Table 4
Levels of toxaphene congeners in the composite food samples and dietary intakes.

	Year (No. of pooled diets)		#26 (pg g fw^{-1})	#50 (pg g fw^{-1})	#62 (pg g fw^{-1})	Total (ng d^{-1})	Total (ng kg bw^{-1} d^{-1})	HSD test[a]	Intake/TDI (‰)
Beijing	1993 (n = 5)	Range (n > MDL)	<3.3 (0)	<3.6 (0)	<28.0 (0)	N.A.	N.A.	-	N.A.
	2009 (n = 5)	Range (n > MDL)	<3.3–4.9 (1)	<3.6–4.0 (1)	<28.0 (0)	22.0[b]	0.3[b]	_	1.6[b]
Seoul	1994 (n = 5)	Range (n > MDL)	<3.3–12.0 (1)	<3.6–14.0 (3)	<28.0 (0)	6.1–45.9	0.1–0.8		0.6–4.1
		Mean ± SD	-	5.4 ± 5.0	-	16.1 ± 16.9	0.3 ± 0.3		1.4 ± 1.5
		GM (GSD)	-	4.0 (2.4)	-	11.6 (2.3)	0.2 (2.3)	A	1.0 (2.3)
		P95 estimate	-	16.0	-	45.3	0.80		4.0
	2007 (n = 5)	Range (n > MDL)	34.1–59.2 (5)	43.9–67.4 (5)	<28.0 (0)	157.8–286.3	3.0–5.2		14.9–25.8
		Mean ± SD	41.1 ± 10.5	52.3 ± 9.1	-	194.0 ± 52.5	3.6 ± 0.9		18.1 ± 4.4
		GM (GSD)	40.2 (1.3)	51.7 (1.2)	-	189.2 (1.3)	3.6 (1.2)	B	17.7 (1.2)
		P95 estimate	58.5	67.7	-	260.0	5.10		25.4
Hokkaido	1992, 1995 (n = 7)	Range (n > MDL)	<3.3–32.5 (6)	<3.6–37.1 (6)	<28.0 (0)	8.0–168.5	0.1–3.1		0.7–15.4
		Mean ± SD	16.7 ± 11.7	17.7 ± 14.0	-	78.5 ± 60.3	1.4 ± 1		7.2 ± 5.4
		GM (GSD)	12.0 (2.8)	12.0 (2.9)	-	54.0 (2.9)	12.0 (2.9)	AB	5.0 (2.9)
		P95 estimate	65.0	69.5	-	310.5	5.80		29.0
	2009 (n = 7)	Range (n > MDL)	4.3–55.0 (7)	<3.6–60.6 (6)	<28.0 (0)	11.5–208.6	0.2–4.1		1.1–20.5
		Mean ± SD	18.0 ± 17.5	21.5 ± 20.8	-	74.9 ± 68.4	1.5 ± 1.3		1.5 ± 1.37
		GM (GSD)	12.7 (2.4)	13.0 (3.3)	-	50.0 (2.8)	0.0 (2.8)	AB	5.0 (2.8)
		P95 estimate	54.0	94.1	-	273.4	5.40		27.0
Kyoto	1996, 1997 (n = 6)	Range (n > MDL)	3.9–145.7 (6)	5.3–146.4 (6)	<28.0–123.5 (1)	16.0–542.0	0.4–11.0		1.7–54.3
		Mean ± SD	33.2 ± 55.5	37.3 ± 54.4	-	130.7 ± 204.2	2.6 ± 4.1		13.0 ± 20.5
		GM (GSD)	14.4 (3.5)	18.8 (3.3)	-	61.0 (3.5)	1.2 (3.5)	AB	6.0 (3.5)
		P95 estimate	115.2	136.0	-	468.0	9.20		46.0
	2009 (n = 6)	Range (n > MDL)	<3.3–16.8 (3)	<3.6–22.5 (3)	<28.0 (0)	5.2–61.6	0.10–1.2		0.5–6.1
		Mean ± SD	7.6 ± 7.3	9.8 ± 9.3	-	27.1 ± 25.4	0.5 ± 0.5		2.7 ± 2.5
		GM (GSD)	4.6 (3.2)	5.6 (3.5)	-	16.0 (3.3)	0.3 (3.3)	A	1.6 (3.3)
		P95 estimate	30.3	43.6	-	115.0	2.30		11.3
Okinawa	1992, 1995 (n = 7)	Range (n > MDL)	<3.3–18.7 (5)	<3.6–22.8 (6)	<28.0 (0)	6.8–138.0	0.1–2.8		0.6–13.7
		Mean ± SD	8.6 ± 6.9	10.6 ± 7.8	-	54.1 ± 47.2	1.1 ± 0.9		5.4 ± 4.7
		GM (GSD)	6.0 (2.7)	7.9 (2.4)	-	36.3 (2.8)	0.7 (2.9)	AB	3.6 (2.9)
		P95 estimate	31.3	34.0	-	201.0	3.40		17.0
	2009 (n = 7)	Range (n > MDL)	<3.3–28.0 (6)	<3.6–47.4 (6)	<28.0 (0)	6.6–139.7	0.1–1.8		0.4–9.0
		Mean ± SD	12.0 ± 9.7	18.2 ± 17.0	-	54.5 ± 47.9	0.7 ± 0.6		3.5 ± 3.1
		GM (GSD)	8.4 (2.7)	11.3 (3.1)	-	36.4 (2.9)	0.5 (2.9)	A	2.3 (2.9)
		P95 estimate	42.4	74.2	-	206.1	4.10		20.3

N.A.: not applicable; MDL: method detection limit; SD: standard deviation; GM: geometric mean; GSD: geometric standard deviation.
P95 estimates were calculated by multiplying the GM by the GSD to the power of 1.64.
[a] GMs with different letters differ significantly (p < 0.05, Tukey–Kramer HSD test). For example, the letters A and B indicate that the corresponding values differ significantly at p < 0.05, while A and AB or AB and B indicate that the corresponding values do not differ significantly. The samples from Beijing were excluded because of the low detection rates.
[b] Data are only shown for the one sample with a detectable level.

time periods were above the values of 624 ng d^{-1} for a 60-kg person in Taiwan (Doong et al., 1999) and 114.4 ng d^{-1} in Texas (Schecter et al., 2010).

Ratio of endosulfan isomers was also analyzed. There were significant differences between time periods in Beijing and Seoul (p < 0.05 by t-test). Technical endosulfan consists of 70% α-isomer and 30% β-isomer and conversion occurs from β- to α-isomer favorably (Schmidt et al., 1997). Samples from Beijing in 2009 showed comparatively lowest ratio of α- to β-isomer. Current use of endosulfan might concentrate β-isomer. In Seoul, ratio of isomer in 2007 was higher than in 1994. Past use of endosulfan might lead to concentrate α-isomer converted from β-isomer. α-isomer is also more volatile (vapor pressure: 0.006 mm Hg) compared to β-isomer (0.003 mm Hg) (Guerin and Kennedy, 1992) which may result in difference in transboundary pollution.

3.3. Trends of toxaphene in the daily consumed food samples

In the present study, toxaphene Parlars #26 and #50 were comparably detected in the majority of the food composites (40/60 and 42/60, respectively) (Table 4). Parlar #62 was only found in one sample from Japan in the 1990s.

Toxaphene was detected in 33 of 40 samples from Japan. The dietary intake of toxaphene (sum of Parlars #26, #50 and #62) as GM ranged from 0.3 to 1.2 ng kg bw^{-1} d^{-1} and no temporal trend was observed (p > 0.05, Tukey–Kramer HSD test). The toxaphene intake in Seoul increased significantly from 1994 (GM: 0.2 ng kg bw^{-1} d^{-1}) to 2007 (GM: 3.6 ng kg bw^{-1} d^{-1}) (p < 0.05, Tukey–Kramer HSD test). Only one of the 10 samples from Beijing contained a detectable level of toxaphene in 2009 (0.3 ng kg bw^{-1} d^{-1}), and this value was only slightly above the MDL, indicating negligible contamination.

Toxaphene residues have been documented in seafood samples in the United States (Maruya et al., 2001), Canada (Chan and Yeboah, 2000) and the Netherlands (van der Valk and Wester, 1991), and some of these studies have documented that the risks associated with particular groups of people are substantial. The estimated levels in Seoul and Japan in the 2000s were above the value of 11.3 ng d^{-1} in Texas (Schecter et al., 2010). Seafood consumption is higher in Japan and Korea (166.53 and 144.27 g $capita^{-1}$ d^{-1} in 2007, respectively) (FAOSTAT, 2010) than in China and the United States (72.49 and 65.90 g $capita^{-1}$ d^{-1} in 2007, respectively), which may partly explain the differences in exposure. However, the temporal increase in the samples in Seoul, as shown in Fig. 2, needs further explanation because there was no significant difference in seafood consumption in Korea (138.37 g $capita^{-1}$ d^{-1} in 1994) (FAOSTAT, 2010). Compared with a high-exposure population in north Greenland (4080 ng d^{-1} in 2004) (Deutch et al., 2006), the levels in East Asian countries have a large margin of exposure.

As reviewed by Simon and Manning (2006), the three congeners represent 22% of total weathered toxaphene. In Japan, the range of total toxaphene intake was estimated 1.45–5.50 ng kg bw^{-1} d^{-1}, and in Seoul 0.95 ng kg bw^{-1} d^{-1} in 1994 and 16.13 ng kg bw^{-1} d^{-1} in 2007.

3.4. Dietary intakes of endosulfan and toxaphene and health risks

On the basis of the estimated daily intake values against the ADI/TDI, the potential health risks were evaluated. For the entire study population, the average dietary intakes of both endosulfan and toxaphene in the 1990s and 2007–2009 were below the ADI/TDI of 6 and 0.2 μg kg bw^{-1} d^{-1} for endosulfan (sum of α- and β-isomers) and toxaphene (sum of Parlars #26, #50 and #62), respectively. As shown in Table 3, the upper 95th percentile dietary exposures for endosulfan in Seoul and Beijing in the 2000s were 17.5% and 0.6% of the TDI/ADI value, respectively. Consumers in Seoul and Hokkaido had daily exposures to 2.5% and 2.7% of the TDI/ADI value for toxaphene, respectively (Table 4).

This study provides insights into the trends in human dietary exposure to endosulfan and toxaphene, with implications for the existing food safety regulations in the study regions. A clear limitation of the study was the assumption made for the 2009 Japanese subjects, which may cause bias in the food consumption data as a result, and may lead to underestimation of the actual dietary exposure. This study was also based on pooled 24-h food duplicate samples, which may dilute the contributions and effects of individual samples. The 95th percentile assumption for exposure estimation is another limitation, because these values are less likely to remain constant for lifetime exposure. In view of these limitations, all the assumptions are designed to include a substantial safety margin to ensure the safety of all exposed populations in the regions.

4. Conclusions

This study estimated historical trends concerning dietary exposure to endosulfan and toxaphene in Beijing, Seoul, Kyoto, Okinawa and Hokkaido. The consumers in all the study sites were exposed to some levels of endosulfan and toxaphene, with the exception of Beijing where consumers were exposed to negligible amounts of toxaphene. Although still at a low level, an exponentially increasing trend in endosulfan exposure was observed in Beijing. The significant increase over time for toxaphene in Seoul was also remarkable. It is therefore essential to refine the dietary intake estimates in Seoul by targeting food types and source identification to ensure safe food for consumers.

Overall, the possibility of adverse health effects from the dietary intakes of endosulfan and toxaphene in all the study sites is unlikely.

Acknowledgments

This study was mainly supported by a Grant-in-Aid for Health Sciences Research from the Ministry of Health, Labour and Welfare of Japan (H21-Food-003) and Special Coordination Funds for Promoting Science and Technology (No. 1300001) sponsored by the Japan Science and Technology Agency. The authors have no financial conflicts of interest.

References

Ahmad, R., Salem, N.M., Estaitieh, H., 2010. Occurrence of organochlorine pesticide residues in eggs, chicken and meat in Jordan. Chemosphere 78, 667–671.
ATSDR, 1990. Toxicological Profile for Toxaphene, Atlanta, GA, USA.
ATSDR, 1991. Toxicological Profile for Toxaphene, Atlanta, GA, USA.
ATSDR, 1996. Toxicological Profile for Toxaphene, Atlanta, GA, USA.
Berntssen, M.H., Glover, C.N., Robb, D.H., Jakobsen, J.V., Petri, D., 2008. Accumulation and elimination kinetics of dietary endosulfan in Atlantic salmon (*Salmo salar*). Aquat. Toxicol. 86, 104–111.
Boobis, A.R., Ossendorp, B.C., Banasiak, U., Hamey, P.Y., Sebestyen, I., Moretto, A., 2008. Cumulative risk assessment of pesticide residues in food. Toxicol. Lett. 180, 137–150.
Burgoyne, T.W., Hites, R.A., 1993. Effect of temperature and wind direction on the atmospheric concentrations of alpha-endosulfan. Environ. Sci. Technol. 27, 910–914.
Chan, H.M., Yeboah, F., 2000. Total toxaphene and specific congeners in fish from the Yukon, Canada. Chemosphere 41, 507–515.
de Geus, H.J., Besselink, H., Brouwer, A., Klungsoyr, J., McHugh, B., Nixon, E., Rimkus, G.G., Wester, P.G., de Boer, J., 1999. Environmental occurrence, analysis, and toxicology of toxaphene compounds. Environ. Health Perspect. 107 (1), 115–144.
Deutch, B., Dyerberg, J., Pedersen, H.S., Asmund, G., Moller, P., Hansen, J.C., 2006. Dietary composition and contaminants in north Greenland, in the 1970s and 2004. Sci. Total Environ. 370, 372–381.
Doong, R.A., Lee, C.Y., Sun, Y.C., 1999. Dietary intake and residues of organochlorine pesticides in foods from Hsinchu, Taiwan. J. AOAC Int. 82, 677–682.
Dougherty, C.P., Henricks Holtz, S., Reinert, J.C., Panyacosit, L., Axelrad, D.A., Woodruff, T.J., 2000. Dietary exposures to food contaminants across the United States. Environ. Res. 84, 170–185.
FAOSTAT, 2010. Consumption of Fish and Fishery Products.
Fisk, A.T., Rosenberg, B., Cymbalisty, C.D., Stern, G.A., Muir, D.C.G., 1999. Octanol/water partition coefficients of toxaphene congeners determined by the "slow-stirring" method. Chemosphere 39, 2549–2562.
Guerin, T.F., Kennedy, I.R., 1992. Distribution and dissipation of endosulfan and related cyclodienes in sterile aqueous systems: implications for studies on biodegradation. J. Agric. Food Chem. 40, 2315–2323.
Guo, J.Y., Zeng, E.Y., Wu, F.C., Meng, X.Z., Mai, B.X., Luo, X.J., 2007. Organochlorine pesticides in seafood products from southern China and health risk assessment. Environ. Toxicol. Chem. 26, 1109–1115.
Hamilton, D., Crossley, S., 2004. Pesticide Residues in Food and Drinking Water: Human Exposure and Risks. John Wiley, New York.
Ikeda, M., Zhang, Z.W., Shimbo, S., Watanabe, T., Nakatsuka, H., Moon, C.S., Matsuda-Inoguchi, N., Higashikawa, K., 2000. Urban population exposure to lead and cadmium in east and south-east Asia. Sci. Total Environ. 249, 373–384.
Imanishi, K., Kawakami, M., Shimada, A., Kimura, Y., Chikaishi, K., Murakami, M., Kajiwara, N., Yamada, T., Tanabe, S., 2005. Toxaphene and mirex residues in cetaceans from Japanese waters. J. Environ. Chem. 15, 429–443 (in Japanese).
Jia, H., Li, Y.F., Wang, D., Cai, D., Yang, M., Ma, J., Hu, J., 2009. Endosulfan in China 1-gridded usage inventories. Environ. Sci. Pollut. Res. Int. 16, 295–301.
Jiang, Q.T., Lee, T.K., Chen, K., Wong, H.L., Zheng, J.S., Giesy, J.P., Lo, K.K., Yamashita, N., Lam, P.K., 2005. Human health risk assessment of organochlorines associated with fish consumption in a coastal city in China. Environ. Pollut. 136, 155–165.
Keith, L., Telliard, W., 1979. ES&T special report: priority pollutants: I – a perspective view. Environ. Sci. Technol. 13, 416–423.
Koizumi, A., Yoshinaga, T., Harada, K., Inoue, K., Morikawa, A., Muroi, J., Inoue, S., Eslami, B., Fujii, S., Fujimine, Y., Hachiya, N., Koda, S., Kusaka, Y., Murata, K., Nakatsuka, H., Omae, K., Saito, N., Shimbo, S., Takenaka, K., Takeshita, T., Todoriki, H., Wada, Y., Watanabe, T., Ikeda, M., 2005. Assessment of human exposure to polychlorinated biphenyls and polybrominated diphenyl ethers in Japan using archived samples from the early 1980s and mid-1990s. Environ. Res. 99, 31–39.
Koizumi, A., Harada, K.H., Inoue, K., Hitomi, T., Yang, H.R., Moon, C.S., Wang, P., Hung, N.N., Watanabe, T., Shimbo, S., Ikeda, M., 2009. Past, present, and future of environmental specimen banks. Environ. Health Prev. Med. 14, 307–318.
Li, J., Zhang, G., Guo, L., Xu, W., Li, X., Lee, C.S.L., Ding, A., Wang, T., 2007. Organochlorine pesticides in the atmosphere of Guangzhou and Hong Kong:

regional sources and long-range atmospheric transport. Atmos. Environ. 41, 3889–3903.
Liu, Z., Zhang, H., Tao, M., Yang, S., Wang, L., Liu, Y., Ma, D., He, Z., 2010. Organochlorine pesticides in consumer fish and mollusks of Liaoning Province, China: distribution and human exposure implications. Arch. Environ. Contam. Toxicol. 59, 444–453. doi:10.1007/s00244-010-9504-7.
Lu, F., 1995. A review of the acceptable daily intakes of pesticides assessed by the World Health Organization. Regul. Toxicol. Pharmacol. 21, 352–364.
Maruya, K., Walters, T., Manning, R., 2001. Residues of toxaphene in finfish and shellfish from Terry and Dupree Creeks, Georgia, USA. In: Estuaries and Coasts. Springer, New York, pp. 585–596.
Matolcsy, G., N?dasy, M., Andriska, V., Ter?nyi, S., 1988. Pesticide Chemistry. Elsevier, Elsevier Science Pub. Co. [distributor], Amsterdam, New York, NY, USA.
Oehme, M., Schlabach, M., Kallenborn, R., Haugen, J.E., 1996. Sources and pathways of persistent polychlorinated pollutants to remote areas of the North Atlantic and levels in the marine food chain: a research update. Sci. Total Environ. Mar. Mammals Mar. Environ. 186, 13–24.
POPRC, 2009. Endosulfan. Draft Risk Profile UNEP/POPS/POPRC.5/3.
Saleh, M.A., Casida, J.E., 1978. Reductive dechlorination of the toxaphene component 2,2,5-endo, 6-exo,8,9,10-heptachlorobornane in various chemical, photochemical, and metabolic systems. J. Agric. Food Chem. 26, 583–590.
Schecter, A., Colacino, J., Haffner, D., Patel, K., Opel, M., Papke, O., Birnbaum, L., 2010. Perfluorinated compounds, polychlorinated biphenyls, and organochlorine pesticide contamination in composite food samples from Dallas, Texas, USA. Environ. Health Perspect. 118, 796–802.
Schmidt, W.F., Hapeman, C.J., Fettinger, J.C., Rice, C.P., Bilboulian, S., 1997. Structure and asymmetry in the isomeric conversion of ??- to ??-endosulfan. J. Agric. Food Chem. 45, 1023–1026.
Simon, T., Manning, R., 2006. Development of a reference dose for the persistent congeners of weathered toxaphene based on in vivo and in vitro effects related to tumor promotion. Regul. Toxicol. Pharmacol. 44, 268–281.
Sutherland, T.D., Home, I., Weir, K.M., Russell, R.J., Oakeshott, J.G., 2004. Toxicity and residues of endosulfan isomers. Rev. Environ. Contam. Toxicol. 183, 99–113.
Takazawa, Y., Yoshikane, M., Shibata, Y., 2007. Toxaphene and other organochlorine compounds in pintails (*Anas acuta*) from Saitama Kamoba in Japan during Oct. 2000–Feb. 2002. Bull. Environ. Contam. Toxicol. 79, 87–90.
The National Health and Nutrition Survey in Japan, 2004. Health and Nutritional Information Study Group (Eds.), Ministry of Health, Labour and Welfare, Japan. Dai-Ichi Shuppan Publishers, Tokyo (in Japanese).
Thomson, B.M., Cressey, P.J., Shaw, I.C., 2003. Dietary exposure to xenoestrogens in New Zealand. J. Environ. Monit. 5, 229–235.
Tryphonas, H., Bryce, F., Huang, J., Lacroix, F., Hodgen, M., Ladouceur, D.T., Hayward, S., 2000. Effects of toxaphene on the immune system of cynomolgus (*Macaca fascicularis*) monkeys. A pilot study. Food Chem. Toxicol. 38, 25–33.
van der Valk, F., Wester, P.G., 1991. Determination of toxaphene in fish from Northern Europe. Chemosphere 22, 57–66.
Wang, X., Li, X., Cheng, H., Xu, X., Zhuang, G., Zhao, C., 2007. Organochlorine pesticides in particulate matter of Beijing, China. J. Hazard. Mater. 155, 350–357.
Weber, J., Halsall, C.J., Muir, D., Teixeira, C., Small, J., Solomon, K., Hermanson, M., Hung, H., Bidleman, T., 2009. Endosulfan, a global pesticide: a review of its fate in the environment and occurrence in the Arctic. Sci. Total Environ. 408, 2966–2984.
WHO, 2005. Total Diet Studies: A Recipe for Safer Food.
Wong, M.H., Leung, A.O., Chan, J.K., Choi, M.P., 2005. A review on the usage of POP pesticides in China, with emphasis on DDT loadings in human milk. Chemosphere 60, 740–752.
Yeo, H.-G., Choi, M., Chun, M.-Y., Sunwoo, Y., 2003. Concentration distribution of polychlorinated biphenyls and organochlorine pesticides and their relationship with temperature in rural air of Korea. Atmos. Environ. 37, 3831–3839.
Yeo, H.-G., Choi, M., Sunwoo, Y., 2004. Seasonal variations in atmospheric concentrations of organochlorine pesticides in urban and rural areas of Korea. Atmos. Environ. 38, 4779–4788.

Chemosphere 82 (2011) 25–31

Contents lists available at ScienceDirect

Chemosphere

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

Detection of dicofol and related pesticides in human breast milk from China, Korea and Japan

Yukiko Fujii [a], Koichi Haraguchi [b], Kouji H. Harada [a], Toshiaki Hitomi [a], Kayoko Inoue [a], Yoshiko Itoh [b], Takao Watanabe [c], Katsunobu Takenaka [d], Shigeki Uehara [e], Hye-Ran Yang [f], Min-Young Kim [f], Chan-Seok Moon [g], Hae-Sook Kim [h], Peiyu Wang [i], Aiping Liu [i], Nguyen Ngoc Hung [j], Akio Koizumi [a,*]

[a] Department of Health and Environmental Sciences, Kyoto University Graduate School of Medicine, Yoshida-Sakyo Kyoto 606-8501, Japan
[b] Daiichi College of Pharmaceutical Sciences, 22-1 Tamagawa-cho, Minami-ku, Fukuoka 815-8511, Japan
[c] Miyagi University of Education, Sendai 980-0845, Japan
[d] Department of Neurosurgery, Takayama Red Cross Hospital, Takayama 506-8550, Japan
[e] Department of Obstetrics and Gynecology, Tohoku Kosai Hospital, Sendai 980-0803, Japan
[f] Research Institute of Public Health and Environment, Seoul Metropolitan Government, Yangjae Seoul 137734, Republic of Korea
[g] Department of Industrial Health, Catholic University of Pusan, Keumjeong, Busan 609757, Republic of Korea
[h] College of Nursing Science, Kyung Hee University, Seoul 130701, Republic of Korea
[i] Department of Social Medicine and Health Education, School of Public Health, Peking University, Haidian Beijing 100083, PR China
[j] Department of Science and Technology, Hanoi Medical University, Ton That Tung, Hanoi, SR, Viet Nam

ARTICLE INFO

Article history:
Received 28 June 2010
Received in revised form 27 September 2010
Accepted 9 October 2010
Available online 3 November 2010

Keywords:
Human milk
DDTs
Dicofol
Japan
Korea
China

ABSTRACT

Previously, we demonstrated that the concentrations of DDTs were greater in breast milk collected from Chinese mothers than from Japanese and Korean mothers. To investigate dicofol as a possible source of the DDTs in human breast milk, we collected breast milk samples from 2007 to 2009 in China (Beijing), Korea (Seoul, Busan) and Japan (Sendai, Takarazuka and Takayama). Using these breast milk samples, we quantified the concentrations of dichlorobenzophenone, a pyrolysis product of dicofol (simply referred to as dicofol hereafter), dichlorodiphenyltrichloroethane and its metabolites (DDTs) using GC–MS. Overall, 12 of 14 pooled breast milk samples from 210 mothers contained detectable levels of dicofol (>0.1 ng g^{-1} lipid). The geometric mean concentration of dicofol in the Japanese breast milk samples was 0.3 ng g^{-1} lipid and significantly lower than that in Chinese (9.6 ng g^{-1} lipid) or Korean breast milk samples (1.9 ng g^{-1} lipid) ($p < 0.05$ for each). Furthermore, the ΣDDT levels in breast milk from China were 10-fold higher than those from Korea and Japan. The present results strongly suggest the presence of extensive emission sources of both dicofol and DDTs in China. However, exposure to dicofol cannot explain the large exposure of Chinese mothers to DDTs because of the trace levels of dicofol in the ΣDDTs. In the present study, dicofol was confirmed to be detectable in human breast milk. This is the first report to identify dicofol in human samples.

1. Introduction

Dicofol (trade name, Kelthane) is a pesticide that is used worldwide for agricultural applications (Fig. 1). Since dicofol has a similar structure to DDT (dichlorodiphenyltrichloroethane), it is associated with similar concerns to DDT and its metabolites such as its persistence, bioaccumulation, long-range transport and adverse effects on humans, animals and the environment. Dicofol is manufactured from technical-grade DDT by chlorination to an intermediate, Cl-DDT, followed by hydrolysis to dicofol. Unreacted DDT and Cl-DDT are degraded to *p,p'*-DDE, which remains in the technical-grade dicofol as an impurity (Qiu et al., 2005; Turgut et al., 2009). In Japan, dicofol was used as a pesticide from 1956, and then banned in 2004. However, dicofol has been widely used in agricultural practices in China until the present time and is suspected of being one of the major sources of DDTs in cotton fields (Yang et al., 2008). Dicofol exerts acute toxicity toward humans and is thought to be a human carcinogen (Lessenger and Riley, 1991; Settimi et al., 2003). Regarding its acute toxicity, neurological damage and cognitive and emotional difficulties have been reported (Lessenger and Riley, 1991). A case-control study revealed an association of exposure to DDT and dicofol with prostate cancer (Settimi et al., 2003).

There have been several reports on the levels of ΣDDTs in human breast milk in Asian countries (Konishi et al., 2001; Nakata

* Corresponding author. Address: Department of Health and Environmental Sciences, Kyoto University Graduate School of Medicine, Yoshida Konoe-cho, Sakyo-ku, Kyoto 606-8501, Japan. Tel.: +81 75 753 4456; fax: +81 75 753 4458.
E-mail address: Akio.Koizumi@z06.mbox.media.kyoto-u.ac.jp (A. Koizumi).

0045-6535/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2010.10.036

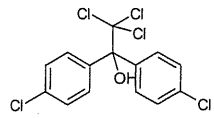


Fig. 1. Structure of dicofol.

et al., 2002; Burke et al., 2003; Minh et al., 2004; Kunisue et al., 2004, 2006; Poon et al., 2005; Wong et al., 2005; Chao et al., 2006; Yu et al., 2006; Zhao et al., 2007; Hui et al., 2008; Haraguchi et al., 2009). The levels of ΣDDTs in human breast milk were reported to be higher in Chinese mothers than in Japanese and Korean mothers (Konishi et al., 2001; Nakata et al., 2002; Kunisue et al., 2004; Poon et al., 2005; Wong et al., 2005; Yu et al., 2006; Kunisue et al., 2006; Zhao et al., 2007; Hui et al., 2008; Haraguchi et al., 2009). Based on the ratio of DDTs (*o,p′*-DDT/*p,p′*-DDT) used as an indicator for the contribution of dicofol to the ΣDDT levels, we suspected that a large proportion of the ΣDDTs in Chinese mothers may be attributable to exposure to dicofol (Haraguchi et al., 2009). Moreover, there have been other reports suggesting that dicofol is a source of DDT atmospheric pollution (Qiu et al., 2005; Qiu and Zhu, 2010; Yang et al., 2008). On the other hand, Liu et al. (2009) demonstrated that DDT pollution in the atmosphere of Chinese cities was attributable to usage of DDT itself, rather than usage of dicofol.

In the present study, we determined dichlorobenzophenone, a pyrolysis product of dicofol (simply referred to as dicofol hereafter) as a surrogate chemical for dicofol in breast milk, using gas chromatography–mass spectrometry (GC–MS). The results of our study were expected to provide direct evidence for whether dicofol can contaminate human breast milk. In addition, we examined whether dicofol could be the major source of ΣDDTs. To achieve this, we analyzed samples collected in various geographic sites in the three Asian countries with the aim of providing insights into the magnitude of pollution with dicofol and ΣDDTs in Asian countries as of 2010.

2. Materials and methods

2.1. Sample collection

Human milk samples were obtained from the Kyoto University Human Specimen Bank (Koizumi et al., 2005, 2009). All of the breast milk samples were collected using a standardized protocol (Koizumi et al., 2009). Fifteen individual breast milk samples (1 mL each) were pooled to obtain 15-mL breast milk samples. Overall, 14 pools were prepared from 210 human breast milk samples (Table 1). The method of pooling samples is efficient when the quantity of individual samples is small or the size of the sample analysis is in small lots. The samples analyzed were collected from 2007 to 2009 from volunteers living in China ($n = 60$ for Beijing in December 2007 and September 2008), Korea ($n = 30$ for Seoul in October 2007; $n = 30$ for Busan from December 2008 to January 2009) and Japan ($n = 30$ for Sendai from April to May 2009; $n = 30$ for Takarazuka in August 2008; $n = 30$ for Takayama from June to October 2008) (Fig. 2). The milk samples were collected manually during breast-feeding at 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. Three distilled water tubes were prepared as operational blanks and tested for possible contamination. The Ethics Committee of Kyoto University approved the protocol of the present study (E25) and appropriate written informed consent was obtained from all the participants.

2.2. Chemicals

$^{13}C_{12}$-labeled 2,3,4,5,6,3′,4′,5′-octachlorobiphenyl (CB-205; AccuStandard Inc., CA, USA) was used as an internal standard for the identification and quantification of dicofol and DDTs. The analytes investigated were *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE), *p,p′*-dichlorodiphenyldichloroethane (*p,p′*-DDD), *o,p′*-

Table 1
Background data for the participants in China, Korea and Japan.

Region		Pool number	N	Age[a]	BMI[b]	Occupation			Number of delivery		Lipid (%)
						Housewife	Clerk	Others	Primiparae	Multiparae	
China											
	Beijing	1	15	25(1.0)[c]	25.2(3.4)[c]	0	6	9	15	0	3.9
	Beijing	2	15	28(1.1)[c]	27.3(4.3)[c]	0	6	9	15	0	4.2
	Beijing	3	15	26(1.1)[c]	26.3(3.2)[c]	0	6	9	15	0	4.3
	Beijing	4	15	28(0.5)[c]	25.6(1.8)[c]	0	8	7	15	0	5.0
	Arithmetic mean (standard deviation)			27(1.7)	26.0(3.3)				15 (0)	0 (0)	4.4 (0.5)
Korea											
	Seoul	1	15	29(3.2)[c]	19.9(2.1)[c]	8	1	6	11	4	3.3
	Seoul	2	15	34(1.6)[c]	19.8(3.3)[c]	6	2	7	10	5	2.6
	Busan	1	15	30(3.6)[c]	19.8(1.8)[c]	10	2	3	9	6	3.1
	Busan	2	15	32(2.5)[c]	20.7(2.0)[c]	8	5	2	8	7	3.3
	Arithmetic mean (standard deviation)			31(3.3)	20.0(2.3)				9.5 (1.3)	5.5 (1.3)	3.1 (0.3)
Japan											
	Sendai	1	15	30(2.9)[c]	22.0(2.4)[c]	7	3	5	9	6	3.4
	Sendai	2	15	37(1.7)[c]	22.4(2.3)[c]	9	4	2	8	7	3.5
	Takayama	1	15	27(2.3)[c]	20.7(3.1)[c]	1	3	11	8	7	3.0
	Takayama	2	15	34(3.3)[c]	19.5(1.2)[c]	0	5	10	10	5	3.5
	Takarazuka	1	15	28(4. 1)[c]	21.5(3.4)[c]	6	2	7	9	6	2.5
	Takarazuka	2	15	35(2.0)[c]	21.1(3.0)[c]	5	5	5	2	13	3.4
	Arithmetic mean (Standard deviation)			32(4.6)	21.2(2.7)				7.7 (2.9)	7.3 (2.9)	3.2 (0.4)

[a] Years.
[b] BMI: Body mass index.
[c] Arithmetic mean (standard deviation) of each pooled sample.

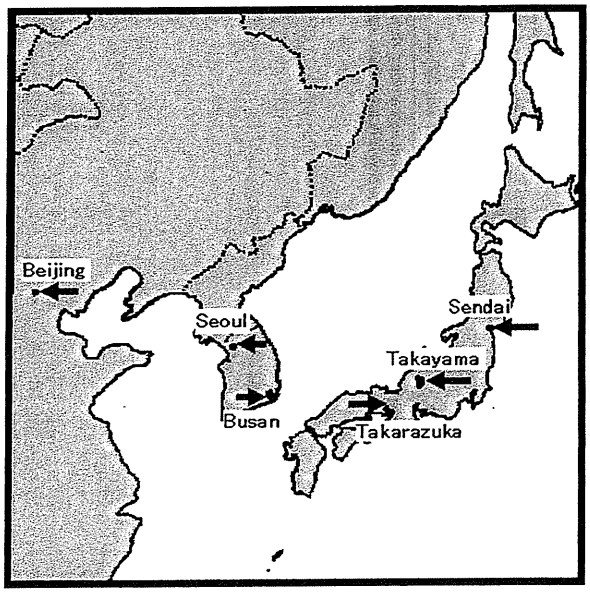


Fig. 2. Map of the sampling locations.

dichlorodiphenyltrichloroethane (*o,p'*-DDT), *p,p'*-dichlorodiphenyltrichloroethane (*p,p'*-DDT) and dicofol. As standard chemicals, the pesticides Mix 1037 and Mix 1111 (Kanto Chemical Co. Inc., Japan) were used for four-point calibration and determination of DDTs and dicofol. A pyrolysis product, 4,4'-dichlorobenzophenone, was measured as a surrogate of dicofol because dicofol is thermally decomposed in the flow of the GC–MS analysis. All solvents used were of pesticide-grade quality.

2.3. Clean-up procedure

Before extraction, each pooled breast milk sample (15 mL) was fortified with ^{13}C-labeled CB-205 (10 ng) as an internal standard. The pooled breast milk samples were then extracted twice using 15 mL of *n*-hexane, after adding 5 mL of 2% potassium oxalate solution and 10 mL of ethanol and diethylether (1:1). Each extract was washed with water and dried over sodium sulfate. After solvent evaporation, a gravimetric lipid determination was performed. Briefly, an aliquot of lipids (200–300 mg) was dissolved in *n*-hexane:dichloromethane (1:1) and subjected to gel permeation chromatography (400 × 45 mm, i.d.; Bio-Beads S-X3; Bio-Rad Laboratories, CA, USA). The eluate containing 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., Japan) by elution with 15 mL of *n*-hexane:dichloromethane (88:12, v/v). The resulting fraction was concentrated to 200 μL prior to GC–MS analysis.

2.4. Instruments and quantification

GC–MS analyses of the samples and reference standards were performed using an Agilent GC/MSD 5973i system (Agilent Technologies, CA, USA) equipped with a 6890 N gas chromatograph. The GC–MS conditions and target ions for the determination of the target chemicals are summarized in Table 2. Dicofol and DDTs were analyzed in the electron ionization mode. All analytes were quantified by comparing the peak areas of the target compounds in the sample extracts with that of the internal standard ($^{13}C_{12}$-labeled CB-205). The limits of quantification calculated by the signal-to-noise ratio ($S/N = 10$) were 0.20 ng g^{-1} lipid for dicofol, 0.02 ng g^{-1} lipid for *p,p'*-DDE, 0.01 ng g^{-1} lipid for *p,p'*-DDD and 0.10 ng g^{-1} lipid for both *o,p'*-DDT and *p,p'*-DDT. The limits of detection (LOD) were one-half of the above values. For quality

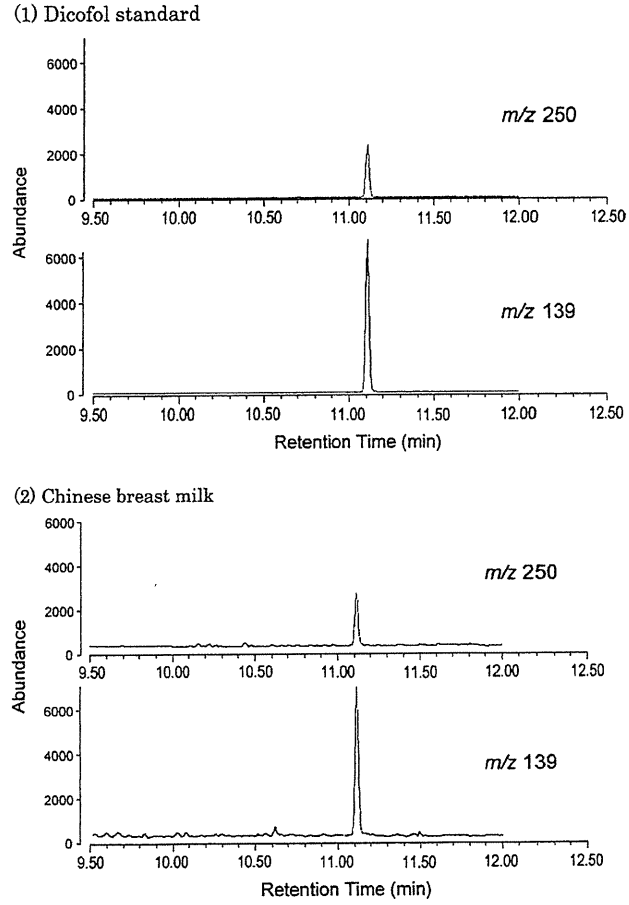


Fig. 3. Typical chromatograms of dicofol obtained in this study. (1) Dicofol standard; (2) Chinese breast milk.

Table 2
GC–MS conditions and selected ions (*m/z*) for determination of the chemicals.

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 mm film thickness, J&W Scientific, CA, USA)	
Oven	70 °C (1.5 min), then 20 °C min^{-1} to 230 °C (0.5 min), and then 4 °C min^{-1} to 280 °C (5 min)	
Temperature	Injector (250 °C), transfer line (280 °C), and ion source (230 °C)	
Target ions	235 (237)[a]	*o,p'*-DDT, *o,p'*-DDT, *p,p'*-DDD
-electron ionization mode	318 (316)[a]	*p,p'*-DDE
	139 (250)[a]	Dichlorobenzophenone (Dicofol pyrolysis product)
	442 (444)[a]	[^{13}C]CB-205 (Internal standard)

[a] Confirmation ion.

Table 3
Concentrations (ng g^{-1} lipid) of dicofol and DDTs in human breast milk samples from China, Korea and Japan.

		Concentration (ng g^{-1} lipid)							Dicofol occupancy (%) Ratio			
		*p,p′-DDE[a]	*p,p′-DDD[a]	*o,p′-DDT[a]	*p,p′-DDT[a]	ΣDDTs[d]	*Dicofol[a]	Total[e]	Dicofol/Total[e]	p,′p-DDE/*p,p′-DDT$_a$	*o,p′-DDT/*p,p′-DDT$_a$	*Dicofol/*p,p′-DDE$_a$	Dicofol/*p,p′-DDT$_a$
China													
	Beijing1	996.00	7.67	4.03	47.50	1055.20	64.0	206.40	0.6	20.97	0.08	0.006	0.13
	Beijing2	1237.30	8.59	7.68	50.41	1303.98	12.08	1316.06	0.9	24.54	0.15	0.010	0.24
	Beijing3	893.80	10.85	6.86	43.06	954.57	5.83	960.40	0.6	20.76	0.16	0.007	0.14
	Beijing4	2308.10	17.63	18.52	173.19	2517.44	19.09	2536.53	0.8	13.33	0.11	0.008	0.11
	GM (GSD)[c]	1262.72(1.5) A	10.60(1.4) A	7.92(1.9) A	65.01(1.9) A	1348.48(1.5) A	9.63(1.7) A	1358.26 (1.6) A	0.7	19.43(1.3) n.s.	0.12(1.4) n.s.	0.008(1.2) AB	0.15(1.4) n.s.
Korea													
	Busan1	86.60	5.17	1.82	21.44	115.03	2.13	117.16	1.8	4.04	0.08	0.025	0.10
	Busan2	156.10	4.33	1.84	10.69	172.96	0.80	173.76	0.5	14.60	0.17	0.005	0.07
	Seoul1	129.30	3.40	1.35	6.53	140.58	2.40	142.98	1.7	19.79	0.21	0.019	0.37
	Seoul2	129.20	3.98	1.73	11.52	146.43	2.96	149.39	20	11.21	0.15	0.023	0.26
	GM (GSD)[c]	122.59(1.3) B	4.17(1.2) B	1.67(1.2) B	144.40(1.2) B	142.26(1.2) B	1.87(1.8) A	144.40 (1.2) B	1.3	10.70(2.0) n.s.	0.14(1.5) n.s.	0.015(2.1) A	0.16(2.2) n.s.
Japan													
	Sendai1	116.50	1.91	0.77	4.84	124.02	0.81	124.83	0.6	24.07	0.16	0.007	0.17
	Sendai2	135.20	2.40	2.4	5.85	144.49	0.99	145.48	0.7	23.12	0.18	0.007	0.17
	Takayama1	75.30	5.25	0.69	7.07	88.31	0.32	88.63	0.4	10.66	0.10	0.004	0.05
	Takayama2	116.60	5.46	1.38	9.21	132.65	2.650	133.05	0.3	12.66	0.15	0.003	0.04
	Takarazuka1	95.00	2.90	1.02	7.46	106.38	<0.1[b]	106.48	0.0	12.74	0.14	0.00053[2)]	0.0067[2)]
	Takarazuka2	117.90	2.99	0.95	6.44	128.28	<0.1[b]	128.38	0.0	18.30	0.15	0.00042[2)]	0.0078[2)]
	GM (GSD)C	107.57(1.2) B	3.23(1.2) B	0.95(1.2) B	6.68(1.2) B	119.17(1.2) B	0.32(3.8) B	119.60(1.2) B	0.3	0.14(1.2) n.s.	16.11(1.4) n.s.	0.003(2.5) B	0.05(3.0) n.s.

[a] *$p<0.05$, Significant difference in the natural logarithms by one-way analysis of variance and multiple comparisons (Tukey's test). GMs with different letters differed significantly from each other countries among three sampling countries. For example, the letters A and B indicate that the corresponding values differ significantly at $p < 0.05$., while A and AB or AB and B indicated that the corresponding values do not differ significantly. n.s.: not significant.

[b] Undetected chemicals (under the limits of detection; dicofol: >0.1 ng g^{-1} lipid) were treated on the one-half scale (dicofol: 0.05 ng g^{-1} lipid) for calculations.

[c] GM: Geometric mean, GSD: Geometric standard deviation.

[d] Four congeners: *p,p′-DDE + *p,p′-DD + *o,p′-DDT + *p,p′-DDT.

[e] Total: dicofol + *p,p′-DDE + *p,p′-DDD + *o,p′-DDT + *p,p′-DDT.

assurance and control, a standard reference material (cod liver oil; NIST; SRM 1588b) was analyzed for DDTs. Our data were in good agreement with the certified values (relative standard deviation, 7.7–10.1%). Typical chromatograms of dicofol obtained in this study are shown in Fig. 3. The recoveries of the analytes were 91 ± 8% for dicofol and 84–94% for DDTs and the internal standard. Procedural blanks were processed in parallel with every batch of ten samples and their findings were negligible. The samples were kept in the dark during the extraction.

2.5. Statistical analysis

The obtained data were analyzed statistically using SPSS software Version 16.0 for Windows 2007 (SPSS Inc., IL, USA). One-way analysis of variance and multiple comparisons (Tukey's HSD test) were used to examine differences in the target chemical concentrations in natural logarithms among the three countries. When the levels of the target chemicals were less than their LODs, we allocated one-half of the LOD as the value for the calculation (0.05 ng g^{-1} lipid for dicofol). Probability values of less than 0.05 were considered to indicate statistical significances.

3. Results

3.1. Demographic characterization of the study population

The age, body mass index, occupation and number of deliveries of the mothers as well as the lipid contents of the breast milk samples are shown in Table 1. Owing to the family planning laws in China, all the Chinese breast milk samples were taken from primiparous women. In the other two countries, the breast milk samples are collected both primiparous and multiparous women.

3.2. Levels of ΣDDTs and dicofol in breast milk samples from Asian mothers

Table 3 shows the lipid-normalized concentrations (ng g^{-1} lipid) of dicofol and DDTs (four congeners: *p,p'*-DDE, *p,p'*-DDD, *o,p'*-DDT and *p,p'*-DDT) in the breast milk samples from six regions in the three countries. Dicofol was detected in the highest number of pooled breast milk samples except for those from Takarazuka in Japan. The geometric mean concentration of dicofol in the Japanese breast milk samples was 0.3 ng g^{-1} lipid and significantly lower than that in Chinese (9.6 ng g^{-1} lipid) or Korean breast milk samples (1.9 ng g^{-1} lipid) ($p < 0.05$ for each). The geometric mean concentration of the total amount (ΣDDTs + dicofol) in the Chinese breast milk samples was 1358 ng g^{-1} lipid ($n = 4$), which was 9–11-fold higher than those in the Korean and Japanese samples (144 and 120 ng g^{-1} lipid, respectively, $p < 0.05$ for each). The geometric means of the ΣDDTs (four congeners) were significantly higher in China than in Korea and Japan ($p < 0.05$ for each). *p,p'*-DDE was the predominant contributor to the ΣDDT composition (74–94%), followed by *p,p'*-DDT (4–18%) and *p,p'*-DDD (0.7–6%) in most cases. No significant differences in the *p,p'*-DDE/*p,p'*-DDT and *o,p'*-DDT/*p,p'*-DDT ratios were observed among the three countries. These findings for DDTs were comparable to the levels reported by Haraguchi et al. (2009).

4. Discussion

4.1. Dicofol profile in breast milk samples

Since dicofol has been widely used in agricultural practices and is lipophilic similar to DDT, we speculated occurrence of dicofol in human breast milk. As expected, we successfully detected dicofol in human breast milk samples. However, the levels of dicofol in the breast milk samples were trace amounts compared with the ΣDDT levels, and were in the order of ng g^{-1} lipid. The highest level of dicofol was observed in the Chinese breast milk samples. This finding may simply reflect the current or past use of dicofol in China. Since the levels of dicofol in the breast milk samples were relatively lower than the levels of DDTs, the current levels of dicofol do not suggest that dicofol is a major source of the ΣDDT levels.

Technical dicofol products in China contains *o,p'*-DDT and *p,p'*-DDT as impurities at an amount of 5–10% of their total composition (Tao et al., 2007). The ratio of that *o,p'*-DDT and *p,p'*-DDT (*o,p'*-DDT/*p,p'*-DDT) is known as 7 (Qiu et al., 2005). However, the ratio of *o,p'*-DDT/*p,p'*-DDT in breast milk samples from Chinese mothers was 0.12, which was as low as from Japanese mothers or Korean mothers (both 0.14). Such a small ratio supports our conclusion, given a shorter half life of dicofol in the environment than that of ΣDDTs (OSPAR Commission, 2002; Howard, 1991).

Spatial differences in the levels of dicofol were observed among the three Asian countries examined, since the levels in Japan were lower than those in China and Korea. Although dicofol has not been used in Japan since 2004, residual dicofol may be present in imported food products from China, Korea and other countries where dicofol is still used.

4.2. Daily intake estimation and hazard assessment for infants

The provisional tolerable daily intake (PTDI) levels were established as 0.02 mg kg^{-1} body weight for DDTs and 0.002 mg kg^{-1} body weight for dicofol by the FAO/WHO Joint Meeting on Pesticide Residues (JMPR, 1992; 2000). The average breast milk consumption rate and body weight for 1-year-old infants were assumed to be 600 g^{-1} d^{-1} and 7.3 kg, respectively. Based on these assumptions, the daily intakes of ΣDDTs and dicofol by 1-year-old infants were estimated (Table 4). For dicofol, the calculated level

Table 4
Daily intake estimations and hazard assessment for 1-year-old infants.

		ΣDDTs[a]		Dicofol	
		Estimated intake (μg kg^{-1}-body weight d^{-1})	%[c]	Estimated intake (μg kg^{-1}-body weight	%[d]
China					
	Beijing 1	3.38	16.9	0.021	1.0
	Beijing 2	4.50	22.5	0.042	2.1
	Beijing 3	3.37	16.9	0.021	1.0
	Beijing 4	10.35	51.7	0.078	3.9
	AM[b]	5.40	27.0	0.039	1.9
Korea					
	Busan1	0.31	1.6	0.006	0.3
	Busan2	0.37	1.8	0.002	0.1
	Seoul1	0.36	1.8	0.006	0.3
	Seoul2	0.40	2.0	0.008	0.4
	AM[b]	0.36	1.8	0.005	0.2
Japan					
	Sendai1	0.35	1.7	0.002	0.1
	Sendai2	0.42	2.1	0.003	0.1
	Takayama1	0.22	1.1	0.001	0.0
	Takayama2	0.38	1.9	0.001	0.1
	Takarazuka1	0.22	1.1	0.000	0.0
	Takarazuka2	0.36	1.8	0.000	0.0
	AM[b]	0.32	1.6	0.001	0.1

[a] Four congeners: *p,p'*-DDE + *p,p'*-DDD + *o,p'*-DDT + *p,p'*-DDT.
[b] AM: Arithmetic mean.
[c] Percent of provisional tolerable daily intake (PTDI: 0.02 mg kg^{-1} body weight) for DDTs by FAO/WHO Joint Meeting on Pesticide Residues (JMPR, 2000).
[d] Percent of provisional tolerable daily intake (PTDI: 0.002 mg kg^{-1} body weight) for dicofol byFAO/WHO Joint Meeting on Pesticide Residues (JMPR, 1992).

was only 0.0–3.9% of the PTDI in all samples. Meanwhile, the calculated levels of ΣDDTs (1.1–51.7%) were much higher than those of dicofol. The highest levels were observed in the Chinese breast milk samples for both dicofol and ΣDDTs. The identification of breast milk samples with >51.7% of the PTDI in this study needs to be addressed. The estimated daily intake of ΣDDTs through breast milk in China was comparable with other studies (Hui et al., 2008; Kunisue et al., 2004). Owing to the limitations of the experimental design of the present study (using pooled samples), we can possibly overlook certain individual samples that may exceed the PTDI for ΣDDTs. Therefore, exposure to DDTs through breast milk requires further monitoring.

4.3. Comparison with other data

Table 5 shows comparisons of the levels of DDTs in breast milk samples reported by previous studies and the present study. During the past decade, the levels of DDTs in all three countries (China, Korea and Japan) have been decreasing. Nevertheless, the levels of ΣDDTs in China were still more than 10 times higher than those in

Table 5
Levels of DDTs and dicofol in human breast milk samples in different regions (ng g^{-1} lipid).

Country	Area	Region (Name)	Year	Number of samples	Representative value	Concentration (ng/g lipid)					Ratio		Reference
						o,p'-DDT	*p,p'*-DDT	*p,p'*-DDE	*p,p'*-DDD	ΣDDTs	Dicofol	*p,p'*-DDE/*p,p'*-DDT	
Japan	North	Sendai	2009	30 (2 pools)	AM[a]	0.9	5	126	2.2	134	0.90	23.5	This study
	North	Sendai	2007	20	AM[a]	1.4	7	250	1.7	260	–[b]	36.2	Haraguchi et al., 2009
	Middle	Takarazuka	2008	30 (2 pools)	AM[a]	1.0	7	106	2.9	117	<0.1	15.3	This study
	Middle	Takayama	2008	30 (2 pools)	AM[a]	1.0	8	96	5.4	110	0.36	11.8	This study
	Middle	Kyoto	2007–2008	20	AM[a]	0.5	6	150	1.5	160	–[b]	23.4	Haraguchi et al., 2009
	Middle	Takayama	2007	20	AM[a]	0.6	4	92	1.1	97	–[b]	23.0	Haraguchi et al., 2009
	Middle	Osaka	1998	49	AM[a]	–[b]	18	270	–[b]	288	–[b]	15.2	Konishi et al., 2001
	Middle	Osaka	1997	47	AM[a]	–[b]	19	299	–[b]	318	–[b]	15.7	Konishi et al., 2001
	Middle	Osaka	1996	57	AM[a]	–[b]	19	264	–[b]	283	–[b]	14.0	Konishi et al., 2001
	Middle	Osaka	1995	59	AM[a]	–[b]	21	353	–[b]	374	–[b]	16.9	Konishi et al., 2001
	Middle	Osaka	1994	61	AM[a]	–[b]	16	443	–[b]	459	–[b]	27.3	Konishi et al., 2001
	South	Fukuoka (Primiparae)	2001–2004	38	AM[a]	–[b]	13	330	1.0	340	–[b]	25.4	Kunisue et al., 2006
	South	Fukuoka (Multiparae)	2001–2004	55	AM[a]	–[b]	10	220	0.7	230	–[b]	22.0	Kunisue et al., 2006
China	North	Beijing	2007–2008	60 (4 pools)	AM[a]	9.3	79	1359	11.2	1458	10.85	17.3	This study
	North	Beijing	2007	25	AM[a]	8.0	38	1250	5.7	1300	–[b]	32.9	Haraguchi et al., 2009
	North	Dalian	2002	20	AM[a]	–[b]	130	2000	6.0	2100	–[b]	15.4	Kunisue et al., 2004
	North	Shenyang	2002	20	AM[a]	–[b]	40	830	1.6	870	–[b]	20.8	Kunisue et al., 2004
	North	Beijing	1998	60	Median	<100	240	1720	–[b]	2040	–[b]	7.2	Yu et al., 2006
	North	Beijing	1993	59	Median	<100	550	3070	–[b]	3590	–[b]	5.6	Yu et al., 2006
	Middle	Pingqiao	2003–2005	16	Median	–[b]	–[b]	1324	–[b]	1324	–[b]	N/A[c]	Zhao et al., 2007
	Middle	Luqiao	2003–2005	5	Median	–[b]	–[b]	1528	–[b]	1528	–[b]	N/A[c]	Zhao et al., 2007
	South	Guangzhou	2004	30	AM[a]	19.9	118	1911	83.1	2464	–[b]	16.2	Quet al2010
	South	Hong Kong	2001–2002	316	Median	14.0	99	1380	6.0	1500	–[b]	13.9	Hui et al., 2008
	South	Hong Kong	1999–2000	26	AM[a]	–[b]	–[b]	–[b]	–[b]	3270	–[b]	N/A[c]	Poon et al., 2005
Taiwan	–	Taiwan	2001–2002	36	AM[a]	–[b]	23	310	–[b]	333	–[b]	13.5	Chao et al., 2006
	Middle	Seoul	2007	30 (2 pools)	AM[a]	1.5	9	129	3.7	144	2.68	14.3	This study
Korea	Middle	Seoul	2007	20	AM[a]	2.0	10	170	2.0	180	–[b]	17.0	Haraguchi et al., 2009
	South	Busan	2008–2009	30 (2 pools)	AM[a]	1.8	16	121	4.8	144	1.47	7.6	This study
	South	Masan	1994–1995	10	AM[a]	–[b]	22	162	4.4	283	–[b]	7.4	Kang et al., 2000

[a] AM: Arithmetic mean.
[b] Not measured.
[c] N/A: Not available.

Korea and Japan in the present study. The concentration ratio of *p,p'*-DDE/*p,p'*-DDT is usually used as an indicator for the residence time of *p,p'*-DDT in the environment. Therefore, lower ratios indicate more recent exposure to *p,p'*-DDT (Wong et al., 2005). In Beijing, China, the ratio was 5.6 in 1993 (Yu et al., 2006), but 32.9 in 2007 (Haraguchi et al., 2009) and 17.3 in this study. These results may suggest that the exposure to *p,p'*-DDT in Beijing has been declining.

4.4. Limitations of this study

One of the major limitations of this study is the sample size. In this study, 14 pools from 210 human milk samples were analyzed in total. This size may be sufficiently large to confirm the presence or absence of dicofol in human breast milk and may allow comparisons of the levels of dicofol among the three countries. However, the high and low levels of each pooled sample were averaged out by the pooling, and this masks potential domestic differences in the countries and significant correlations between each of the chemicals and associated factors such as age, body mass index or parity.

In terms of the analytical method, we determined the concentrations of dichlorobenzophenone as a surrogate chemical for dicofol. It is a pyrolysis product of dicofol during GC analysis as well as a degraded product of dicofol in the environment. Therefore, it is impossible to distinguish dichlorobenzophenone from two sources in this study. Future studies need to develop an analytical method to solve this issue, such as GC–MS with an on-column injection technique or use of liquid chromatography–mass spectrometry.

5. Conclusions

In the present study, we successfully detected dichlorobenzophenone in human breast milk samples. The very small proportions of dicofol in the ΣDDT levels in the breast milk samples exclude a major role in the exposure to ΣDDTs. The level of ΣDDTs in Chinese breast milk samples has decreased from 7700 to 1300 ng g^{-1} lipid during the period from 1983 to 1998 (Haraguchi et al., 2009; Yu et al., 2006). Nevertheless, the large daily intake of ΣDDTs through breast milk needs further monitoring.

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, a Grant-in-Aid for Health Sciences Research from the Ministry of Health, Labour and Welfare of Japan (H21-Food-003) and a Korea-Japan Joint Research Project under the KOSEF-JSPS Cooperative Program. The authors have no financial conflicts of interest.

References

JMPR, 1992. Monographs of Toxicological Evaluations. No. 842. Dicofol, Pesticide Residues in Food: 1992 Evaluations. Part II Toxicology. FAO/WHO Joint Meeting on Pesticide Residues.

JMPR, 2000. Monographs of Toxicological Evaluations. No. 972. DDT, Pesticide Residues in Food: 2000 Evaluations. Part II Toxicology. FAO/WHO Joint Meeting on Pesticide Residues.

Burke, E.R., Holden, A.J., Shaw, I.C., 2003. A method to determine residue levels of persistent organochlorine pesticides in human milk from Indonesian women. Chemosphere 50, 529–535.

Chao, H.R., Wang, S.L., Lin, T.C., Chung, X.H., 2006. Levels of organochlorine pesticides in human milk from central Taiwan. Chemosphere 62, 1774–1785.

Haraguchi, K., Koizumi, A., Inoue, K., Harada, K.H., Hitomi, T., Minata, M., Tanabe, M., Kato, Y., Nishimura, E., Yamamoto, Y., Watanabe, T., Takenaka, K., Uehara, S., Yang, H.R., Kim, M.Y., Moon, C.S., Kim, H.S., Wang, P., Liu, A., Hung, N.N., 2009. Levels and regional trends of persistent organochlorines and polybrominated diphenyl ethers in Asian breast milk demonstrate POPs signatures unique to individual countries. Environ. Int. 35, 1072–1079.

Howard, P.H., 1991. Handbook of Environmental Fate and Exposure Data for Organic Chemicals, vol. 3. Lewis Publishers, Chelsea, MI.

Hui, L.L., Hedley, A.J., Kypke, K., Cowling, B.J., Nelson, E.A., Wong, T.W., van Leeuwen, F.X., Malisch, R., 2008. DDT levels in human milk in Hong Kong, 2001–02. Chemosphere 73, 50–55.

Koizumi, A., Harada, K., Inoue, K., Hitomi, T., Yang, H.-R., Moon, C.-S., Wang, P., Hung, N., Watanabe, T., Shimbo, S., Ikeda, M., 2009. Past, present, and future of environmental specimen banks. Environ. Health Prevent. Med. 14, 307–318.

Koizumi, A., Yoshinaga, T., Harada, K., Inoue, K., Morikawa, A., Muroi, J., Inoue, S., Eslami, B., Fujii, S., Fujimine, Y., Hachiya, N., Koda, S., Kusaka, Y., Murata, K., Nakatsuka, H., Omae, K., Saito, N., Shimbo, S., Takenaka, K., Takeshita, T., Todoriki, H., Wada, Y., Watanabe, T., Ikeda, M., 2005. Assessment of human exposure to polychlorinated biphenyls and polybrominated diphenyl ethers in Japan using archived samples from the early 1980s and mid-1990s. Environ. Res. 99, 31–39.

Konishi, Y., Kuwabara, K., Hori, S., 2001. Continuous surveillance of organochlorine compounds in human breast milk from 1972 to 1998 in Osaka, Japan. Arch. Environ. Contam. Toxicol. 40, 571–578.

Kunisue, T., Nakanishi, S., Oka, N., Sato, F., Tsurumi, M., Tanabe, S., 2006. Dioxins and related compounds in albatrosses from the Torishima Island, Japan: accumulation features by growth stage and toxicological implications. Environ. Sci. Technol. 40, 6919–6927.

Kunisue, T., Someya, M., Kayama, F., Jin, Y., Tanabe, S., 2004. Persistent organochlorines in human breast milk collected from primiparae in Dalian and Shenyang, China. Environ. Pollut. 131, 381–392.

Lessenger, J.E., Riley, N., 1991. Neurotoxicities and behavioral changes in a 12-year-old male exposed to dicofol, an organochloride pesticide. J. Toxicol. Environ. Health 33, 255–261.

Liu, X., Zhang, G., Li, J., Yu, L.L., Xu, Y., Li, X.D., Kobara, Y., Jones, K.C., 2009. Seasonal patterns and current sources of DDTs, chlordanes, hexachlorobenzene, and endosulfan in the atmosphere of 37 Chinese cities. Environ. Sci. Technol. 43, 1316–1321.

Minh, N.H., Someya, M., Minh, T.B., Kunisue, T., Iwata, H., Watanabe, M., Tanabe, S., Viet, P.H., Tuyen, B.C., 2004. Persistent organochlorine residues in human breast milk from Hanoi and Hochiminh City, Vietnam: contamination, accumulation kinetics and risk assessment for infants. Environ. Pollut. 129, 431–441.

Nakata, H., Kawazoe, M., Arizono, K., Abe, S., Kitano, T., Shimada, H., Li, W., Ding, X., 2002. Organochlorine pesticides and polychlorinated biphenyl residues in foodstuffs and human tissues from china: status of contamination, historical trend, and human dietary exposure. Arch. Environ. Contam. Toxicol. 43, 473–480.

OSPAR Commission, 2002. Hazardous Substances Series: Dicofol.

Poon, B.H., Leung, C.K., Wong, C.K., Wong, M.H., 2005. Polychlorinated biphenyls and organochlorine pesticides in human adipose tissue and breast milk collected in Hong Kong. Arch. Environ. Contam. Toxicol. 49, 274–282.

Qiu, X., Zhu, T., 2010. Using the o,p'-DDT/ *p,p'*-DDT ratio to identify DDT sources in China. Chemosphere 34, 56–89. doi:10.1016/j.chemosphere.2010.08.049.

Qiu, X., Zhu, T., Yao, B., Hu, J., Hu, S., 2005. Contribution of dicofol to the current DDT pollution in China. Environ. Sci. Technol. 39, 4385–4390.

Settimi, L., Masina, A., Andrion, A., Axelson, O., 2003. Prostate cancer and exposure to pesticides in agricultural settings. Int. J. Cancer 104, 458–461.

Tao, S., Li, B.G., He, X.C., Liu, W.X., Shi, Z., 2007. Spatial and temporal variations and possible sources of dichlorodiphenyltrichloroethane (DDT) and its metabolites in rivers in Tianjin, China. Chemosphere 68, 10–16.

Turgut, C., Gokbulut, C., Cutright, T.J., 2009. Contents and sources of DDT impurities in dicofol formulations in Turkey. Environ. Sci. Pollut. Res. Int. 16, 214–217.

Wong, M.H., Leung, A.O., Chan, J.K., Choi, M.P., 2005. A review on the usage of POP pesticides in China, with emphasis on DDT loadings in human milk. Chemosphere 60, 740–752.

Yang, X., Wang, S., Bian, Y., Chen, F., Yu, G., Gu, C., Jiang, X., 2008. Dicofol application resulted in high DDTs residue in cotton fields from northern Jiangsu province, China. J. Hazard Mater. 150, 92–98.

Yu, H.F., Zhao, X.D., Zhao, J.H., Zhu, Z.Q., Zhao, Z., 2006. Continuous surveillance of organochlorine pesticides in human milk from 1983 to 1998 in Beijing, China. Int. J. Environ. Health. Res. 16, 21–26.

Zhao, G., Xu, Y., Li, W., Han, G., Ling, B., 2007. PCBs and OCPs in human milk and selected foods from Luqiao and Pingqiao in Zhejiang, China. Sci. Total. Environ. 378, 281–292.

Environment International 37 (2011) 1183–1189

Contents lists available at ScienceDirect

Environment International

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

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

Kouji H. Harada [a], Toshiaki Hitomi [a], Tamon Niisoe [a], Katsunobu Takanaka [b], Sigetosi Kamiyama [c], Takao Watanabe [d], Chan-seok Moon [e], Hye-ran Yang [a], Nguyen Ngoc Hung [f], Akio Koizumi [a,*]

[a] Department of Health and Environmental Sciences, Kyoto University Graduate School of Medicine, Yoshida, Kyoto 606-8501, Japan
[b] Department of Neurosurgery, Takayama Red Cross Hospital, Takayama 506-8550, Japan
[c] Morinomiyako Industrial Health Association, Miyagino, Sendai 983-0031, Japan
[d] Tohoku Bunkyo College, Katayachi, Yamagata 990-2316, Japan
[e] Department of Industrial Health, Catholic University of Pusan, Busan 609757, Republic of Korea
[f] Department of Science and Technology, Hanoi Medical University, Ton That Tung, Hanoi, Viet Nam

ARTICLE INFO

Article history:
Received 23 January 2011
Accepted 16 April 2011
Available online 19 May 2011

Keywords:
Perfluorocarboxylate
Perfluorooctanoic acid
Serum
Temporal trend
East Asia

ABSTRACT

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

1. Introduction

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

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

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

* Corresponding author at: Department of Health and Environmental Sciences, Kyoto University Graduate School of Medicine, Yoshida Konoe, Sakyo, Kyoto 606-8501, Japan. Tel.: +81 75 753 4456; fax: +81 75 753 4458.
E-mail address: koizumi.akio.5v@kyoto-u.ac.jp (A. Koizumi).

0160-4120/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.envint.2011.04.011

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

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

2. Material and methods

2.1. Experimental design and study population

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

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

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

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

2.2. Reagents

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

2.3. Determination of PFCAs in serum

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

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

Table 1
Study area and study population.

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

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

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

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

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

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

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

2.4. Quality assurance

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

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

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

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

2.5. Statistical analysis

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

3. Results

3.1. Temporal changes in PFCA concentrations in Japan

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

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

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

Table 3
Serum concentrations of PFCAs in Japan.

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

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

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

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

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

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

3.4. Correlations among PFCA levels and factor analysis

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

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

Table 4
Serum concentrations of PFCAs in Korea.

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

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

Table 5
Serum concentrations of PFCAs in Hanoi, Vietnam.

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

GM: geometric mean; GSD: geometric standard deviation.

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

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

4. Discussion

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

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

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

Table 6
Correlation between different chain length PFCAs.

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

ρ: Spearman's rank correlation coefficient.

Table 7
Factor analysis among PFCAs.

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

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

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

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

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

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

Acknowledgments

We are grateful to Dr. Anna Kärrman (Örebro University, Sweden) for valuable instructions for quality assurance. 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 authors have no financial conflicts of interest.

Appendix A. Supplemental data

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

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

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

References

Berenson ML, Levine DM, Goldstein M. Intermediate statistical methods and applications: a computer package approach. Englewood Cliffs, N.J.: Prentice-Hall; 1983

Ericson I, Gomez M, Nadal M, van Bavel B, Lindstrom G, Domingo JL. Perfluorinated chemicals in blood of residents in Catalonia (Spain) in relation to age and gender: a pilot study. Environ Int; 2007.

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.

Furdui VI, Helm PA, Crozier PW, Lucaciu C, Reiner EJ, Marvin CH, et al. Temporal trends of perfluoroalkyl compounds with isomer analysis in lake trout from Lake Ontario (1974–2004). Environ Sci Technol 2008;42:4739–44.

Guruge KS, Taniyasu S, Yamashita N, Wijeratna S, Mohotti KM, Seneviratne HR, et al. Perfluorinated organic compounds in human blood serum and seminal plasma: a study of urban and rural tea worker populations in Sri Lanka. J Environ Monit 2005;7:371–7.

Harada KH, Koizumi A. Environmental and biological monitoring of persistent fluorinated compounds in Japan and their toxicities. Environ Health Prev Med 2009;14:7-19.

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.

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.

Harada KH, Yang HR, Moon CS, Hung NN, Hitomi T, Inoue K, et al. Levels of perfluorooctane sulfonate and perfluorooctanoic acid in female serum samples from Japan in 2008, Korea in 1994–2008 and Vietnam in 2007–2008. Chemosphere 2010;79:314–9.

Haug LS, Thomsen C, Becher G. Time trends and the influence of age and gender on serum concentrations of perfluorinated compounds in archived human samples. Environ Sci Technol 2009;43:2131–6.

Houde M, Martin JW, Letcher RJ, Solomon KR, Muir DC. Biological monitoring of polyfluoroalkyl substances: a review. Environ Sci Technol 2006;40:3463–73.

Joensen UN, Bossi R, Leffers H, Jensen AA, Skakkebaek NE, Jorgensen N. Do perfluoroalkyl compounds impair human semen quality? Environ Health Perspect 2009;117:923–7.

Kärrman A, van Bavel B, Jarnberg U, Hardell L, Lindstrom G. Perfluorinated chemicals in relation to other persistent organic pollutants in human blood. Chemosphere 2006;64:1582–91.

Kärrman A, Harada KH, Inoue K, Takasuga T, Ohi E, Koizumi A. Relationship between dietary exposure and serum perfluorochemical (PFC) levels—a case study. Environ Int 2009;35:712–7.

Kato K, Calafat AM, Wong LY, Wanigatunga AM, Caudill SP, Needham LL. Polyfluoroalkyl compounds in pooled sera from children participating in the National Health and Nutrition Examination Survey 2001–2002. Environ Sci Technol 2009;43:2641–7.

Keller JM, Calafat AM, Kato K, Ellefson ME, Reagen WK, Strynar M, et al. Determination of perfluorinated alkyl acid concentrations in human serum and milk standard reference materials. Anal Bioanal Chem 2010;397:439–51.

Koizumi A, Yoshinaga T, Harada K, Inoue K, Morikawa A, Muroi J, et al. Assessment of human exposure to polychlorinated biphenyls and polybrominated diphenyl ethers in Japan using archived samples from the early 1980 s and mid-1990 s. Environ Res 2005;99:31–9.

Koizumi A, Harada K, Inoue K, Hitomi T, Yang H-R, Moon C-S, et al. Past, present, and future of environmental specimen banks. Environ Health Prev Med 2009;14: 307–18.

Kuklenyik Z, Reich JA, Tully JS, Needham LL, Calafat AM. Automated solid-phase extraction and measurement of perfluorinated organic acids and amides in human serum and milk. Environ Sci Technol 2004;38:3698–704.

Liao C, Wang T, Cui L, Zhou Q, Duan S, Jiang G. Changes in synaptic transmission, calcium current, and neurite growth by perfluorinated compounds are dependent on the chain length and functional group. Environ Sci Technol 2009;43:2099–104.

Lindstrom G, Karrman A, van Bavel B. Accuracy and precision in the determination of perfluorinated chemicals in human blood verified by interlaboratory comparisons. J Chromatogr A 2009;1216:394–400.

Martin JW, Mabury SA, Solomon KR, Muir DCG. Bioconcentration and tissue distribution of perfluorinated acids in rainbow trout (*Oncorhynchus mykiss*). Environ Toxicol Chem 2003;22:196–204.

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.

Niisoe T, Harada KH, Ishikawa H, Koizumi A. Long-term simulation of human exposure to atmospheric perfluorooctanoic acid (PFOA) and perfluorooctanoate (PFO) in the Osaka urban area, Japan. Environ Sci Technol 2010;44:7852–7.

Olsen GW, Mair DC, Church TR, Ellefson ME, Reagen WK, Boyd TM, et al. Decline in perfluorooctanesulfonate and other polyfluoroalkyl chemicals in American Red Cross adult blood donors, 2000–2006. Environ Sci Technol 2008;42:4989–95.

Pan Y, Shi Y, Wang J, Cai Y, Wu Y. Concentrations of perfluorinated compounds in human blood from twelve cities in China. Environ Toxicol Chem 2010;29:2695–701.

Powley CR, George SW, Ryan TW, Buck RC. Matrix effect-free analytical methods for determination of perfluorinated carboxylic acids in environmental matrixes. Anal Chem 2005;77:6353–8.

Prevedouros K, Cousins IT, Buck RC, Korzeniowski SH. Sources, fate and transport of perfluorocarboxylates. Environ Sci Technol 2006;40:32–44.

Renner R. Scotchgard scotched—following the fabric protector's slippery trail to a new class of pollutant. Sci Am 2001;284:18.

Roosens L, D'Hollander W, Bervoets L, Reynders H, Van Campenhout K, Cornelis C, et al. Brominated flame retardants and perfluorinated chemicals, two groups of persistent contaminants in Belgian human blood and milk. Environ Pollut 2010;158:2546–52.

Steenland K, Fletcher T, Savitz DA. Epidemiologic evidence on the health effects of perfluorooctanoic acid (PFOA). Environ Health Perspect 2010;118:1100–8.

Toms LM, Calafat AM, Kato K, Thompson J, Harden F, Hobson P, et al. Polyfluoroalkyl chemicals in pooled blood serum from infants, children, and adults in Australia. Environ Sci Technol 2009;43:4194–9.

Upham BL, Deocampo ND, Wurl B, Trosko JE. Inhibition of gap junctional intercellular communication by perfluorinated fatty acids is dependent on the chain length of the fluorinated tail. Int J Cancer 1998;78:491–5.

ENVIRONMENTAL Science & Technology

ARTICLE

pubs.acs.org/est

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

Kouji H. Harada,[†] Takumi Takasuga,[‡] Toshiaki Hitomi,[†] Peiyu Wang,[§] Hidenori Matsukami,[‡] and Akio Koizumi*[,†]

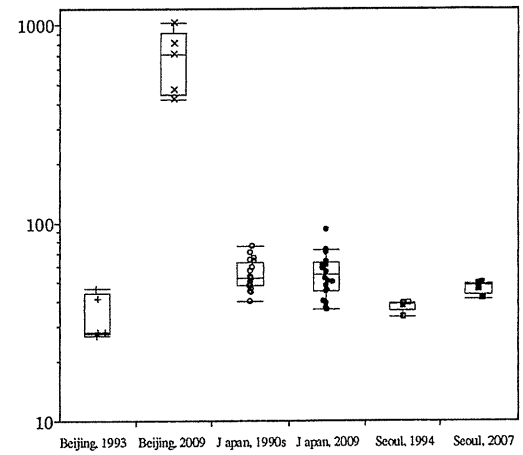
[†]Department of Health and Environmental Sciences, Kyoto University Graduate School of Medicine, Yoshida, Kyoto 606-8501, Japan

[‡]Shimadzu Techno-Research Incorporated, Nishinokyo, Kyoto 604-8435, Japan

[§]Department of Social Medicine and Health Education, School of Public Health, Peking University, Haidian, Beijing 100083, PR China

Supporting Information

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



INTRODUCTION

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

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

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

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

Received: February 19, 2011
Accepted: July 11, 2011
Revised: June 30, 2011
Published: July 11, 2011

■ MATERIALS AND METHODS

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

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

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

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

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

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

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

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

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

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

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

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

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

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

RESULTS

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

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

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

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

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

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

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