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Mycotoxin Detection in Urine Samples from Patients with Chronic Kidney Disease of Uncertain Etiology in Sri Lanka

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Abstract This was a screening study that aimed to determine the presence of nephrotoxic mycotoxins in urine samples from patients with chronic kidney disease of uncertain etiology in the North Central Province of Sri Lanka. The percentage detection of aflatoxins, ochratoxins and fumonisins in 31 patients were 61.29%, 93.5% and 19.4%, respectively. Geometric means of urinary aflatoxins and ochratoxins were 30.93 creatinine and 34.62 ng/g creatinine in chronic kidney disease of uncertain etiology stage 1–2 patients and 84.12 ng/g creatinine and 63.52 ng/g creatinine in unaffected relatives of patients. In chronic kidney disease of uncertain etiology stage 3–5 patients, geometric means of urinary aflatoxins and ochratoxins were 10.40 and 17.08 ng/g creatinine, respectively. Non-affected relatives of patients (n = 6) had comparable levels of these mycotoxins, but healthy Japanese individuals (n = 4) had lower levels than in Sri Lanka. The higher detection rate of urinary ochratoxins in Sri Lankans indicates that exposure is common in the region.

Keywords Chronic kidney disease of uncertain etiology · Sri Lanka · Urine sample · Aflatoxin · Ochratoxin · Fumonisin

High prevalence of chronic kidney disease of uncertain etiology (CKDue) in the North Central Province of Sri Lanka has been reported. The disease predominantly affects male farming communities. Several hypotheses have been made to explain the causal associations between the high prevalence of CKDue in the region and existing environmental factors (Chandrajith et al. 2010; Illeperuma et al. 2009).

Mycotoxins, such as aflatoxins (AFLs) (Glahn et al. 1994), ochratoxins (OTs) (Sauvant et al. 2005) and fumonisins (FBs) (Badria et al. 1996) are dietary contaminants that are known to possess nephrotoxicity. Animal studies demonstrated ochratoxin (Baudrimont et al. 2001; Berndt et al. 1980) and AFB_1 (Grosman et al. 1983) induced proteinurea and decrease of glomerular filtration rate and tubular reabsorption of glucose. Detection of OT associated with the incidence of endemic nephropathy in other regions has been reported (Castegnaro et al. 2006; Domijan et al. 2009). A recent study by Wanigasuriya et al. (2008) has reported that the concentration of OT A in selected food items in the study region was low. Food analysis, in some instances, might not be sufficient to establish a relationship with occurrence of diseases due to heterogeneity of toxin distribution over time, and even within a particular food product, casts doubt on the feasibility of sampling plans (Parsons et al. 2007. For instance, in some studies (Castegnaro et al. 2006), the relationship of

This study is conducted for the Chronic Kidney Disease of Uncertain Etiology Consortium.
Please refer the Appendix section for the full list of members.

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an increase in OTA intake was not found in agreement with the immediate increase of its elimination in urine. In an attempt to overcome this problem and to validate the actual exposure, we screened urinary excretion levels of AFL, OTs and FBs in patients and their relatives living in a CKD endemic community.

Materials and Methods

Ethical approval for this study was obtained from the Ethical Committee of Kyoto University, Japan and the Ethical Review Committees of the Faculty of Medicine, University of Peradeniya, Sri Lanka. The urine samples were originally collected at Medawachchiya and Girandrukotte, Sri Lanka in August 2009 (106 patients and 87 unaffected relatives of CKDue patients) and stored at –30°C in the Kyoto University Human Specimen Bank (Koizumi et al. 2009). A total of 41 urine samples, 31 from stage 1–5 CKDue patients, six from unaffected relatives, and four from healthy Japanese individuals as controls, were randomly selected from each stratum. Definition of CKD and further classification of the stages were made according to the Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines. Patients with a history and current treatment of diabetes mellitus, severe hypertension, urological disease of known etiology, glomerulonephritis, or snake bite were excluded. Creatinine concentration in urine sample was measured by enzyme assay using creatinine amidohydrolase (SRL, Tokyo, Japan).

Urine samples were thawed and centrifuged at 15,000 rpm for 10 min to remove any cellular debris, and the supernatant was used for the determination of mycotoxin level. One milliliter of urine was diluted with 3 mL PBS (pH 7.4). The mixed sample was directly passed through analyte-specific immunoaffinity columns (R-Biopharm AG, Darmstadt, Germany) at a flow rate of 1–2 drops/s. The column was washed with 20 mL PBS and air was passed through the column for 1 min. The bound mycotoxin was eluted with 3 mL methanol and the eluate was evaporated to dryness using a nitrogen evaporator. The residue was reconstituted with 100 μL 10% methanol in water, and analyzed for each mycotoxin with the specific competitive ELISA kits (RIDASCREEN FAST Mycotoxins; R-Biopharm AG) using a microplate spectrophotometer (infinite M200 Pro; Tecan, Tokyo, Japan) at 450 nm. ELISA kits for AFL, OTs and FBs recognized aflatoxins B1, B2, G1, G2 and M1; ochratoxins A, B and C, and fumonisins B1, B2 and B3, respectively. External standards of different concentrations and all urine samples were run in duplicate.

Mycotoxin concentration was computed based on the absorbance of five standard solutions provided by the immunoassay kit for each mycotoxin. Mean recoveries of AFLs, OTs and FBs from the fortified samples were 79%, 105% and 92%, and the corresponding coefficients of variation were 11, 13 and 15, respectively. Detection limits for AFLs, OTs and FBs in the urine samples were 0.005, 0.005 and 0.035 ng/mL, respectively. For values below the detection limit, half of the limit of detection value was assigned. Mycotoxin concentrations are presented in ng/mL and ng/g Cr (creatinine). Statistical significance of differences between groups was tested by using non-parametric methods (χ^2 test and Wilcoxon two-sample test; $p < 0.05$).

Results and Discussion

Study subjects comprised 20 men and 21 women (Table 1). The mean (range) age regardless of disease stage (31, stage 1–5) was 41.32 ± 15.55 (9–65) years, whereas that of unaffected relatives and Japanese controls was 20.67 (6–34) years and 45.25 (42–53) years, respectively.

Results of urinary AFL, OT and FB levels are shown in Table 2. The percentage detection of AFLs, OTs and FBs in patients was 61.29%, 93.5% and 19.4%, respectively. The detection rate of all mycotoxins in stage 1 disease was the highest. Disease stages were classified as early (stage 1 and 2) and late (stage 3–5) for examination of concentration differences during disease progression. Detection rates of AFLs in the early and late stages were 78.57% and 47.06%, respectively ($\chi^2 = 9.323$; $p < 0.001$). OTs were detected in all of the urine samples from 14 patients with early stage disease, whereas the rate of detection at the late stage was 88.24% (n = 17) ($\chi^2 = 23.516$, $p < 0.001$). Both AFLs and OTs were detected in all of the relatives of CKDue patients, but only OTs were detected in the Japanese controls.

The highest AFL concentration in urine samples from CKDue patients was 0.8 ng/mL, whereas 90% of the samples had a concentration <0.044 ng/mL (397.1 ng/g Cr). The 90th percentile for OTs was 0.098 ng/mL (60.85 ng/g Cr). The geometric means of urinary AFLs and OTs were 0.033 ng/mL (30.93 ng/g Cr) and 0.037 ng/mL (34.62 ng/g Cr) in the early stage, and 0.008 ng/mL (10.40 ng/g Cr) and 0.012 ng/mL (17.08 ng/g Cr) in the late stage of the disease. Mean concentration difference for urinary OT level was observed between the early and late stages of the disease (Wilcoxon test, $p = 0.008$). In contrast, comparable concentrations of OTs and AFLs were also observed in the unaffected relatives of CKDue patients ($p > 0.05$ compared with all patients). The concentration

Table 1 Baseline characteristics of CKDue patients in Sri Lanka, 2009

Disease stages	Sex Male/female (total)	Age (year) Mean (range)
Stage 1 (slight)	3/4 (7)	24.14 (9–40)
Stage 2 (mild)	6/1 (7)	48.00 (39–59)
Stage 1–2 (early stage)	9/5 (14)	36.07 ± 15.19†
Stage 3 (moderate)	3/3 (6)	41.00 (11–60)
Stage 4 (severe)	3/3 (6)	47.50 (35–58)
Stage 5 (end stage)	3/2 (5)	49.00 (30–65)
Stage 3–5 (late stage)	9/8 (17)	45.65 ± 14.90†
Total (CKDue patients)	18/13 (31)	41.32 ± 15.55
Relatives of CKDue patients	2/4 (6)	20.67 (6–34)†
Japanese controls	0/4 (4)	45.25 (42–53)

† Mean ± SD

Table 2 Urine concentration of AFL, OT and FB in CKDue patients in Sri Lanka, 2009

Subjects		AFL		OT		FB	
		ng/mL	ng/g Cr	ng/mL	ng/g Cr	ng/mL	μg/g Cr
Stage 1 (n = 7)	Range (n > MDL)	ND–0.800(6)	ND–734.00	0.013–0.360 (7)	17.63–93.90	ND–0.042 (4)	ND–0.14
	Mean	0.359	230.21	0.044	39.67	<MDL	<MDL
	GM	0.092	87.41	0.035	33.33	<MDL	<MDL
Stage 2 (n = 7)	Range (n > MDL)	ND–0.037 (5)	ND–53.05	0.006–0.058 (6)	11.87–74.81	ND–0.036 (1)	ND–0.07
	Mean	0.018	19.58	0.085	65.07	–	–
	GM	0.012	10.95	0.039	35.95	–	–
Stage 1–2	GM	0.033	30.93	0.037	34.62*	–	–
Stage 3 (n = 6)	Range (n > MDL)	ND–0.039 (4)	ND–44.74	ND–0.028 (5)	8.57–41.25	ND–0.130 (1)	ND–0.19
	Mean	0.023	25.57	0.022	21.76	–	–
	GM	0.022	18.75	0.016	19.36	–	–
Stage 4 (n = 6)	Range (n > MDL)	ND–0.800 (4)	ND–991.57	ND–0.019 (4)	ND–34.27	–	–
	Mean	0.140	174.82	0.016	18.75	ND	ND
	GM	0.009	12.71	0.012	17.07	–	–
Stage 5 (n = 5)	Range (n > MDL)	ND	ND	0.010 (4)	ND–27.06	ND	ND
	Mean	–	–	0.044	16.56	–	–
	GM	–	–	0.080	14.72	–	–
Stage 3–5	GM	0.008	10.40	0.012	17.08*	–	–
Stage 1–5	GM	0.012	17.01	0.020	23.50	–	–
Relatives controls (n = 6)	Range (n > MDL)	0.020–0.800 (6)	5.9–1000.00	0.032–0.223 (6)	28.63–278.00	ND–0.093 (1)	ND–0.14
	Mean	0.298	249.09	0.104	88.95	–	–
	GM	0.112	84.12	0.085	63.52	–	–
Japanese controls (n = 4)	Range (n > MDL)	ND	ND	0.005–0.012 (4)	4.4–19.40	ND	ND
	Mean	–	–	0.007	9.69	–	–
	GM	–	–	0.007	8.14	–	–

ND not detected, *MDL* method detection limit, *GM* geometric mean

* Wilcoxon test for mean OT concentration difference between early and late stages ($p = 0.008$)

Table 3 Urine mycotoxin level in other countries

Mycotoxin type	Detection rate	Mean (range)	Study subjects	Country	References
AFL	61.29%	17.0 (ND–991.6) ng/gCr	CKDue patients	Sri Lanka	Present study
	58%	391.0 (19.0–19,219.0) pg/g Cr	General population	Czech Republic	(Malir et al. 2004)
OT A	100%	37.1 (12.4–360.0) pg/mL	CKDue patients (early stage)	Sri Lanka	Present study
	88.24%	12.0 (ND–58.2) pg/mL	CKDue patients (late stage)	Sri Lanka	Present study
	100%(n = 6)	85.0 (32.0–223.0) pg/mL	Relatives of CKDue patients	Sri Lanka	Present study
	61%	13.0 (6.0–65.0) pg/mL	Healthy individuals	Hungary	(Fazekas et al. 2005)
	43%	7.0 (5.0–15.0) pg/mL	Endemic nephropathy	Croatia	(Domijan et al. 2009)
	92.20%	22.0 (ND–69.0) pg/mL	General population	Portugal	(Duarte et al. 2010)
	88%	50.8 (1.0–330.0) pg/mL	Endemic nephropathy	Bulgaria	(Castegnaro et al. 2006)
	97.6%	191.7 (1.0–191.0) pg/mL	Endemic nephropathy	Bulgaria	(Castegnaro et al. 2006)
FB	19.4%	(ND–130.0 pg/mL)	CKDue patients	Sri Lanka	Present study
	0% (LOD = 5 ng/mL)		General population	Portugal	(Silva et al. 2010)
	75%	70.1 (ND–9312.0) pg/mL	General population	Mexico	(Gong et al. 2004)

of OTs in the healthy Japanese individuals was 0.007 ng/mL (8.14 ng/g Cr) which was lower than in the Sri Lankan individuals.

The small sample size of the control subjects and their characteristic differences with the patients limit the comparability of the results. However, the high detection frequency and urinary levels of OTs and AFLs among CKDue patients and their relatives demonstrated the potential human exposure in the region. Findings were also discussed in relation to similar studies in other countries (Table 3). The average AFL concentration in urine samples from CKDue patients was markedly higher, by over an order of magnitude, than the level of 0.391 ng/g Cr in the Czech Republic (Malir et al. 2004). An FB exposure study in two Portuguese populations has shown no detectable level in urine samples (Silva et al. 2010) and in Mexico 75% detection frequency was observed (Gong et al. 2004), whereas minute level of FBs was detected at the early stage of the disease in the present study.

Higher detection of OTs was observed compared with the 61% detection rate among healthy individuals in Hungary and 43% in the endemic nephropathy area in Croatia (Domijan et al. 2009), whereas the detection was comparable with the 88–97.8% in the endemic nephropathy region of Bulgaria (Castegnaro et al. 2006). Although the mean OT level in CKDue patients in our study was higher than the 0.007 ng/mL in Croatia (Domijan et al. 2009) and 0.013 ng/mL in Hungary (Fazekas et al. 2005), and was comparable to the 0.022 ng/mL in Portugal (Duarte et al. 2010), the urine concentration levels in half of our CKDue patients were <0.017 ng/mL (n = 15). Therefore, the potential sources of exposure to OTs in the region need to be clarified.

Animal studies have demonstrated the possibility of higher concentrations of OT A in kidney tissues and low levels in the urine (Zepnik et al. 2003). Likewise, an increase in OT A intake in humans in the region of endemic nephropathy did not result in an immediate increase in its elimination (Castegnaro et al. 2006). OT A is characterized by high plasma protein binding potential, therefore, its removal efficiency might be low (Petzinger and Weidenbach 2002; Ringot et al. 2006), and it is possible that OT A may accumulate in renal tissue. It is worth noting that the cumulative effect of long-term consumption of products that contain low levels of mycotoxins could contribute to a gradual deterioration of organ function.

This study is believed to be the first to determine the presence of AFLs, OTs and FBs in urine samples from CKDue patients and their relatives living in communities with CKDue. The higher detection rate of OTs in Sri Lanka has led to a working hypothesis that these mycotoxins could be common in the region, which corroborates the need for further exposure assessment, associated with disease occurrence.

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Appendix

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Historical trends in human dietary intakes of endosulfan and toxaphene in China, Korea and Japan

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ABSTRACT

Recently, the Stockholm Convention prohibited the use of toxaphene and has been reviewing endosulfan. The historical use of these pesticides may contaminate food and tend to accumulate in the food chain. In this study, to evaluate the spatial and temporal trends of food contamination, the endosulfan and toxaphene levels were measured 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. Endosulfan was detected in 32 of 40 samples from Japan, but its levels (sum of α- and β-isomers) were low in both the 1990s and 2009 (range as geometric mean (geometric standard deviation) [GM (GSD)]: 0.96 (1.6)–1.42 (1.4) ng kg^{-1} d^{-1}). The dietary intakes of endosulfan in Seoul as GM (GSD) were 38.68 (1.3) ng kg bw^{-1} d^{-1} in 1994 and 92.17 (4.4) ng kg bw^{-1} d^{-1} in 2007, and significantly higher than those in Japan ($p < 0.05$). The samples from Beijing showed a 50-fold increase in the endosulfan levels from 1993 (GM: 0.58 ng kg^{-1} d^{-1}) to 2009 (GM: 24.91 ng kg bw^{-1} d^{-1}) ($p < 0.05$). Toxaphene was detected in 33 of 40 samples from Japan. The dietary intake of toxaphene in Japan (sum of Parlars #26, #50 and #62) was 0.32–1.21 ng kg bw^{-1} d^{-1} (range as geometric mean) and no temporal trend was observed. The dietary intake of toxaphene in Seoul increased significantly from 0.2 ng kg bw^{-1} d^{-1} (GM) in 1994 to 3.6 ng kg bw^{-1} d^{-1} (GM) in 2007 ($p < 0.05$). Only one of 10 pooled samples from Beijing contained a detectable level of toxaphene (0.3 ng kg bw^{-1} d^{-1}). For the entire population, the risk of adverse health effects from dietary intakes of endosulfan and toxaphene is unlikely. However, the concentrations of endosulfan in several samples exceeded 10% of the acceptable daily intake limit value of 6 μg kg bw^{-1} d^{-1} set by the World Health Organization (WHO). It appears important to refine dietary intake estimates targeting food types and source identification to ensure safe food for consumers.

1. Introduction

Non-occupational human exposure to organochlorine pesticides is mainly attributed to past and present consumption of foods contaminated by these chemicals (Dougherty et al., 2000; Jiang et al., 2005; WHO, 2005; Boobis et al., 2008). In real time, chronic exposure to these chemical contaminants and their potential subtle health effects as a consequence may not be noticed. Endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methylene-2,3,4-benzodioxathiepin-3-oxide) and toxaphene (polychlorinated 2,2-dimethyl-3-methylenebicyclo[2,2,1]heptane) are notable examples of organochlorine pesticides classified by the WHO and the United States Environmental Protection Agency (USEPA) as priority pollutants (Keith and Telliard, 1979). These chemicals occur in many environmental compartments and accumulate through the food chain owing to their persistence and semi-volatility (Saleh and Casida, 1978; ATSDR, 1990, 1991; Burgoyne and Hites, 1993; Weber et al., 2009).

The widespread use of endosulfan as an insecticide around the world (approximately 409 000 metric tons from 1946 to 1974), with technical formulations of α and β-isomers, has been reported (Matolcsy et al., 1988; Weber et al., 2009). Agricultural use of endosulfan in Japan started in 1960 and the total shipping volume of endosulfan from 2003 to 2007 was about 80 tons (POPRC, 2009). An inventory report by Jia et al. (2009) documented that 25 700 tons of endosulfan was used in China from 1994 to 2004 (Jia et al., 2009). Likewise, widespread environmental contamination by this chemical has been reported in China (Li et al., 2007), South Korea (Yeo et al., 2003) and urban Seoul (Yeo et al., 2004).

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Guo et al. (2007) reported the exposure levels to this chemical via seafood products in China (Guo et al., 2007).

Toxaphene is a complex mixture of polychlorinated monoterpenes and was the most heavily used insecticide to replace DDT (Saleh and Casida, 1978). More than 1.3 million tons of toxaphene have been applied throughout the world (Oehme et al., 1996). A report on toxaphene practice indicated that this pesticide was registered in Korea (Wong et al., 2005) and China (de Geus et al., 1999). Although toxaphene has never been registered as a pesticide in Japan (Imanishi et al., 2005; Takazawa et al., 2007). Takazawa et al. (2007) hypothesized about the potential spread of this chemical in Far Eastern Countries including Japan. While the possibility of high-level contamination of toxaphene is low, since it is no longer used, there is a concern regarding health hazards attributable to long-term exposure to low levels of this chemical (ATSDR, 1996; Tryphonas et al., 2000).

Although countries differ in their histories of pesticide production, application and regulation, more importantly, changes in the consumption patterns and the growing international trades in food track these chemicals that could challenge global food safety. Owing to these possible changes in exposure to these chemicals over time, the temporal and regional variations in dietary residue levels need to be investigated. To the best of our knowledge, there are no documented studies assessing the historical trends of these chemicals in China, Korea and Japan. The present study estimated the trends of the dietary intakes of endosulfan and toxaphene in adult populations from these selected countries across specified time periods.

2. Materials and methods

2.1. Food sampling and preparation

The Ethics Committee of Kyoto University approved this study and appropriate written informed consent was obtained from all the research participants. Food samples from the Kyoto University Human Specimen Bank (Koizumi et al., 2005, 2009) were used for the analyses.

A total of 200 duplicate 24-h food samples were collected from Hokkaido in 1992 and 1995, Okinawa in 1992 and 1995, Kyoto in 1996 and 1997, Beijing in 1993 and 2009 and Seoul in 1994 and 2007 (Ikeda et al., 2000; Koizumi et al., 2009). Women participants were selected purposively to examine implication for future generation. In addition, for the 2009 samples in Japan, for practical reason, a 100-d meal and water supply was purchased by volunteers from markets in Kyoto, Okinawa and Hokkaido owing to the difficulty of finding individuals who often do not consume food away from home. From the total of 300 homogenized composite food samples, randomly selected five samples (30 g of each) were then pooled into 60 samples, as illustrated in Fig. 1. Therefore, the food samples from five subjects were treated as one pooled sample weighing 150 g. The samples were stored in a glass bottles at −30 °C until analysis.

2.2. Chemicals

The analytes investigated were α-endosulfan, β-endosulfan and toxaphene Parlars #26, #50 and #62 (Supelco Inc., Bellefonte, PA). $^{13}C_9$-α-endosulfan and $^{13}C_9$-β-endosulfan (Cambridge Isotope Laboratories, Andover, MA) were used as internal standards for α-endosulfan and β-endosulfan, respectively. $^{13}C_{10}$-trans-chlordane (Cambridge Isotope Laboratories) was used as the internal standard for toxaphene. $^{13}C_{12}$-2,2′,3,3′,5,5′,6-heptachlorobiphenyl and $^{13}C_{12}$-2,3,3′,5,5′-pentachlorobiphenyl (Cambridge Isotope laboratories) were used to monitor the recovery of the internal standards for endosulfan and toxaphene, respectively. Acetone, hexane, dichloromethane and sodium sulfate were purchased from Kanto Chemical Co. Inc. (Reagents and solvents for residual pesticides test and PCB test).

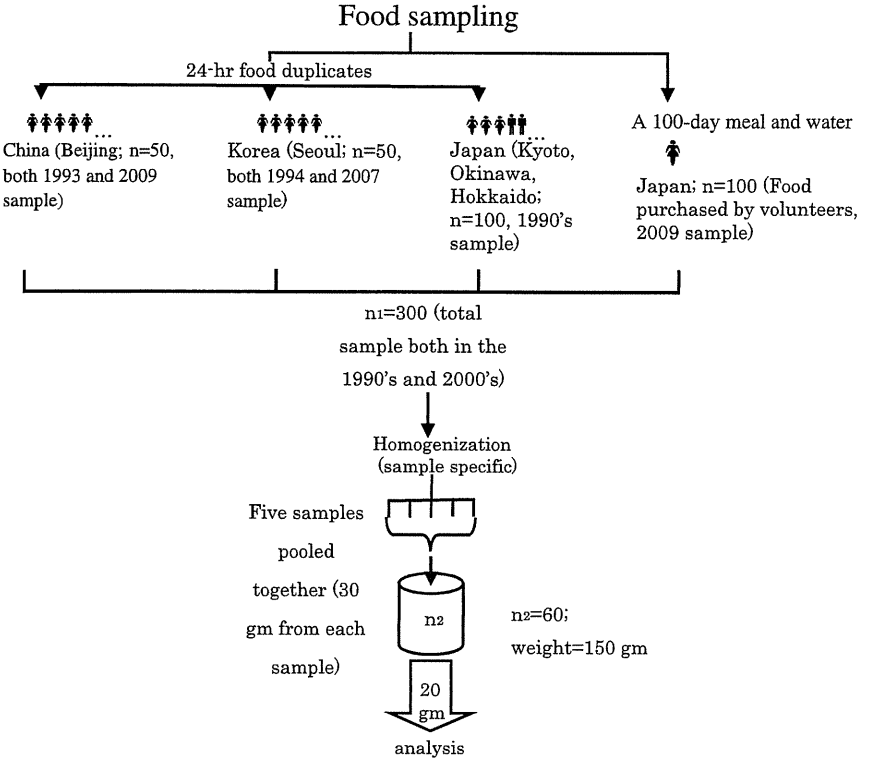

Fig. 1. Schematic presentation of the food sampling process.

2.3. Extraction

Composite samples (20 g) were extracted with 100 mL of 1:1 acetone/hexane (50% v/v). The extracts were filtered and the residues were extracted again with 100 mL of 1:1 acetone/hexane. The extracts were combined and washed with 500 mL of hexane-washed distilled water. The water layer was extracted twice with 50 mL of hexane. The organic layers were combined and washed with 100 mL of hexane-washed distilled water. The organic fraction was dried with anhydrous sodium sulfate and concentrated to ca. 20 mL on a rotary evaporator.

A 2 mL aliquot of crude extract was spiked with internal standards 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 dichloromethane/hexane. Toxaphene was eluted with 90 mL of 1:4 dichloromethane/hexane and spiked with $^{13}C_{12}$-2,3,3′,5,5′-pentachlorobiphenyl. The eluate was concentrated to 0.1 mL of decane prior to gas chromatography–mass spectrometry (GC–MS) analysis. α + β-Endosulfan was then eluted with 150 mL of dichloromethane and concentrated in the same manner as toxaphene.

2.4. Instruments and quantification

A high resolution gas chromatography/high resolution mass spectrometry in electron capture negative ionization mode (HRGC/ECNI-HRMS) system was used, which comprised a Hewlett–Packard 6890 Series Gas Chromatograph connected to a Thermoelectron Finningan MAT-95XL (Thermo Electron Corporation, Yokohama, Japan). The separation conditions and target ions are shown in Table 1.

2.5. Quality control

The calibration curves used for quantification consisted of five points covering 0.1–20 ng mL^{-1} in standard solution and were plotted using a linear fit ($r > 0.999$). The method detection limits (MDLs) were 3.0, 1.9, 3.3, 3.6 and 28 pg g^{-1} wet weight for α-endo-

Table 1
GC-MS conditions and selected ions (*m/z*) for the determination of endosulfan and toxaphene.

Target analytes	Endosulfan	Toxaphene
Capillary column	DB-17MS (J&W) 30 m × 0.25 mm (id) 0.25 μm	DB-5MS (J&W) 60 m × 0.32 mm (id) 0.25 μm
Oven temperature	120 °C (1 min)–20 °C min^{-1}–200 °C–10 °C min^{-1}–300 °C (10 min)	120 °C (1 min)–20 °C min^{-1}–200 °C–3 °C min^{-1}–275 °C–20 °C min^{-1}–300 °C min^{-1} (10 min)
Helium gas flow	1.0 mL min^{-1}	1.0 mL min^{-1}
Injection method	On column injection	On column injection
Inlet temperature	120 °C–100 °C min^{-1}–300 °C (10 min)	120 °C–100 °C min^{-1}–300 °C (15 min)
Injection volume	2 μL	2 μL
Ionization	Negative chemical ionization	Negative chemical ionization
Reaction gas	Methane	Methane
Ionizing voltage	70 eV	90 eV
Interface temperature	270 °C	275 °C
Ion source temperature	130 °C	130 °C
	Monitor ions	
	Quantification	Confirmation
Endosulfan	405.8140	403.8169
$^{13}C_9$-endosulfan	414.8436	412.8465
$^{13}C_{12}$-#111-PeCB	337.9207	339.9178
Toxaphene Parlar #26	376.8573	378.8544
Toxaphene Parlar #50	412.8154	414.8124
Toxaphene Parlar #62	376.8387	374.8416
$^{13}C_{10}$-trans-chlordane	419.8254	417.8283
$^{13}C_{12}$-#138-HpCB	407.8398	405.8428

Table 2
Demographic characteristics and average food intakes of the study participants in the 1990s and 2007–2009.

Area	Year	*n*	Sex	Age	HSD test[a]	Height	Weight	BMI	Food intake		HSD test[a]
			male/female	(yr)		(cm)	(kg)		(g d^{-1})	(g kg bw^{-1} d^{-1})	
Beijing	1993	25	0/25	35.5 ± 2.3	A	158.7 ± 2.7	55.0 ± 3.5	21.8 ± 0.9	2249 ± 408	41.0 ± 2.6	AB
	2009	25	0/25	26.5 ± 0.9	B	163.8 ± 2.3	69.8 ± 3.5	26.0 ± 1.9	3054 ± 365	43.8 ± 5.6	A
Seoul	1994	25	0/25	37.8 ± 5.7	A	161.7 ± 0.6	56.3 ± 3.9	21.5 ± 1.6	1777 ± 457	31.7 ± 2.0	C
	2007	25	0/25	35.8 ± 4.0	A	158.5 ± 3.1	53.4 ± 1.7	21.3 ± 0.8	2062 ± 152	38.7 ± 3.1	ABC
Hokkaido	1992, 1995	35	0/35	51.7 ± 4.9	C	150.9 ± 1.6	54.5 ± 2.2	24.0 ± 1.2	2249 ± 274	41.3 ± 5.7	AB
	2009	35[b]	N.A.	N.A.	-	N.A.	N.A.	N.A.	1901 ± 161	37.3 ± 3.2	ABC
Kyoto	1996, 1997	30	0/30	21.5 ± 0.4	B	158.4 ± 1.3	50.7 ± 4.2	20.2 ± 1.9	1740 ± 335	34.4 ± 6.4	BC
	2009	30[b]	N.A.	N.A.	-	N.A.	N.A.	N.A.	1575 ± 73	30.9 ± 1.4	C
Okinawa	1992, 1995	35	15/20	49.4 ± 4.4	C	155.1 ± 6.8	61.8 ± 4.7	25.7 ± 1.8	2614 ± 433	42.4 ± 6.5	AB
	2009	35[b]	N.A.	N.A.	-	N.A.	N.A.	N.A.	1845 ± 137	36.3 ± 2.7	ABC

BMI: body mass index; N.A.: not applicable because of differences in the sampling methods.
Data are presented as the mean ± standard deviation.
[a] Means 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.
[b] Food samples were collected by five volunteers.

sulfan, β-endosulfan and toxaphene Parlars #26, #50 and #62, respectively.

Procedural blanks were processed in parallel to every batch of seven samples to check for interference or contamination by solvents and glassware. There were no detectable residues in any of the procedural blanks ($n = 9$).

The recovery rates (mean ± standard deviation) of $^{13}C_9$-α-endosulfan, $^{13}C_9$-β-endosulfan and $^{13}C_{10}$-trans-chlordane were 114.2 ± 14.3%, 108.3 ± 38.4% and 105.0 ± 5.7%, respectively.

2.6. Exposure estimations

A duplicate portion technique was employed to collect food samples. To calculate the actual dietary intakes of endosulfan and toxaphene, the following equation was used:

$$Qx = \sum Cx_i * D \quad (1)$$

where Qx is the total dietary intake of chemical × per d (ng kg bw^{-1} d^{-1}), Cx is the residue level of chemical × (endosulfan or toxaphene) in the food composites (pg g^{-1}), i is the congener or isomer and D is the daily food consumption level (g kg bw^{-1} d^{-1}).

For the 2009 food samples from Japan, the average weight of a Japanese female was used for the exposure estimation. The estimates were based on the 95th percentile of the daily exposure. The estimated intake values were then compared with the acceptable daily intake/tolerable daily intake (ADI/TDI) value of that chemical. We used an ADI value of 6 μg kg bw^{-1} d^{-1} for endosulfan set by the FAO/WHO (Lu, 1995) and an ADI value of 0.2 μg kg bw^{-1} d^{-1} for toxaphene set by the USEPA.

2.7. Data analysis

Data values below the MDL were assumed to have concentrations equal to one-half of the MDL for calculating summary statistics and performing statistical comparisons, except for toxaphene Parlar #62. For values where the proportion of non-detects was small, particularly for endosulfan (10/60 non-detects), the incorporation of these values was assumed to have limited influence on the purpose of our study. However, the non-detect value for Parlar #62 was critical (59/60 non-detects) and we equated it to a zero value, considering the high detection sensitivity of our method and the consistent non-detect values across all groups. All statisti-

Table 3
Levels of endosulfan isomers in the composite food samples and dietary intakes.

	Year (No. of pooled diets)		α-Isomer (pg g fw^{-1})	β-Isomer	Total (ng d^{-1})	Total (ng kg bw^{-1} d^{-1})	HSD test[a]	Intake/TDI (‰)
Beijing	1993 ($n = 5$)	Range ($n >$ MDL)	<3.0–24.2 (3)	<1.9–34.1 (3)	5.5–131.1	0.1–2.3		0.02–0.4
		Mean ± SD	12.5 ± 10.4	16.1 ± 14.8	64.1 ± 56.3	1.20 ± 1.0		0.2 ± 0.2
		GM (GSD)	7.0 (4.1)	6.8 (6.1)	31.6 (5.0)	0.6 (5.2)	A	0.1 (5.2)
		P95 estimate	71.0	133.0	438.0	8.60		1.4
	2009 ($n = 5$)	Range ($n >$ MDL)	180.5–269.3 (5)	288.4–475.3 (5)	1459.5–2242.0	19.2–33.0		3.2–5.5
		Mean ± SD	218.4 ± 41.2	365.5 ± 92.5	1755.6 ± 296.3	25.3 ± 5.0		4.2 ± 0.8
		GM (GSD)	215.4 (1.2)	356.5 (1.3)	1737.0 (1.2)	25.0 (1.2)	B	4.2 (1.2)
		P95 estimate	292.0	534.4	2259.0	34.00		5.7
Seoul	1994 ($n = 5$)	Range ($n >$ MDL)	372.2–544.2 (5)	427.5–1149.1 (5)	1421.0–2999.0	25.6–54.5		4.3–9.1
		Mean ± SD	461.6 ± 76.8	811.4 ± 316.8	2262.3 ± 696.4	40.0 ± 11.2		6.7 ± 1.9
		GM (GSD)	456.5 (1.2)	759.3 (1.5)	2174.4 (1.4)	39.0 (1.3)	B	6.4 (1.3)
		P95 estimate	600.0	1504.0	3664.0	62.00		10.4
	2007 ($n = 5$)	Range ($n >$ MDL)	170.0–7980.0 (5)	170.7–9304.2 (5)	770.0–36 736.5	13.9–714.7		2.3–119.1
		Mean ± SD	2406.0 ± 3198.0	2817.2 ± 3745.0	10 775.0 ± 14 828.7	206.5 ± 289.5		34.4 ± 48.2
		GM (GSD)	1124.0 (4.3)	1262.0 (4.6)	4916.0 (4.3)	92.0 (4.4)	B	15.4 (4.4)
		P95 estimate	12 419.0	15 492.0	54 286.0	1050.00		175.0
Hokkaido	1992, 1995 ($n = 7$)	Range ($n >$ MDL)	<3.0–77.2 (6)	<1.9–156.3 (6)	5.4–446.1	0.1–7.7		0.01–1.3
		Mean ± SD	23.8 ± 25.1	36.4 ± 53.7	128.0 ± 148.5	2.3 ± 2.6		0.4 ± 0.4
		GM (GSD)	14.6 (3.3)	16.5 (4.5)	70.3 (3.8)	1.3 (3.8)	A	0.2 (3.8)
		P95 estimate	104.0	197.0	641.0	11.40		2.0
	2009 ($n = 7$)	Range ($n >$ MDL)	12.1–31.2 (7)	12.5–29.1 (7)	47.9–98.1	0.9–1.9		0.2–0.3
		Mean ± SD	19.0 ± 6.4	20.2 ± 6.1	75.4 ± 22.4	1.5 ± 0.4		0.2 ± 0.1
		GM (GSD)	18.2 (1.4)	19.5 (1.4)	72.3 (1.4)	1.42 (1.4)	A	0.2 (1.4)
		P95 estimate	30.0	32.0	121.5	2.40		0.4
Kyoto	1996, 1997 ($n = 6$)	Range ($n >$ MDL)	<3.0–44.0 (4)	9.7–29.7 (6)	17.8–150.0	0.36–2.94		0.1–0.5
		Mean ± SD	18.9 ± 15.0	17.5 ± 8.1	65.3 ± 46.9	1.29 ± 0.92		0.2 ± 0.2
		GM (GSD)	12.6 (3.2)	15.9 (1.6)	52.7 (2.1)	1.04 (2.1)	A	0.2 (2.1)
		P95 estimate	86.0	34.4	175.0	3.40		0.6
	2009 ($n = 6$)	Range ($n >$ MDL)	<3.0–38.9 (4)	7.6–26.9 (6)	14.1–97.3	0.3–1.9		0.05–0.3
		Mean ± SD	15.6 ± 12.6	16.6 ± 6.6	50.2 ± 27.0	1.0 ± 0.5		0.2 ± 0.1
		GM (GSD)	11.0 (2.9)	15.4 (1.5)	43.5 (1.9)	0.9 (1.9)	A	0.1 (1.9)
		P95 estimate	64.3	31.2	122.3	2.40		0.4
Okinawa	1992, 1995 ($n = 7$)	Range ($n >$ MDL)	<3.0–49.8 (5)	7.8–50.5 (7)	20.4–293.1	0.3–4.4		0.1–0.7
		Mean ± SD	16.1 ± 16.3	21.0 ± 14.9	104.5 ± 95.6	1.6 ± 1.4		0.3 ± 0.2
		GM (GSD)	9.1 (3.7)	17.4 (1.9)	72.0 (2.7)	1.2 (2.6)	A	0.2 (2.6)
		P95 estimate	79.1	50.7	356.0	5.60		1.0
	2009 ($n = 7$)	Range ($n >$ MDL)	<3.0–26.9 (6)	9.2–21.2 (7)	20.0–81.5	0.4–1.6		0.1–0.3
		Mean ± SD	13.4 ± 7.4	15.1 ± 4.2	52.2 ± 18.6	1.0 ± 0.4		0.2 ± 0.1
		GM (GSD)	10.6 (2.5)	14.6 (1.3)	48.7 (1.6)	1.0 (1.6)	A	0.2 (1.6)
		P95 estimate	47.1	23.4	100.0	2.00		0.3

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.

cal analyses was performed using JMP (Version 4; SAS Institute Inc., Cary, NC, USA). The mean, range and geometric mean (GM) were calculated. Since there was a large variation in the concentrations of toxaphene and endosulfan among the groups, the values were log-transformed and differences between the mean values were tested by the Tukey–Kramer honestly significant difference (HSD) test after ANOVA. Residue intake based on the upper 95th percentile limits were calculated by multiplying the GM by the geometric standard deviation (GSD) to the power of 1.64, according to a log-normal distribution. Correlations were tested by Spearman's rank correlation coefficient. Differences were considered to be statistically significant at $p < 0.05$.

3. Results and discussion

3.1. Characteristics of the study participants and pooled sample

A total of 300 samples were collected from the five study sites, as indicated in Fig. 1 and Table 2. A food duplicate sample technique was used to collect the samples, except for the 2009 samples from Japan. The Japanese samples from 2009 were purchased in markets by volunteers, and we assumed average body weight of female aged 26–29 in 2007 in Japan (50.9 kg, National Health and Nutrition Survey in Japan). Apart from a few subjects ($n = 15$) in Okinawa, all the other participants were females. The mean (±SD) age of the participants was 36.5 ± 11.5 years. There were significant differences in the mean ages between time periods in Beijing ($p < 0.05$, Tukey–Kramer HSD test) and these subjects from 2009 appear to be obese (BMI > 25). Food consumption variation was observed among individuals in the groups, which might be a reflection of differences in age, weight and financial situation. These factors may have influenced the choice and amount of food that the subjects consume. However, the intake estimation based on the chemical residues in the food that the subject eats can minimize the introduction of errors, which is a characteristic advantage of the approach used in this study (Hamilton and Crossley, 2004).

Fat content was measured and comparisons were made between time periods because POPs are commonly lipophilic and considered as major source of dietary intake (Fisk et al., 1999). Between time periods, the differences were within twofold's. Among three countries, China had higher fat content (3.90%) in 2009; however, this figure was not consistent with the temporal changes in the intake of endosulfan.

3.2. Trends of endosulfan in the daily consumed food samples

The total detection frequencies of α- and β-endosulfan were 83.3% and 95%, respectively (Table 3). β-Endosulfan was found at relatively high concentrations, which supports the idea that this isomer is more persistent (Sutherland et al., 2004; Berntssen et al., 2008).

The profile of total endosulfan in both time periods in this study was highest in Seoul with a detection frequency of 100% ($n = 10$). The GMs of the α- and β-isomer concentrations were 1124 and 1262 pg g^{-1} in the pooled food composites from Seoul in 2007, respectively. The GMs of the dietary intake of endosulfan in Seoul were 39 ng kg bw^{-1} d^{-1} in 1994 and 92 ng kg bw^{-1} d^{-1} in 2007, and significantly higher than those in Japan ($p < 0.05$, Tukey–Kramer HSD test). The samples from Beijing showed a 50-fold increase in the level of endosulfan from 1993 (GM: 0.6 ng kg bw^{-1} d^{-1}) to 2009 (GM: 25 ng kg bw^{-1} d^{-1}) ($p < 0.05$, Tukey–Kramer HSD test). Among the three areas in Japan, there were no differences in the dietary intake of endosulfan in both time periods ($p > 0.05$, Tukey–Kramer HSD test). Fig. 2 shows log-transformed endosulfan and toxaphene levels in food composite samples where 95% of the values lie inside the boundary of the ellipse. From the figure, it is of interest to note similar increasing pattern of endosulfan in Beijing and Seoul, while a decreasing trend was observed in Japan.

Endosulfan intake in Japan showed a gradual decrease, which may result from reduced agricultural consumption in the country (POPRC, 2009). However, the daily intakes of endosulfan showed increasing trends in Seoul and Beijing by twofold and an order of magnitude in the GMs, respectively. Liu et al. (2010) reported a detected mean endosulfan level of 1.5 ng g^{-1} with variation from non-detects to 13.1 ng g^{-1} in fish samples in Northeastern China. These figures are likely to show high exposure via sea food consumption in the country. The detection of endosulfan in this region was replicated in another study (Guo et al., 2007). In the present study, the significant rise observed in Beijing was in agreement with the endosulfan production history, following its production in 1994 and agricultural application in the country (Jia et al., 2009). Therefore, more importantly, agricultural use could explain the increasing trend. Likewise, Wang et al. (2007) determined high levels of endosulfan in other environmental media in the Northeastern part of Beijing where agricultural land existed.

On the other hand, the concentrations (GM ± GSD) in both Beijing (1737.0 ± 1.2 ng d^{-1}) and Seoul (4916 ± 4.3 ng d^{-1}) were above the 130 ng d^{-1} value in New Zealand (Thomson et al., 2003). China, Korea and New Zealand had been among the remaining users of endosulfan. The food trade between these countries and the Korean reliance on food imports along with the continued use of endosulfan could be another possibility that may somewhat explain the food safety issue. Since food trade partner countries have usually different histories of pesticide use, evaluation of the residue levels and contributions of imported foodstuffs is also worth considering. The variation in concentrations among the pooled samples could also suggest another possibility that individual food types have different contributions, meaning that some groups of people with selective food preferences may be exposed to acutely high-dose.

High levels of endosulfan detection were also reported in Taiwan (Doong et al., 1999), whereas residues of both the α- and β-isomers were not detected in any samples in Jordan (Ahmad et al., 2010). The estimates in Beijing in 2009 and Seoul in both

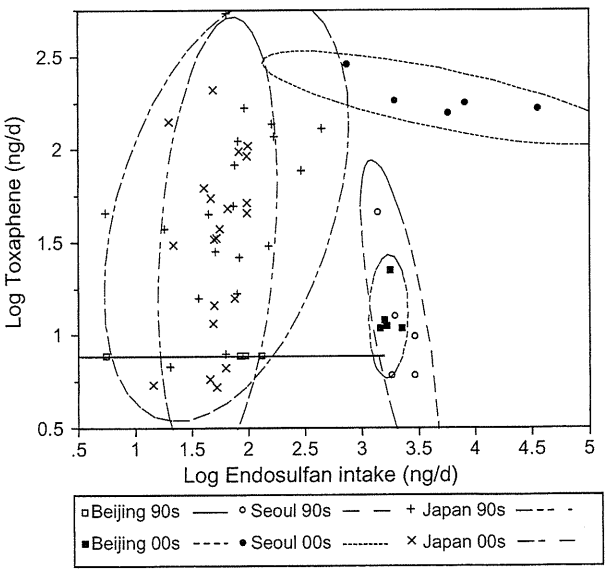

Fig. 2. Plot of the log-transformed endosulfan and toxaphene levels in food composite samples the 1990s and 2000s. Overall, 95% of the values lie inside the boundary ellipse.

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, #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.

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

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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

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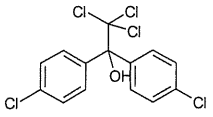

Fig. 1. Structure of dicofol.

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.

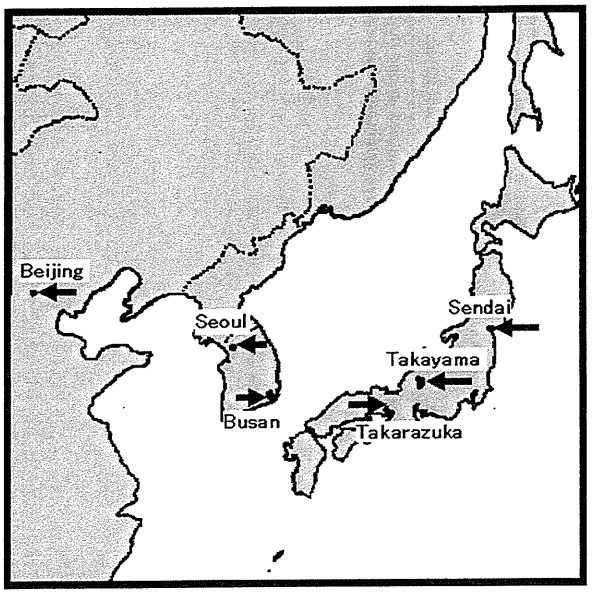

Fig. 2. Map of the sampling locations.

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

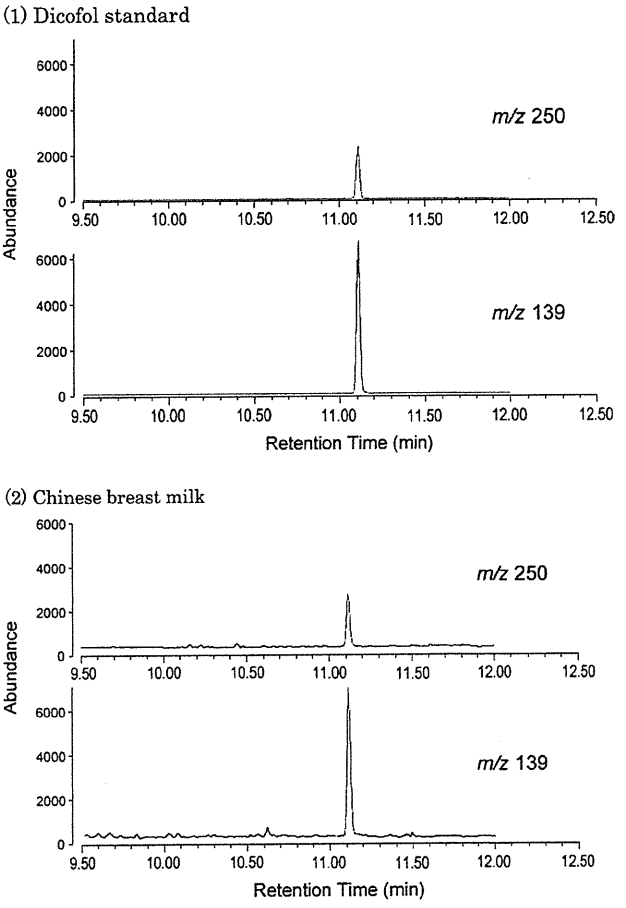

Fig. 3. Typical chromatograms of dicofol obtained in this study. (1) Dicofol standard; (2) Chinese breast milk.

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.

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