(1.5)
	P90	106	29	15	42	11	40	189
China	n > MDL(%)	19(63.3)	21(70.0)	4(13.3)	17(56.7)	3(10.0)	7(23.3)	28(93.3)
Beijing	Median	51(<40–122)B*	15(<10–47)B*	<15(<15–29)B*	15(<10–47)B*	<10(<10–25) n.s.	<10(<10–43)B*	84(<10–200.8)B*
	Mean	51.6 ± 30.6	15.3 ± 9.6	<15	16.0 ± 12.9	<10	<10	87.8 ± 54.9
	GM(GSD)	43.0(1.9)	12.6(2.0)	<15	11.7(2.3)	<10	<10	68.8(2.2)
	P90	103	27	18	42	10	22	164

MDL: method detection limit; GM: geometric mean; GSD: geometric standard deviation; P90: 90th percentile.
* Medians among different sites differ significantly ($p < 0.05$, Steel–Dwass test). For example, the letters A and B indicate that the corresponding values differ significantly at $p < 0.05$, while A and A or B and B indicate that the corresponding values do not differ significantly.

Table 4
Factor analysis among PFCAs.

	Initial solution		Varimax rotated	
	F1	F2	F1	F2
Eigenvalue	2.60	1.14		
Cumulative contribution (%)	43.3	62.3		
Eigenvector				
PFOA	0.387	−0.511	**0.818**	−0.135
PFNA	0.472	−0.375	**0.857**	0.060
PFDA	0.480	−0.020	**0.668**	0.390
PFUnDA	0.518	0.261	**0.563**	**0.677**
PFDoDA	0.114	0.430	−0.086	0.488
PFTrDA	0.340	0.587	0.135	**0.822**
*Factor score (mean ± SD)**				
		Beijing	−0.5 ± 0.6^{B}	−0.2 ± 0.7
		Kyoto	0.9 ± 1.1^{A}	0.2 ± 1.4
		Seoul	−0.4 ± 0.6^{B}	0.1 ± 0.8

* Means among countries differ significantly ($p < 0.05$, Steel–Dwass test). For example, the letters A and B indicate that the corresponding values differ significantly at $p < 0.05$, while A and A or B and B indicate that the corresponding values do not differ significantly.

were no significant differences ($p > 0.05$). Regarding the total PFCAs in the milk samples, PFOA accounted for 48%, 54%, and 61% in Japan, Korea, and China, respectively. Among the long-chain PFCAs, odd-numbered PFCAs were more frequently detected than even-numbered PFCAs, except for PFDA in Japan.

PFOA was only significantly correlated with PFNA (ρ coefficient:>0.4) (Supplemental Table 1). There were also significant correlations between PFNA and PFUnDA, PFDA and PFUnDA, and PFUnDA and PFTrDA (ρ coefficients: >0.4). In general, the PFCA concentrations showed strong correlations between PFCAs of similar (i.e. adjacent) chain lengths.

The factor analysis revealed that two potential factors, F1 and F2, accounted for 43.3% and 19.0% of the total variance (with eigenvalues of >1), respectively (Table 4). After varimax rotation, F1 indicated higher eigenvectors for PFOA, PFNA, PFDA, and PFUnDA, while F2 had positive eigenvectors for PFUnDA and PFTrDA. The mean factor scores of each sampling site are also shown in Table 4. Although the F1 score was higher in Kyoto than in the other two sites ($p < 0.05$, Steel–Dwass test), there were no significant differences in the F2 scores among all the sampling sites ($p > 0.05$, Kruskal–Wallis test).

3.2. PFCA concentrations in commercially available infant formulas in Japan and China

The PFCA concentrations in the infant formulas are shown in Table 5. PFOA, PFNA, and PFDA were frequently detected in both Japan and China, but there were no significant differences between the two countries. PFUnDA was detected at 40.7 pg mL^{-1} in one sample in Japan. PFDoDA and PFTrDA were not detected in any of the formula samples. Compared with the breast milk samples,

Table 5
Concentrations of PFCAs in infant formulas.

Sampling site	Sample No.	Concentration (pg mL^{-1})[a]						
		PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	∑PFCAs
Japan	1	<20	<5	<7	<5	<5	<5	<5
	2	35.8	27.0	<7	<5	<5	<5	62.8
	3	30.8	8.0	12.1	<5	<5	<5	50.9
	4	<20	8.6	11.5	<5	<5	<5	20.1
	5	22.5	92.0	19.8	40.7	<5	<5	175.0
	Meant ± SD	21.8 ± 11.8	27.6 ± 37.2	10.1 ± 6.9	10.1 ± 17.1	<5	<5	66.4 ± 65.6
China	1	35.4	50.4	14.0	<5	<5	<5	99.7
	2	<20	15.2	<7	<5	<5	<5	15.2
	3	37.1	12.2	12.9	<5	<5	<5	62.2
	4	29.9	11.6	13.9	<5	<5	<5	55.4
	Meant ± SD	28.1 ± 12.4	22.4 ± 18.8	11.1 ± 5.1	<5	<5	<5	61.5 ± 29.3

[a] A 4-mL aliquot of each infant formula was analyzed.

the PFOA levels were 4-fold and 2-fold lower in the formula samples in Japan and China, respectively. The total PFCA concentrations in the infant formulas were lower than those in the breast milk samples in Japan ($p < 0.05$, Kruskal–Wallis test), but not in China ($p > 0.05$, Kruskal–Wallis test).

3.3. Relationships between the PFCA levels and the participants' characteristics

To evaluate the influence of the participants' characteristics on the PFCA concentrations in the human breast milk samples, Spearman's correlation analyses were performed (Supplemental Table 2). PFDoDA was positively correlated with the mother's age in Korea ($p < 0.05$) and PFNA was negatively correlated the mother's age in China ($p < 0.05$). However, these correlations were not consistent among the three countries. In several epidemiological studies (Steenland et al., 2010), the PFC concentrations in the cord blood or maternal pregnancy serum were reported to be associated with the child birth weight. In our study subjects, the correlations between the PFCA concentrations and the child birth weights were not significant. The lactation period was also examined for correlations with PFCAs in the milk samples. PFDA was correlated with the lactation period in Japan ($p < 0.05$), but not in Korea. Among the PFCAs, there were no clear trends in the correlation coefficients. Although consumption of fish was one of the sources of exposure to PFCAs, no significant associations were observed between the PFCA levels in the milk samples and the fish intake ($p > 0.05$). Non-smoking mothers in Japan had relatively higher PFCAs levels than other mothers, but the difference was not significant ($p > 0.05$). The PFCA levels in the milk samples were compared between non-drinking mothers and other mothers. The PFTrDA and PFNA levels were lower in non-drinking mothers in Japan and Korea ($p < 0.05$, Mann–Whitney test).

3.4. Daily intake estimation and hazard assessment for infants

The tolerable daily intake (TDI) for PFOA was established to be 1500 ng kg body weight^{-1} d^{-1} by the Scientific Panel on Contaminants in the Food Chain requested by the European Food Safety Authority (EFSA, 2008). The average breast milk consumption rate and body weight for 1-year-old infants were assumed to be 600 g d^{-1} and 7.3 kg, respectively (Schecter, 1994). Based on these assumptions, the daily intakes of PFCAs by 1-year-old infants were estimated (Supplemental Table 3). For the infant formulas, the calculated mean levels were only 0.1–0.2% of the TDI. Meanwhile, the calculated levels for the human breast milk samples (means: 0.3–

Table 6
Comparisons of the PFCA concentrations in human breast milk with reported data (pg mL^{-1}).

Country	Region	Year	*n*		PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	Reference
Japan	Kyoto	2010	30	Mean	93.5	32.1	21.3	36.6	<10	15.2	This study
				Range	<40–194	<10–72	<15–65	<10–100	<10–29	<10–91	
	Hokkaido	NA	51	Mean	89	35					Nakata et al. (2009)
				Range	<12–339	<4–150					
	Ehime	1999	24	Mean	77.7						Tao et al. (2008b)
				Range	<42.5–170	<8.82–23.9					
Korea	Seoul	2010	30	Mean	64.5	14.7	<15	19.6	<10	16.8	This study
				Range	<40–173	<10–41	<15–19	<10–51	<10–41	<10–43	
		2007	17	Mean	41						Kim et al. (2011)
				Range	<43–77	<8.8	<18	<24	<13		
China	Beijing	2008–2009	30	Mean	51.6	15.3	<15	16.0	<10	<10	This study
				Range	<40–122	<10–47	<15–29	<10–47	<10–25	<10–43	
	Zhoushan	2004	19	Mean	106.3	18.1	7.2	19.1			So et al. (2006)
				Range	47–210	6.3–62	3.8–15	7.6–56			
	12 provinces	2007	1237	Mean	116.0	16.2	9.9	37.6			Liu et al. (2010)
				Range (24 pooled samples)	<14.15–814	6–76	<1.44–63	<1.30–196			
Vietnam	Hanoi, Ho Chi Minh	2000, 2001	40	Range	<42.5–89.2	<8.82–10.9					Tao et al. (2008b)
Cambodia	Phnom Penh	2000	24	Range	<42.5–132	<8.82–12.3					Tao et al. (2008b)
Philippines	Quezon	2000, 2004	24	Range	<42.5–183	<8.82–25.0					Tao et al. (2008b)
Malaysia	Penang	2003	13	Range	<42.5–90.4	<8.82–14.9					Tao et al. (2008b)
Indonesia	Jakarta, Purwakarta	2001	20	Range	<42.5	<8.82–135					Tao et al. (2008b)
India	Chidambaram Kolkata, Chennai	2002, 2004, 2005	39	Range	<42.5–335	<8.82					Tao et al. (2008b)
USA	Unknown	2003	2	Range	<200						Kuklenyik et al. (2004)
	Massachusett	2004	45	Mean	43.8	7.26					Tao et al. (2008a)
				Range	<30.1–161	<5.2–18.4					
Sweden	Uppsala	2004	12	Range	<209–492	<5–20	<8	<5			Kärrman et al. (2007)
		1996–2004	9	Range (Pooled annual composite milk sample)	<209	<5–28	<8	<5			
Germany	NA	2006	38	Range (Archived samples + 19 fresh samples)	201–460						Völkel et al. (2008)
	North Rhine Westphalian	NA	203	Range	25–610						Bernsmann and Furst (2008)
Spain	Tarragona	2007	10	Range	<500	<30	<60	<30	<30		Kärrman et al. (2010)
	Barcelona	2008	20		<15.2–907	<11.5	<85.5–1095				Llorca et al. (2010)

0.5% of the TDI; 90th percentiles: 0.6–0.9% of the TDI) were higher than those for the infant formulas. As of 2011, there is no established TDI for PFCAs that are longer than PFOA.

4. Discussion

In the present study, we first demonstrated contamination of human breast milk with PFDoDA and PFTrDA in Asian countries. Simultaneously, we confirmed similar long-chain PFCA profiles in East Asian breast milk samples, as previously reported (Liu et al., 2010, 2011; Kim et al., 2011). A characteristic PFCA composition was observed for PFUnDA and PFTrDA (both odd-numbered PFCAs) with residual PFDoDA and PFDA (both even-numbered PFCAs). These findings indicated that odd-numbered PFCAs predominated over even-numbered PFCAs in East Asian breast milk samples. The PFCAs with longer chains than PFOA reached 47% of the total PFCAs for the average of the three countries. This finding suggests that infants are exposed to not only classical PFOA but also long-chain PFCAs in East Asia. Indeed, a factor analysis demonstrated two potential factors, F1 and F2, as sources of PFCAs. F1 had loading on medium-chain PFCAs, of which the factor score was significantly higher in Kyoto than in Beijing or Seoul. Kyoto is located in the Hanshin area, where there is a large emission source of PFOA and its related by-products (Niisoe et al., 2010). Thus, F1 may represent a local emission source of PFCAs. On the other hand, F2 had strong associations with long-chain PFCAs. The factor scores for F2 in the three large cities did not differ, suggesting that there are similar sources of long-chain PFCAs (>C10) in the three counties. Therefore, PFCA (C10–C13) exposure through the breast milk is likely to commonly occur in East Asian countries. We are the first to document this possibility.

The sources of long-chain PFCAs are still unknown. Odd-numbered PFCAs predominated in the PFCAs in this study. As previously reported (Harada et al., 2011), odd-numbered PFCAs also predominated in serum samples collected from Asian women. A review by Prevedouros et al. (2006) indicated that odd-numbered PFCAs have been manufactured in Japan via oxidation of fluorotelomer olefins. Industrial application of these odd-numbered PFCAs might contribute to the pattern of PFCAs in breast milk samples collected from East Asian women. Although FTOHs are possible precursors of PFCAs, biodegradation of FTOHs preferentially yields even-numbered PFCAs (Fasano et al., 2009). Therefore, FTOHs are unlikely to be the main exposure source for Asian populations. Further investigations into the sources and exposure routes are needed to predict the future trajectory of these PFCA levels.

Although data concerning the PFC levels in human breast milk are not as abundant as those in blood samples, we can still find several reports for PFCs in human breast milk from Asia, the United States, and Europe. The related data are summarized in Table 6. In Japan, the PFOA levels in three regions were comparable (Tao et al., 2008b; Nakata et al., 2009). In Korea, PFOA had a higher value in the present study compared with earlier research in Seoul (Kim et al., 2011) (mean: 63.8 *vs.* 41 pg mL^{-1}, range: 14.7–172.1 *vs.* 21–77 pg mL^{-1}). This increase may be consistent with the increasing trend in the PFOA level in serum samples by 1.27-fold from 2000 to 2007 in Korea (Harada et al., 2010).

In China, the concentrations of PFOA in Zhoushan ranged from 47 to 210 pg mL^{-1} (So et al., 2006) and in 12 different provinces of China, the mean PFOA level was 116 pg mL^{-1} (Liu et al., 2010). The PFOA levels showed large variations within China, although the other PFCAs were comparable among two previous studies and this study. In Southeast Asian developing countries, most of the milk samples did not contain detectable PFCAs (Tao et al., 2008b), which might result from differences in industrialization. In the United States and European countries, PFOA and PFNA were detected in human breast milk samples, but long-chain PFCAs were not observed (Kuklenyik et al., 2004; Kärrman et al., 2007, 2010; Bernsmann and Furst, 2008; Tao et al., 2008a; Völkel et al., 2008; Llorca et al., 2010). The occurrence of long-chain PFCAs in East Asian countries is likely to be a fingerprint of the sources of exposure.

Infant formulas were also evaluated in this study. The compositions of PFCAs in the infant formulas were different from those in the breast milk samples. In Japan, the levels of PFCAs in the infant formulas were lower than those in the breast milk samples. These findings probably reflect differences in the bioaccumulation potential between humans and cows.

In our study, we found no evident relationships between the mother's characteristics and the PFCA concentrations. Although there were statistically significant differences for some of the PFCAs, no consistent trends were observed among the three countries.

The estimated daily intakes of PFOA were much lower than the TDI in this study. These observations may indicate that the health risks for PFOA intake from breast milk and infant formulas are limited. However, infants have different susceptibilities to adults with regard to their dynamic growth and developmental processes (Sly and Flack, 2008). In addition, the toxicokinetics and toxicities of long-chain PFCAs are still unclear, although these PFCAs comprised 48% of the total PFCAs in this study. These uncertainties necessitate more comprehensive toxicological studies on long-chain PFCAs, including PFOA.

The limitations of this study are the sample sizes and the sample selection method. It should be noted that these findings were based on a relatively small number of non-randomly selected volunteer samples. Moreover, the sampling times for the Chinese donors were uncertain, although it is known that the profiles of chemicals may change during the lactation period. Considering these limitations, a future extended study is required for confirmation of these findings,

In conclusion, various PFCAs were detected in human breast milk samples from East Asian countries. Further studies are needed to evaluate the exposure to long-chain PFCAs and the health risks in infants.

Acknowledgments

This study was mainly supported by Special Coordination Funds for Promoting Science and Technology (No. 1300001) sponsored by the Japan Science and Technology Agency, and a Grant-in-Aid for Health Sciences Research from the Ministry of Health, Labour and Welfare of Japan (H21-Food-003). The funding bodies had no roles in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. The authors have no financial conflicts of interest.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chemosphere.2011.10.035.

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Environ Health Prev Med
DOI 10.1007/s12199-011-0251-9

REGULAR ARTICLE

Preliminary assessment of ecological exposure of adult residents in Fukushima Prefecture to radioactive cesium through ingestion and inhalation

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Received: 21 October 2011 / Accepted: 23 October 2011

Abstract
Objective This study aims to estimate the ecological exposure of adult residents of Fukushima Prefecture to ^{134}cesium (Cs) and ^{137}Cs through ingestion and inhalation between July 2 and July 8, 2011.
Methods Fifty-five sets of meals with tap water, each representing one person's daily intake, were purchased in local towns in Fukushima Prefecture. Locally produced cow's milk (21 samples) and vegetables (43 samples) were also purchased. In parallel, air sampling was conducted at 12 different sites using a high-volume sampler. Nineteen sets of control meals were collected in Kyoto in July 2011. ^{134}Cs and ^{137}Cs levels in the samples were measured using a germanium detector.
Results Radioactivity was detected in 36 of the 55 sample meals from Fukushima, compared with one of 19 controls from Kyoto. The median estimated dose level (μSv/year) was 3.0, ranging from not detectable to 83.1. None of the cow's milk (21) or vegetable (49) samples showed levels of contamination above the current recommended limits (Bq/kg) of 200 for milk and 500 for vegetables. The total effective dose levels by inhalation were estimated to be <3 μSv/year at nine locations, but samples at three other locations close to the edge of the 20-km radius from the crippled nuclear power plant showed higher levels of contamination (μSv/year): 14.7 at Iitate, 76.9 at Namie, and 27.7 at Katsurao.
Conclusions Levels of exposure to ^{134}Cs and ^{137}Cs in Fukushima by ingestion and inhalation are discernible, but generally within recommended limits.

Keywords ^{134}Cs · ^{137}Cs · Exposure assessment · Fukushima Daiichi nuclear power plant accident · Ingestion · Inhalation

Electronic supplementary material The online version of this article (doi:10.1007/s12199-011-0251-9) contains supplementary material, which is available to authorized users.

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Introduction

Following the Tohoku earthquake and tsunami on March 11, 2011, the Fukushima Daiichi nuclear power plant exploded on March 15, 2011, releasing massive amounts of radionuclides, including iodine, cesium (Cs), strontium, and plutonium into the northern part of Japan and the Pacific Ocean, being the second largest nuclear accident, after the Chernobyl disaster [1, 2]. The total amount of ^{137}Cs released into the environment by the Fukushima Daiichi nuclear plant from March 11 to April 15

Published online: 10 November 2011

(1.3×10^{16} Bq) [3] has been estimated to be 10% of that emitted by the Chernobyl disaster in 1986 [1, 2].

Residents living within a 20-km radius of the nuclear power plant were evacuated soon after the disaster, but people in Fukushima Prefecture have continued to live outside this evacuation zone. Although the direct threat from the radioactive plume is over, it is important to continuously assess the exposure doses due to deposited radioactivity. Contamination with ^{137}Cs has been reported in residential areas in Fukushima Prefecture [4], and the internal doses resulting from inhalation of resuspended deposits [5] and ingestion of contaminated foods need to be monitored.

Residents in particular, but also people in remote areas, are seriously concerned about their levels of internal exposure to radionuclides through ingestion of contaminated food and drink. The ingested dose should be evaluated on the basis of the level of radioactivity contained in complete meals consumed (Bq/day/person), rather than on the radioactive content of an individual item (Bq/kg).

To evaluate potential post-accident internal doses, we conducted a field survey in July 2011, focusing on estimated exposures of adult residents of Fukushima Prefecture to ^{134}Cs and ^{137}Cs through ingestion and inhalation.

Materials and methods

Field survey

We tested whole-day meals, vegetables from local food venders, tap water, and air samples from cities at various distances from the nuclear power plant between July 2 and July 8, 2011 (Fig. 1). In the cities denoted as "M" and "V" in Fig. 1, we purchased whole-day meals and vegetables from local food venders, respectively. Tap water was also collected in the same towns or cities. In the cities denoted by "A," we conducted air sampling using a high-volume sampler (HV-1000F; Sibata, Saitama, Japan) and soil sampling (mixed soil samples from depth of 0–5 cm). We also collected continuous air samples at a fixed point in Fukushima City using a low-volume sampler (SL-30; Sibata, Saitama, Japan) with an eight-stage Andersen cascade impactor sampler (AN-200; Tokyo Dylec Co., Tokyo, Japan).

Food collection and processing for radioactivity determinations

Five male researchers (aged 32–68 years) visited one of the most popular local grocery stores in each city or town and purchased several sets of whole-day meals, according to their personal preferences, as reported previously [6]. A set of whole-day meals comprised prepackaged breakfast, lunch, and dinner, as well as desserts, snacks, and

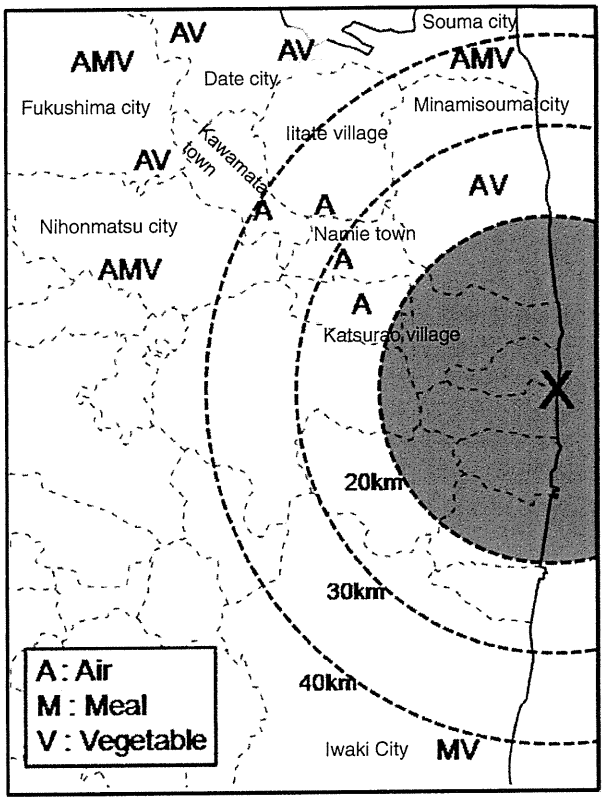

Fig. 1 Geographical locations of the field study areas. "A" represents sites where air sampling was conducted. "M" represents grocery stores where meals were purchased. Tap water (12 L) was collected in the same towns where meals were purchased. "V" represents commercial vender where vegetables were purchased. "X" represents the Fukushima Daiichi nuclear power plant. The *symbols* approximately represent actual geographical positions

beverages. A total of 12 L geographically matched tap water per town was donated by residents of the towns where the grocery stores were located. Locally produced vegetables and cow's milk were also purchased in the same towns. All items were transported daily to Kyoto University at 4°C for processing and analysis.

Daily whole-day meal sets were homogenized with locally collected tap water (approximately 1 L), together with desserts and snacks. The final volumes were recorded, and approximately 1 L of each homogenate was processed for freeze-drying. Vegetables and cow's milk were also freeze-dried. Control meals consisted of whole-day meals collected by 19 females using the food duplicate method, as previously reported [6]. Control meals were collected in July 2011 in Uji, Kyoto, which is located from 540 km to the southwest of the Fukushima nuclear power plant.

Air sampling and determination of radioactivities

A high-volume air sampler was used to collect dust in the air on a quartz membrane filter. A minimum of 50 m^3 was

inspired at all sampling sites at a height of 1.5 m above ground. An Andersen low-volume sampler was used to collect dust of various aerodynamic diameters to estimate the respirable portion of dust in Fukushima Prefecture. This sampler was fixed at a sampling site in Fukushima City. Dust samples were weighed, and their radioactivities were measured.

Determination of ^{137}Cs and ^{134}Cs

Aliquots of 100–200 g from each sample of food and cow's milk (dry weight), and soil (fresh weight) were weighed and sealed in cylindrical plastic containers. Filters from aerosol sampling were pressed into small cylindrical plastic containers. Radiometric determinations were performed using a high-purity, low-background, high-resolution germanium detector (0.7 keV). The detector was protected by a lead shield, 10 cm thick internally, covered with 0.5 mm electrolytic copper. A multichannel analyzer (4,096 channels, range 0–3,000 keV, MCA8000; Princeton Gamma Technologies, NJ, USA) was used for gamma-spectrum acquisition and processing. Characteristic gamma-ray energies were monitored to identify and quantify the radionuclides (^{134}Cs 604.7 and 795.9 keV, ^{137}Cs 661.7 keV). The detector was calibrated using a gamma-ray reference source from the Japan Radioisotope Association (Tokyo, Japan). The gamma spectrum of each sample was measured for >20,000 s for food and dust samples and for >2,000 s for soil samples. The lower limits of detection were 0.05 Bq/kg, 0.2 Bq/kg, 0.2 Bq/kg, 0.2 mBq/m^3, and 1 Bq/kg for food, vegetable, milk, dust, and soil samples, respectively. All samples were assumed to be in radioactive equilibrium. All activities were corrected to March 15, 2011 using physical half-lives (^{134}Cs 2.06 years, ^{137}Cs 30.1 years).

Effective dose coefficients for exposures by ingestion and inhalation

Radioactivities were converted into effective doses using effective dose coefficients of 0.019 μSv/Bq for ^{134}Cs and 0.013 μSv/Bq for ^{137}Cs by ingestion, respectively [7]. For inhalation, we assumed that a standard adult resident inhaled 20 m^3 air per day and used the effective dose coefficients of 0.02 μSv/Bq for ^{134}Cs and 0.039 μSv/Bq for ^{137}Cs for inhalation [7]. For the two routes of exposure, we postulated conservatively that all the radionuclides were retained in the body or in the lung, with no elimination.

Results and discussion

A total of 74 sets of whole-day meals were collected and analyzed. Their menus and components are presented in

Table 1 Dietary intake of radioactive cesium in Fukushima Prefecture

Sampling site	*n*		Food volume (g/day)	Water content (%)	Daily intake (Bq/day)		Estimated dose (μSv/year)
					^{134}Cs	^{137}Cs	
Fukushima total	55	*n* > MDL (%)	–	–	36 (65.5)	35 (63.6)	
		Median (range)	2,053 (1,100–3,145)	80.8 (73.3–97.6)	0.2 (ND–7.2)	0.3 (ND–7.0)	3.0 (ND–83.1)
		Mean ± SD	2,178 ± 400	81.9 ± 4.5	0.5 ± 1.1	0.6 ± 1.0	6.4 ± 12.5
Iwaki	10	*n* > MDL (%)	–	–	9 (90.0)	9 (90.0)	
		Median (range)	2,241 (1,879–2,690)	82.1 (76.8–86.1)	0.4 (ND–2.5)	0.7 (ND–1.6)	6.5 (ND–24.7)
		Mean ± SD	2,238 ± 272	81.5 ± 3.3	0.7 ± 0.8	0.7 ± 0.5	8.6 ± 7.8
Souma	10	*n* > MDL (%)	–	–	7 (70.0)	8 (80.0)	
		Median (range)	2,451 (2,044–2,795)	80.5 (73.3–87.1)	0.6 (ND–7.2)	0.9 (ND–7.0)	8.2 (ND–83.1)
		Mean ± SD	2,395 ± 293	80.1 ± 4.2	1.4 ± 2.2	1.6 ± 2.2	17.4 ± 25.3
Nihonmatsu	10	*n* > MDL (%)	–	–	5 (50.0)	4 (40.0)	
		Median (range)	2,611 (1,964–3,145)	79.4 (75.1–82.6)	0.1 (ND–0.9)	ND (ND–0.9)	1.7 (ND–10.4)
		Mean ± SD	2,529 ± 423	78.9 ± 2.3	0.3 ± 0.4	0.2 ± 0.3	2.9 ± 3.6
Fukushima	25	*n* > MDL (%)	–	–	15 (60.0)	14 (56.0)	
		Median (range)	1,954 (1,100–3,051)	83.7 (77.9–97.6)	0.1 (ND–0.8)	0.2 (ND–1.3)	1.3 (ND–11.3)
		Mean ± SD	1,927 ± 308	84.1 ± 4.8	0.2 ± 0.2	0.2 ± 0.3	2.6 ± 3.1
Kyoto (Uji)	19	*n* > MDL (%)	–	–	1 (5.3)	1 (5.3)	–
		Maximum	–	–	0.4	0.5	5.3
		Mean ± SD	2,955 ± 652	87.2 ± 2.5	–	–	–

Estimated dose is the total for doses attributable to exposure to ^{134}Cs and ^{137}Cs. The effective dose coefficients for ^{134}Cs and ^{137}Cs by oral route were 0.019 and 0.013 μSv/Bq, respectively

MDL method detection limit, *ND* less than MDL

Table 2 Radioactive cesium in local commercial products purchased in Fukushima Prefecture

Sampling site	n		Weight (g)	Radioactivity (Bq/kg)			Recommended standard[a] (Bq/kg)
				^{134}Cs	^{137}Cs	Total	
Milk							200
Fukushima total	21	n > MDL (%)	–	20 (95.2)	19 (90.5)	–	
		Median (range)	–	1.8 (ND–4.9)	1.9 (ND–5.5)	4.1 (ND–10.1)	
		Mean ± SD	985 ± 119	2.1 ± 1.7	2.4 ± 1.9	4.5 ± 3.6	
Iwaki	3	n > MDL (%)	–	3 (100.0)	3 (100)		
		Median (range)	–	0.9 (0.6–1.2)	1.2 (1.1–1.3)	2.0 (1.9–2.3)	
		Mean ± SD	752 ± 202	0.9 ± 0.3	1.2 ± 1.1	2.1 ± 0.2	
Souma	6	n > MDL (%)	–	6 (100.0)	6 (100.0)	–	
		Median (range)	–	3.1 (1.4–3.8)	3.1 (1.9–4.4)	6.1 (3.3–8.2)	
		Mean ± SD	1,019 ± 29	2.8 ± 1.0	3.1 ± 1.0	5.9 ± 1.9	
Nihonmatsu	3	n > MDL (%)	–	3 (100.0)	1 (33.3)		
		Median (range)	–	0.2 (0.2–1.3)	ND (ND–1.1)	0.2 (0.2–2.4)	
		Mean ± SD	1,047 ± 15	0.5 ± 0.7	0.4 ± 0.6	0.9 ± 1.3	
Fukushima	9	n > MDL (%)	–	8 (88.9)	8 (88.9)	–	
		Median (range)	–	3.4 (ND–4.9)	3.9 (ND–5.5)	7.3 (0.2–10.1)	
		Mean ± SD	1,021 ± 18	2.6 ± 2.0	2.3 ± 4.4	5.6 ± 4.4	
Kyoto (Uji)	3	n > MDL (%)	–	1 (33.3)	1 (33.3)	–	
		Median (range)	–	ND (ND–0.7)	ND (ND–0.7)	ND (ND–1.4)	
		Mean ± SD	1,037 ± 21	0.2 ± 0.4	0.2 ± 0.4	0.5 ± 0.8	

	Weight (g)	Radioactivity (Bq/kg weight)			Recommended standard[a] (Bq/kg)
		^{134}Cs	^{137}Cs	Total	
Vegetable/fruit					500
Kyoto (Uji)					
Spinach	1,249	ND	ND	ND	
Japanese mustard spinach	3,044	ND	ND	ND	
Fukushima (n = 43)					
Date					
Japanese mustard spinach	1,828	2.6	2.2	4.8	
Spinach	1,677	0.2	0.3	0.5	
New Zealand spinach	1,097	29.9	32.7	62.6	
Ceylon spinach	826	2.1	3.1	5.2	
Cucumber	1,643	3.4	4.5	7.9	
Welsh onion	1,770	3.3	2.8	6.1	
Kawamata					
Mizuna	504	5.9	7.7	13.7	
Shiitake	1,012	140.4	164.2	304.6	
Ceylon spinach	503	4.4	3.0	7.4	
Cucumber	1,007	1.3	1.6	2.8	
Broccoli	831	6.4	6.6	12.9	
Chinese chives	704	7.2	4.5	11.7	
Partially dried Japanese persimmon	332	1.8	1.7	3.5	
Welsh onion	1,455	5.7	6.6	12.3	
Fukushima					
Chinese chives	436	1.9	2.0	3.9	
Cucumber	493	2.9	3.9	6.8	

Table 2 continued

	Weight (g)	Radioactivity (Bq/kg weight)			Recommended standard[a] (Bq/kg)
		^{134}Cs	^{137}Cs	Total	
Iwaki					
Spinach	1,903	0.5	0.9	1.4	
Snap bean	860	3.5	3.6	7.1	
Shiitake	89	ND	ND	ND	
Green onion	571	7.3	8.5	15.8	
Chinese chives	615	2.8	3.5	6.3	
Broccoli	1,479	0.9	1.1	2.0	
Ceylon spinach	1,079	1.5	2.6	4.0	
Garlic	691	0.8	0.5	1.3	
Souma					
Welsh onion	1,543	4.1	2.6	6.7	
Peach	794	9.3	7.9	17.2	
Cherry	244	29.3	37.3	66.6	
Broad beans	418	4.9	6.0	10.9	
Onion (large)	835	0.5	0.6	1.1	
Onion (small)	430	9.1	9.2	18.3	
Red onion (large)	589	3.3	5.0	8.3	
Red onion (small)	524	9.6	11.6	21.3	
Garlic	256	9.4	7.2	16.6	
Potato	1,258	1.0	0.8	1.8	
Minamisouma					
Carrot	1,271	1.4	2.1	3.5	
Shiitake	417	127.1	154.7	281.8	
Bell pepper	502	ND	ND	ND	
Nihonmatsu					
Asparagus	637	1.3	1.5	2.8	
Bell pepper	390	12.0	10.7	22.7	
Ceylon spinach	1,533	1.7	3.2	4.9	
Cucumber	2,064	3.6	4.3	7.9	
Welsh onion	1,309	5.4	5.0	10.5	
Cherry	352	24.5	28.5	52.9	

MDL method detection limit, *ND* less than MDL

[a] Recommended by Ministry of Health, Labor, and Welfare of Japan [8]

Table S1. Radioactivity per daily intake (Bq/day) is also summarized in Table 1. ^{134}Cs or ^{137}Cs was detected in 36 of 55 whole-day meal samples from Fukushima Prefecture, compared with only one of 19 from Kyoto. The estimated median dose levels was 3.0 μSv/year, ranging from not detectable (ND) to 83.1 μSv/year in Fukushima, while the maximum dose level in Kyoto was 5.3 μSv/year.

The levels of ^{134}Cs and ^{137}Cs in cow's milk and vegetables were also determined (Table 2). The median total activity in milk from Fukushima Prefecture was 4.1 Bq/kg, ranging from ND to 10.1, which was an order of magnitude lower than the recommended limit set by the Ministry of Health, Labor, and Welfare of Japan [8]. Trace radioactivity was detected in only one sample from Kyoto. No vegetables in Fukushima Prefecture exceeded 100 Bq/kg, except for shiitake mushrooms (*Lentinula edodes*), which contained relatively high levels of radioactivity, up to 60% of the recommended limit (Table 2). Radioactivities in shiitake at Kawamata or Minamisouma were larger than at Iwaki, indicating that a radioactive plume was transferred by northeasterly winds from the nuclear plant. No radioactivity was detected in vegetables from Kyoto. These results indicate that the levels of radioactive Cs ingested were well below the recommended limits [8] in various towns in Fukushima Prefecture, except in the case of shiitake.

Table 3 Particle size distribution and respiratory deposition estimate for radioactive cesium in Fukushima Prefecture

Sampling site		Date (2011)	Andersen low-volume sampler, 224 m^3			
			Fraction (μm)	Dust amount (mg)	Radioactivity (mBq/m^3-air)	
					^{134}Cs	^{137}Cs
Fukushima	37°45′42″N 140°28′18″E	7/2–7/8	100–11.4	0.7	0.4	0.3
			11.4–7.4	1.1	0.3	0.3
			7.4–4.9	1	1.0	0.4
			4.9–3.3	0.9	0.5	0.6
			3.3–2.2	0.6	0.3	0.2
			2.2–1.1	0.8	0.3	0.2
			1.1–0.7	1.3	0.8	0.4
			0.7–0.46	1.3	1.5	1.1
			<0.46	0.9	1.5	1.3
		Total		8.6	6.5	4.7
		Respirable	<4.9	5.8	4.8	3.8

Sampling site		Date (2011) (weather)	High-volume air sampler							Ambient dose rate	Radioactivity in soil (Bq/kg)		
			Air volume sampled (m^3)	Dust amount (mg)	Radioactivity in air (mBq/m^3-air)		Estimated dose[a] (μSv/year)						
					^{134}Cs	^{137}Cs	^{134}Cs	^{137}Cs	Total	(μSv/h)	^{134}Cs	^{137}Cs	*n*
Fukushima	37°45′42″N 140°28′18″E	2011/7/2 (F)	473	6.8	1.9	3.0	0.3	0.8	1.1	1.2	NA	NA	
Date	37°47′10″N 140°33′26″E	2011/7/3 (CL)	94	3.5	7.9	6.4	1.1	1.8	3.0	0.9	3,232 ± 2,666	3,855 ± 3,047	5
Fukushima	37°39′26″N 140°32′11″E	2011/7/3 (CL)	83	1.9	4.7	1.5	0.7	0.4	1.1	1.0	2,515 ± 859	3,059 ± 1,077	5
Fukushima	37°45′42″N 140°28′18″E	2011/7/4 (R)	450	8	1.6	1.5	0.2	0.4	0.6	1.2	NA	NA	
Souma	37°46′1″N 140°57′2″E	2011/7/5 (F)	88	0.7	0.6	0.2	0.1	0.1	0.1	0.5	1,710 ± 2,365	2,116 ± 2,976	5
Minami-Souma	37°38′29″N 140°55′30″E	2011/7/5 (F)	84	2.4	0.7	1.1	0.1	0.3	0.4	0.9	1,772 ± 411	2,151 ± 546	5
Souma	37°46′8″N 140°43′1″E	2011/7/5 (F)	84	1.3	1.1	2.3	0.2	0.7	0.8	1.6	1,723 ± 1,792	2,047 ± 2,174	5
Fukushima	37°45′42″N 140°28′18″E	2011/7/5 (F)	220	4	2.9	3.4	0.4	1.0	1.4	1.2	NA	NA	
Nihonmatsu	37°33′21″N 140°27′34″E	2011/7/6 (F)	93	0.1	0.6	0.6	0.1	0.2	0.3	1.2	12,184 ± 12,170	14,202 ± 14,025	5
Nihonmatsu	37°33′21″N 140°30′43″E	2011/7/6 (F)	53	0.3	4.2	7.3	0.6	2.1	2.7	1.9	1,895 ± 674	2,244 ± 755	5
Kawamata	37°36′14″N 140°38′49″E	2011/7/6 (CL)	72	0.4	6.3	6.1	0.9	1.7	2.7	2.0	3,931 ± 4,856	4,741 ± 5,929	5
Fukushima	37°45′42″N 140°28′18″E	2011/7/6 (CL)	246	4	5.3	7.6	0.8	2.2	2.9	1.2	NA	NA	
Fukushima	37°45′42″N 140°28′18″E	2011/7/7 (CL)	259	5.3	1.9	2.5	0.3	0.7	1.0	1.2	NA	NA	
Iitate	37°36′44″N 140°44′52″E	2011/7/7 (CL)	84	1.7	24.6	38.9	3.6	11.1	14.7	9.0	18,531 ± 11,235	23,185 ± 15,664	5
Namie	37°33′38″N 140°45′39″E	2011/7/7 (CL)	84	1.7	148.2	194.2	21.6	55.3	76.9	13.0	13,548 ± 10,469	16,216 ± 12,653	5
Katsurao	37°31′33″N 140°48′21″E	2011/7/7 (CL)	84	1.5	65.0	64.0	9.5	18.2	27.7	10.0	16,332 ± 11,170	16,799 ± 10,058	5

CL cloudy, *F* fine, *R* rainy, *NA* not available

[a] It was assumed that radioactive cesium was in respirable fraction and that a standard human inhales 20 m^3 air

We collected 16 dust samples using the high-volume sampler (Table 3; Fig. 1). Data obtained with the low-flow-volume sampler suggested that a large proportion of the radionuclides from the crippled Fukushima nuclear power plant was in the respirable fraction: 74% (4.8/6.5) of the total ^{134}Cs and 81% (3.8/4.7) of the total ^{137}Cs (Table 3). To estimate the exposure doses for humans, we therefore selected a conservative scenario whereby all ^{134}Cs and ^{137}Cs activities in the dust samples collected using the high-volume sampler were allocated to the respirable fraction (aerodynamic diameter <4.9 μm). The highest dose level of 76.9 μSv/year was recorded in a sample collected at Namie. However, this value was still less than one-tenth of the permissible dose level of 1 mSv/year [8]. The estimated dose levels for ^{137}Cs were significantly correlated with ambient dose rate (μSv/h) ($n = 10$, $r^2 = 0.79, p < 0.05$) but not with mean radioactivity levels in soil (Bq/kg) ($n = 11$, $r^2 = 0.32, p > 0.05$).

Given that the samples in this study were obtained in early July, about 4 months after the major release of radioactivity, airborne radioactivity was likely to represent resuspended deposited radioactivity, rather than direct transport from the source. Several studies have investigated resuspension from a flat surface [5], but information on resuspension from ecological systems including forests and paddy fields is scant.

We demonstrated the radioactivity levels due to ^{134}Cs and ^{137}Cs in Fukushima Prefecture in July 2011. The maximum total exposure dose through inhalation and ingestion was estimated to be 160 μSv/year (83.1 by ingestion and 76.9 by inhalation) in zones outside a 20-km radius of the crippled Fukushima nuclear power plant.

The amounts of radioactivity in the daily meals consumed by residents of the study regions were well below the regulation limit. However, many food items are now imported globally, such that a high portion of foodstuffs comes from uncontaminated areas. It is possible that the radioactivity in some highly contaminated foodstuffs may be diluted by other "clean" foods. However, the ingested doses estimated in the present study would underestimate the exposure of residents whose daily foods are mostly supplied locally from within the contaminated areas. The conclusions of this study may therefore not be applicable to people in such a situation. Furthermore, the current study only utilized air monitoring in a few, geographically limited areas. All meal samples were obtained from outside a 30-km radius of the nuclear power plant, because no commercial venders were present between 20 and 30 km from the power plant, which had been defined as the planned emergency evacuation zone. In addition to the small number of air samples collected, the survey was conducted in the rainy season when "resuspension" is relatively low. The current study is thus subject to the above limitations and biases. However, the conservative approach adopted in this study maximized the estimated dose levels and would thus partially mitigate the effects of any biases and limitations. In conclusion, the estimated dose levels in residents of Fukushima Prefecture as a result of ingestion and inhalation were much lower than the 1 mSv/year, recognized as a publicly permissible dose [8]. Further studies are needed to perform qualitative risk assessments based on more accurate exposure estimates.

Acknowledgments This study was supported by a Grant-in-Aid for Health Sciences Research from the Ministry of Health, Labor, and Welfare of Japan (H21-Food-003), an urgent collaborative research grant from the Disaster Prevention Research Institute, Kyoto University (23U-01), and Tokyo Kenbikyoin Foundation.

Conflicts of interest The authors declare that there are no conflicts of interest.

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Environ Health Prev Med (2012) 17:170–172
DOI 10.1007/s12199-012-0269-7

INTERNATIONAL FORUM

Asian forum on environmental health policy: challenges and perspectives of environmental health problems in the region in the next 30 years

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Published online: 16 February 2012

Introduction

Asia is the world's most populous continent, accomodating circa 3.9 billion people in 48 countries. It is an important player in terms of the environment and world economy, with the second largest gross domestic product of all continents, after Europe. This geographical entity includes many developed countries, such as Japan, South Korea, and Singapore, and a number of very high growth nations, such as China and India. Its climate is very diverse, ranging from the very moist (southeastern parts) to dry (interior), and very cold (e.g., Siberia) to tropical (southern regions). The countries of this continent not only share—to varying degrees—historical experiences but also the effects of environmental pollution, such as Asian dust storms (ADS). The recent Fukushima Daiichi nuclear disaster in Japan has delivered the clear message that although an environmental challenge may be initiated in one specific location, many countries in the region may suffer from the consequences. Global climate change also underlines the fact that environmental issues are often global and, consequently, multinational collaboration is not an option but a necessity. Environmental health problems cannot be properly resolved if the counter-measures are restricted only to within the political borders. Accurate assessment of the problems and the development of appropriate solutions can best be achieved on a broader, regional scale, by communication and collaboration among those scientists and policy-makers facing the same issues.

The past of one country can mirror the present or future situation of another country. Japan, one of the most developed countries in the world, and South Korea, now considered to be a developed country, have experienced very fast economic growth, which has been accompanied by worsening health of its population due to environmental pollution. Ageing has for some time been an important issue in Japanese society and is now also a challenging problem in South Korea. China is the largest economy in Asia and the second largest economy in the world. However, it is now recognizing the adverse consequences of rapid industrialization and development in terms of environmental health. The experiences of South Korea or Japan could help China better prepare for the potential challenges to be faced in terms of environmental health.

Such recognition has led to the organization of a long-term platform for communication and collaboration among environmental health experts representing the diverse geographical areas of Asia. The first International Forum for Environmental Health Policy and Science was held in

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Seoul in November 25, 2011, and delegates from leading academic institutes in Bangkok, Kyoto, Seoul, Taipei, and Wuhan participated. The editorial presents a summary of the motivation for holding this forum, the perspectives of each participant, and future directions that were agreed on, with the intention to encourage other scientists around the region to join in and contribute.

Issues and agendas by delegation

Japan

First of all, we deeply express our sincere thanks for the warm support we have received from Asian countries that may have been directly or indirectly affected by the consequences of the East Japan Earthquake. We also have to admit that serious environmental contamination did take place due to the escape of radionuclides from the crippled Fukushima Daiichi nuclear power plant. International environmental monitoring for radionuclides will be needed for the next 30 years. In addition, we have urged the Japanese government to make information on the crippled plant accessible to the international community.

In the current, rather complex global circumstances, policy-making by any one nation on trans-boundary environmental problems cannot be independent of national interests in making profits because the latter are closely linked to the national economy. However, it is obvious that too much emphasis on the national economy when challenged with trans-boundary environmental problems will only increase conflicts among neighboring countries. Thus, we need to find a way to harmonize policy-making among countries and should establish clear and simple mechanisms to delineate the responsibility of stakeholder countries. One such a way is to visualize the trans-boundary flows of environmental contaminants among Asian countries. Such visualization will increase the transparency to the general populations of Asian countries, facilitating decision-making on the basis of national consensus. The Asian platform is expected to function as a task force for the visualization.

Korea

Demands for a cleaner and healthier environment have become very high in South Korea after several decades of rapid industrialization and development. These demands reflect the increased awareness among the general public of the potential deleterious health effects from exposure to environmental contamination. While serious environmental pollution events due to industrial incidents or by accidental release have since the mid-1990s no longer been frequently occurred, recent experiences, such as the Hebei-Spirit oil spill of 2007, still demonstrate the potential for such events to occur at any time. Therefore, the importance of emergency preparedness cannot be emphasized enough within the framework of environmental health management. In addition to such accidental episodes, the most important health issues related to environmental problems include the consequences of long-term low-dose exposures to multiple environmental contaminants, and environmental health inequity among susceptible populations, such as children and the elderly. Emerging environmental issues associated with global climate change and new technologies (e.g., nanomaterials) also deserve special attention. The Center for Disease Control of South Korea recently reported that the epidemic of acute interstitial pneumonia and the several resulting casualties were likely the result of exposure to a number of disinfectants used in humidifiers. This incidence increased public skepticism on the safety of chemicals that are used in normal daily activities. However, it also provides a chance to critically review the systems for ensuring chemical safety, not only before the release of such chemicals onto the market, but also during the marketing period.

China

China is the world's fastest growing major economy, with annual growth rates of approximately 10% for almost three decades. Since the 1980s China has witnessed increased pollution and degradation of natural resources and now recognizes increasing public health problems due to such pollution. Chronic, non-communicable diseases account for about 80% of deaths and 70% of disability-adjusted life-years lost in China, which is in part related to changes in the environment, lifestyle and diet, as well as an increased life expectancy. Air pollution has emerged as the most important environmental cause of cardiopulmonary diseases and adverse health risks. Lifestyle and diet changes, environmental pollution, and their interaction with genetics or epigenetic factors are involved in endemic, chronic non-communicable diseases. One of the major scientific challenges for the next few decades will be to gain an understanding of the interaction between genetic susceptibility and environmental factors on the etiology of not only cardiopulmonary diseases, such as heart disease, asthma, lung cancer, and chronic obstructive pulmonary diseases, but also on early damage, such as genetic damage, decreased lung function, and heart rate variability. Environment-wide and genome-wide association studies on chronic non-communicable diseases can best be carried out by collaborations and through the exchange of ideas within the region that shares common environmental problems.