図１．計算領域。東西 4500km、南北 3600km、水平解像度 90km の領域１（黒枠）と、東西南北 450km、水平解像度 9km の領域２（赤枠）を結合した。

図２．ハイボリュームエアサンプラー設置の様子。2011 年 2 月 10 日京都大学防災研究所宇治川オープンラボラトリー構内（Fushimi）。

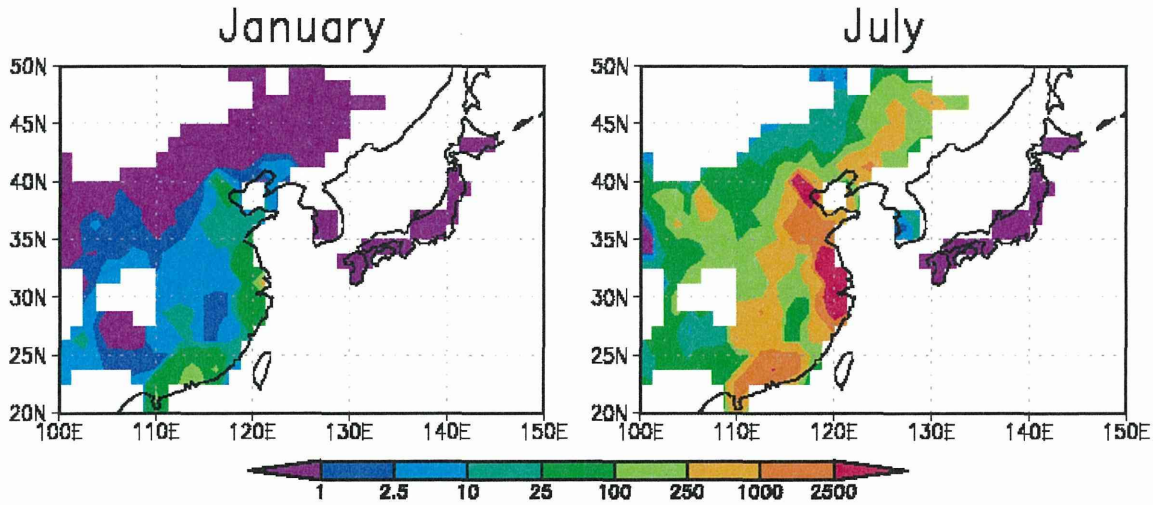

図３．１月と７月における日本、韓国、中国からの大気への SCCPs 推定排出分布（μg m^{-2} mon^{-1}）。国ごとの排出量はそれぞれ 0.08 t yr^{-1}、5.2 t yr^{-1}、12000 t

yr^{-1}。

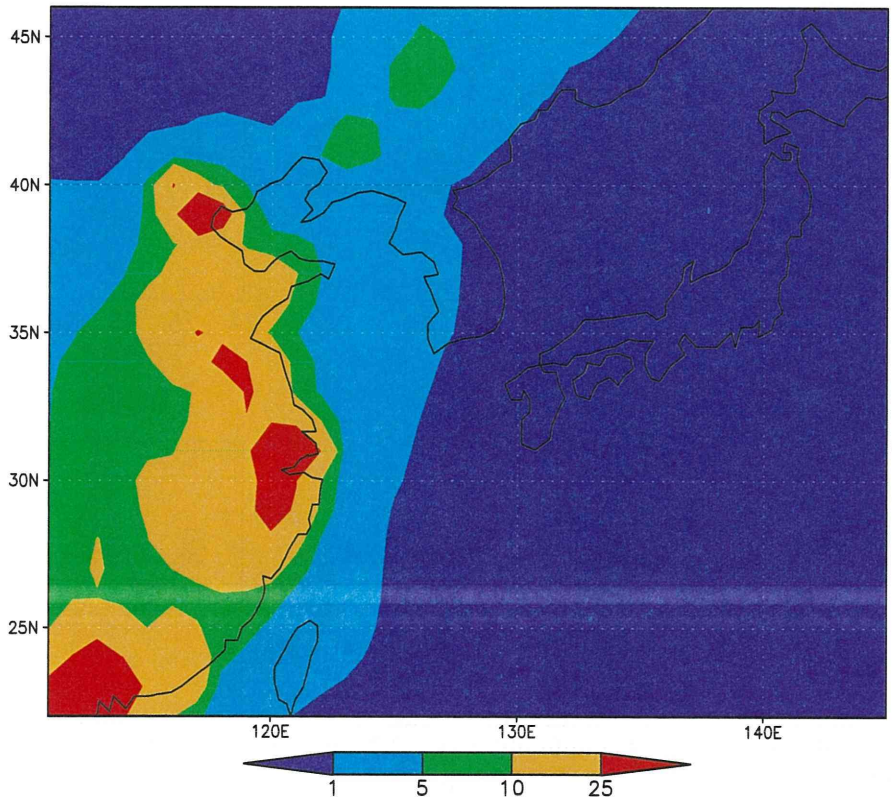

図４．従来の知見に従って推定した排出量（図３）をモデルに与えて計算した2012年１月における地表付近の大気中SCCPsの月平均濃度分布（ng m^{-3}）。日本全体が１ng m^{-3}に満たない領域にある。

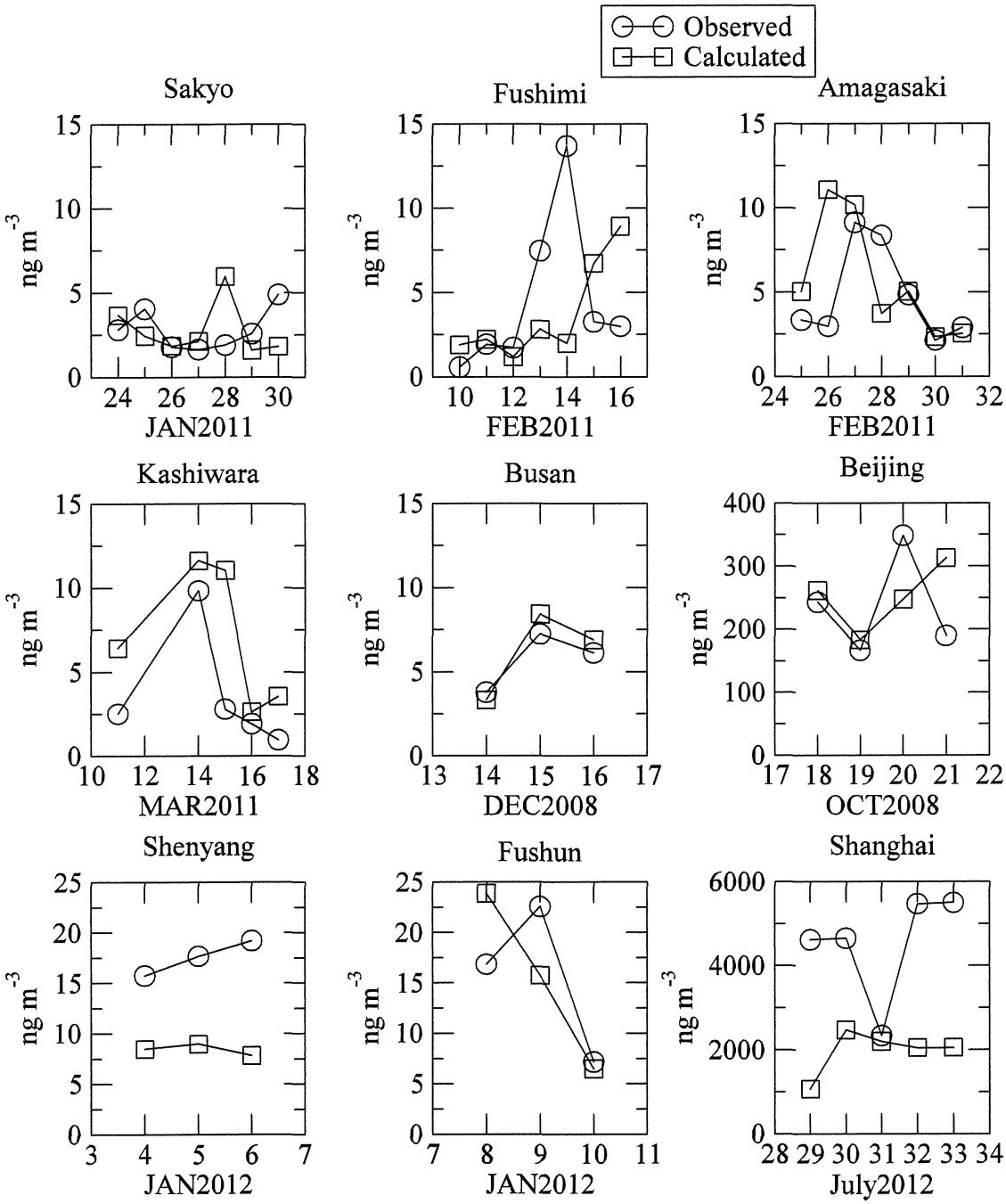

図５．大気中 SCCPs 濃度の日平均値（ng m^{-3}）の計算値（□）と実測値（○）との比較。

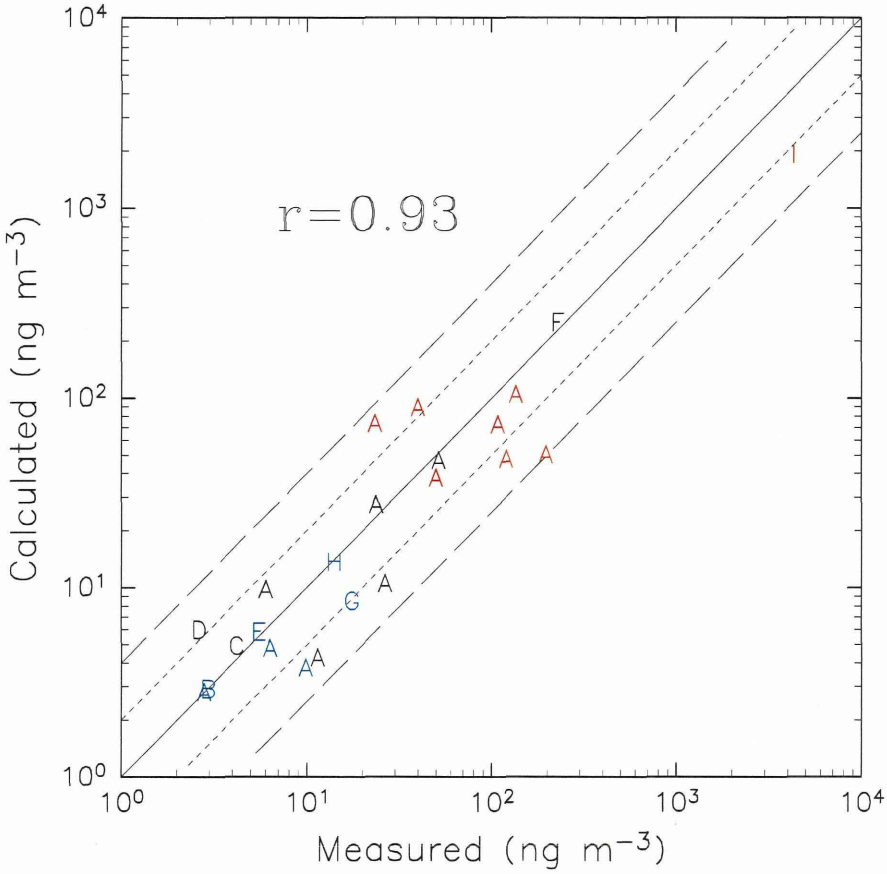

図６．地表面大気中 SCCPs 濃度（ng m^{-3}）の計算値と実測値との比較。表１，２に示した連続測定の期間中の平均値を用いた。実線と破線はそれぞれ factor 2 および 4 の誤差を表す。A: Sakyo、B: Fushimi、C: Amagasaki、D: Kashiwara、E: Busan、F: Beijing、 G: Shenyang、 H: Fushun、 I: Shanghai。赤は夏季、青は冬季の測定であることを示す。r は相関係数（p = 1.37x10^{-10}）。

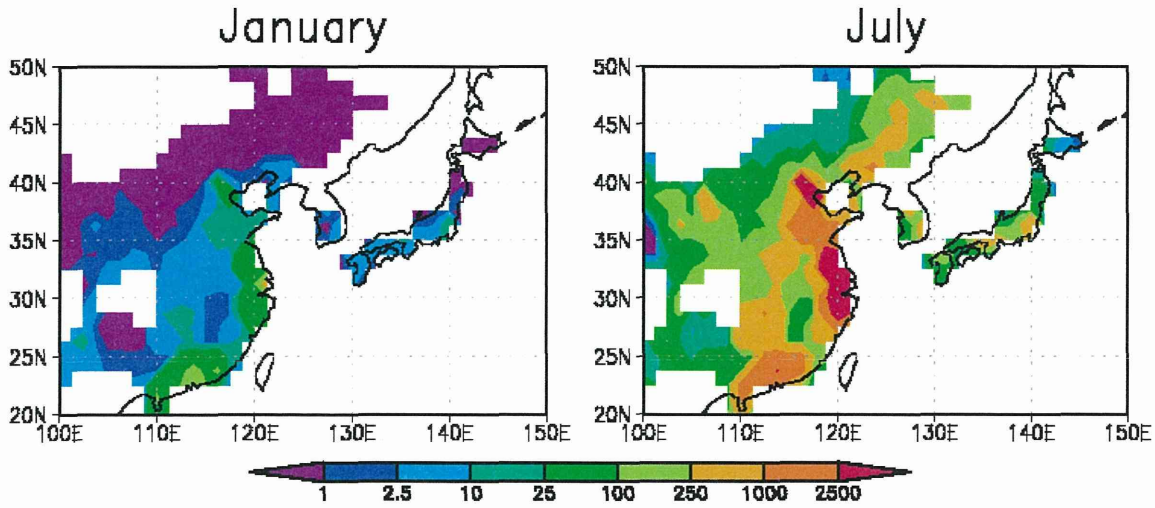

図７．モデルにより最適化したSCCPs排出量（μg m^{-2} mon^{-1}）。日本、韓国からの年間排出量はそれぞれ320 t yr^{-1}、100 t yr^{-1}となった。

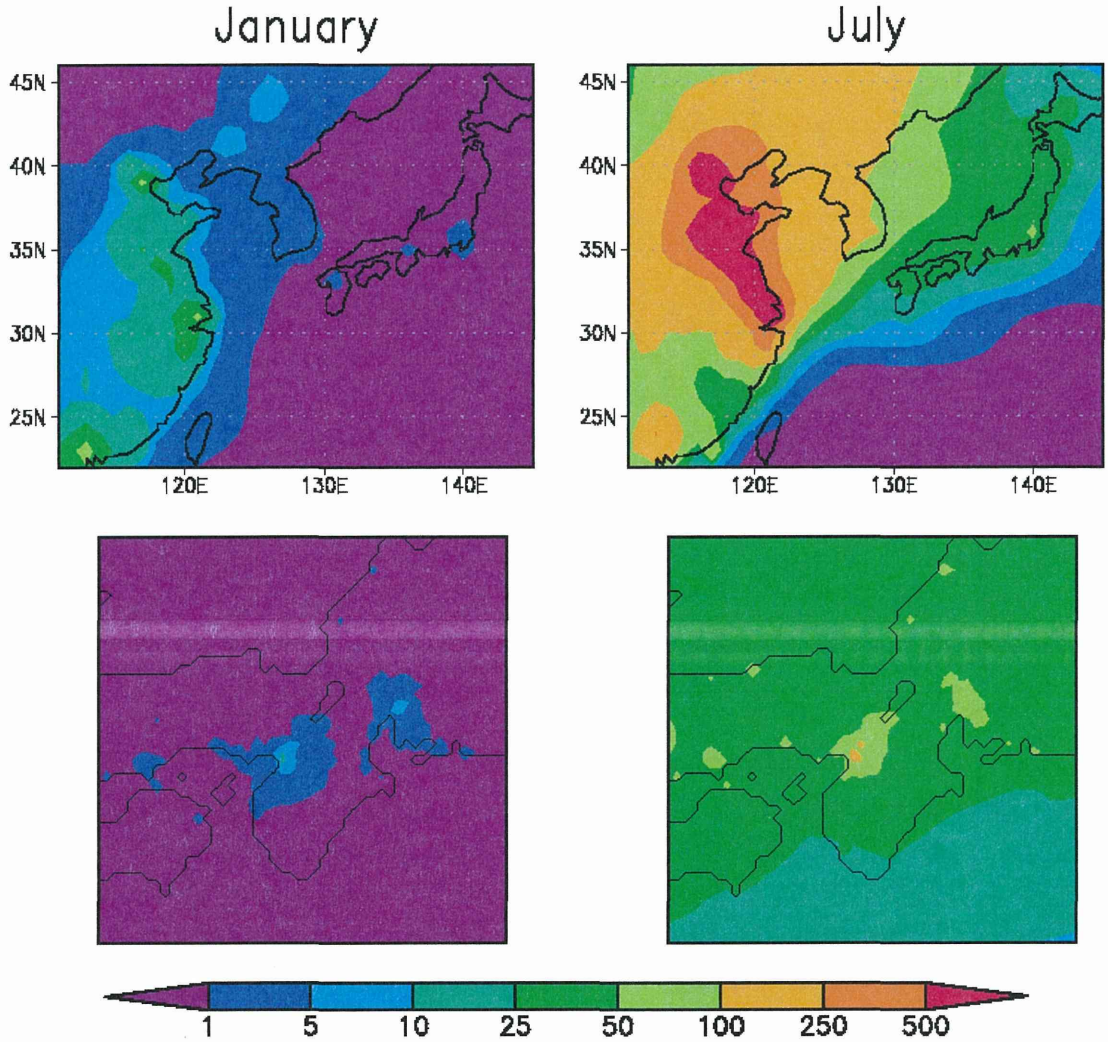

図８．2012年1月および7月における地表面付近の大気中SCCPs濃度の月平均値（ng m^{-3}）。上段は領域１、下段は領域２による計算結果。

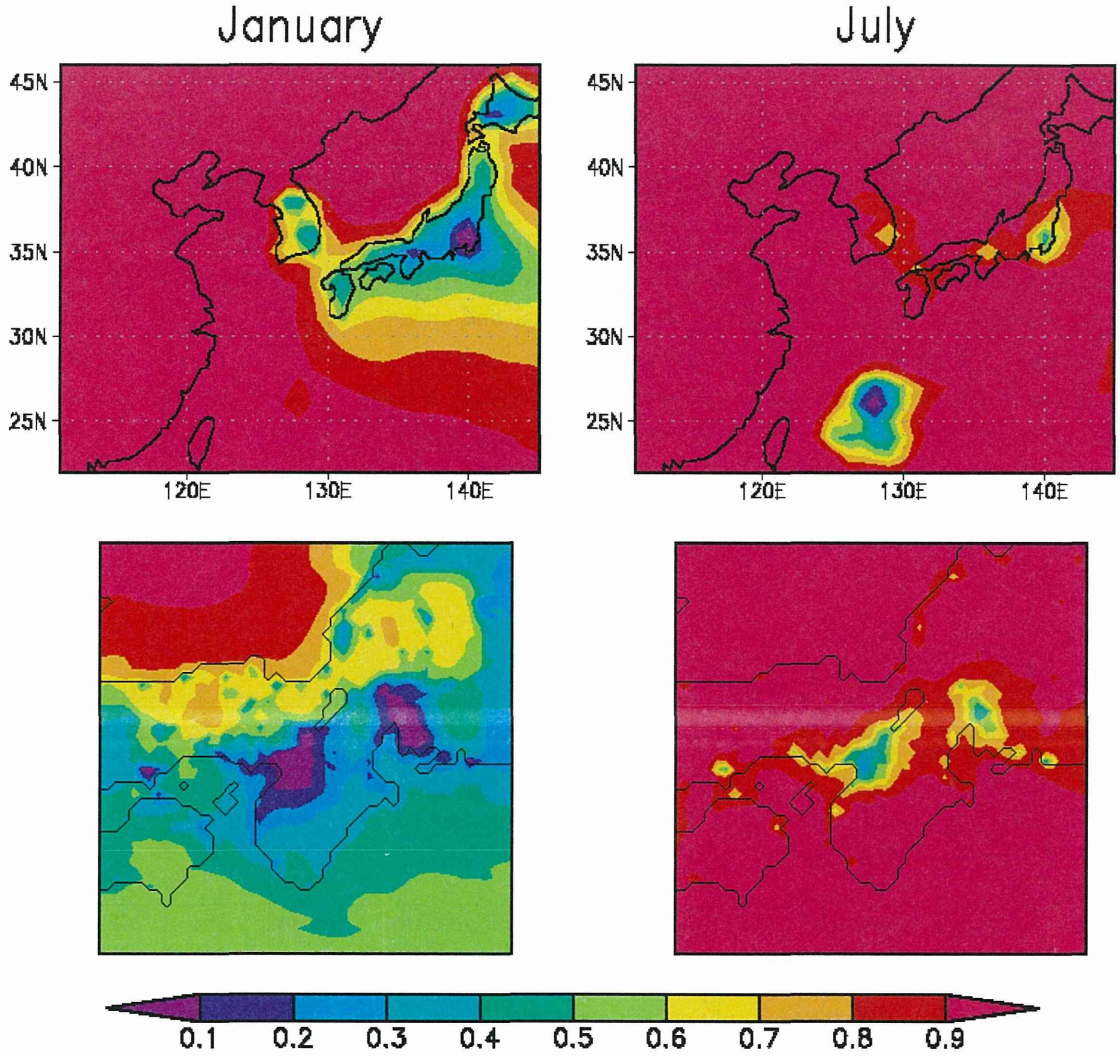

図９．2012年1月および7月における、地表付近の大気中SCCPs全体に対する中国起源成分の寄与。上段は領域１、下段は領域２による計算結果。

別紙４

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
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Yan JX, Inoue K, Asakawa A, Harada KH, Watanabe T, Hachiya N, Koizumi A.	Methylmercury Monitoring Study in Karakuwacho, a Peninsula Area in Japan	Bull. Environ. Contam. Toxicol.	93(1)	36-41	2014
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IV. 研究成果の刊行物・別刷

※主要なもののみ

Environmental Science & Technology

Article

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Analysis of Perfluoroalkyl Carboxylic Acids in Composite Dietary Samples by Gas Chromatography/Mass Spectrometry with Electron Capture Negative Ionization

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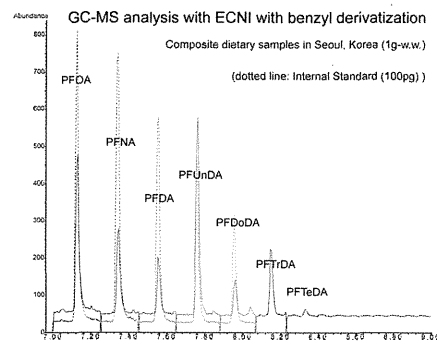
Supporting Information

ABSTRACT: A gas chromatography—mass spectrometry and electron-capture negative ionization (ECNI) method was developed to quantify perfluorinated carboxylic acids (PFCAs) in composite dietary samples. Benzyl esterification was used for pretreatment before PFCAs analysis. This stabilized the benzyl radical leaving group preferentially, and gave carboxylic anions of the PFCAs with ECNI. The method had a low detection limit (0.3—10 pg g^{-1}) and good recoveries (98—90%) for PFCAs with 8—14 carbon atoms (C8 to C14). The method was applied to 24-h dietary samples from subjects in Japan (Hokkaido, Kyoto, and Okinawa; 1992 to 2007, and 2009), Korea (Seoul; 1994 and 2007), and China (Beijing; 1993 and 2009). The levels of the PFCAs were between 39 and 169 ng day^{-1} in Korea, 58 and 71 ng day^{-1} in China, and 56 and 67 ng day^{-1} in Japan. Between the two sampling years, the total levels of PFCAs (C8 to C14) increased significantly ($p < 0.05$). The interaction between the sampling location in Korea and year was significant ($p < 0.05$).



1. INTRODUCTION

Perfluorochemicals such as perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are environmental contaminants of public health concern because of their persistence and bioaccumulation in the environment. They have been detected in human serum and breast milk samples in many areas.[1] However, the exposure routes are not well characterized. The 3M Company was a major manufacturer of PFOS, but phased out its production in 2002.[2] Since then, concern has shifted from PFOS to PFOA. However, in Japan, perfluorinated carboxylic acid (PFCA) emissions contain perfluorononanoic acid (PFNA) and perfluoroundecanoic acid (PFUnDA) in addition to PFOA. [3] In 2000, 25 and 7 t (1 t = 1000 kg) of PFNA and PFUnDA were emitted, respectively.[3] In an earlier study, we detected PFNA and PFUnDA at concentrations comparable to PFOA in serum samples from Japanese, Korean, and Vietnamese adults.[4] The levels of these chemicals have continued to increase in humans, even after production of PFOS was halted in 2002,[4] but the exposure routes that are contributing to these increases are still unknown.

Although dietary intake is thought to be a major route of exposure for PFCAs,[5] there is little data for long-chain PFCA levels in food items because of analytical difficulties with method development in liquid chromatography and mass spectrometry (LC/MS).[6] Quantitative analyses of PFCAs at low pg g^{-1} concentrations in complex matrices such as food require rigorous and complicated cleanup procedures to eliminate matrix effects.[7] Alternatively, gas chromatography—mass spectrometry (GC/MS) can be used as the matrix suppression effect caused by coeluting material is relatively low compared with LC/MS.[8] Electron-capture negative ionization (ECNI) reportedly improves the detection limits of PFCA anilides,[9] and could be coupled with GC/MS.

The aim of the present study was to develop a simple but sensitive quantification method for PFCAs in foods. GC/MS was coupled with ECNI for PFCAs analysis. ECNI improved the sensitivity for PFCAs benzyl esters compared with electron impact ionization (EI). PFCAs in composite dietary samples were quantified by this method, and historical trends in the dietary intake of PFCAs in three Asian countries were analyzed.

2. MATERIALS AND METHODS

2.1. Chemicals. Acetone (LC-MS grade), sodium carbonate (>99.5%), and distilled water (LC-MS grade) were obtained from Kanto Chemicals (Tokyo, Japan). Benzyl bromide, tetrabutylammonium hydrogen sulfate, 11H-perfluoroundecanoic acid, and methyl tertiary-butyl ether (MTBE, HPLC grade) were purchased from Wako Pure Chemical Industries (Osaka, Japan). A mixture of $^{13}C_2$-labeled perfluorohexanoic acid, $^{13}C_4$-labeled PFOA, $^{13}C_5$-labeled PFNA, $^{13}C_2$-labeled PFDA, $^{13}C_2$-labeled PFUnDA, and $^{13}C_2$-labeled perfluorododecanoic acid (PFDoDA) was obtained from Wellington Laboratories (Guelph, ON, Canada). $^{13}C_{12}$-2,3,3′,5,5′-penta-

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Table 1. Quality Assurance for PFCAs Analysis in Food Samples

compound (carbon atoms)		quantification (confirmation) ECNI	quantification (confirmation) EI	instrument detection limit[a] (pg) ECNI	instrument detection limit[a] (pg) EI	recovery and (reproducibility) % (SD%)[b] (n = 10, fortified)[c]	relative detector response[d] % (SD %) (n = 6, fortified)[d]	procedural blank (SD) (pg g^{-1}), n = 6	method detection limit[e] (pg g^{-1})
PFOA	(C8)	413 (414)	504 (485)	0.003	0.2	97 (16)	95 (2.8)	5 (0.4)	10
PFNA	(C9)	463 (464)	554 (535)	0.003	0.2	98 (19)	93 (3.9)	2 (0.3)	4
PFDA	(C10)	513 (514)	604 (585)	0.004	0.2	91 (17)	94 (4.9)	1 (0.3)	2
PFUnDA	(C11)	563 (564)	654 (635)	0.004	0.2	94 (18)	92 (6.3)	1.5 (0.4)	3
PFDoDA	(C12)	613 (614)	704 (685)	0.005	0.4	90 (18)	89 (7.7)	1 (0.2)	2
PFTrDA	(C13)	663 (664)	754 (735)	0.005	0.4	93 (16)		1 (0.2)	2
PFTeDA	(C14)	713 (714)	785 (786)	0.007	2	93 (17)		1 (0.4)	2

[a] 1 μL injection. [b] SD: standard deviation. [c] The recoveries of the PFCAs were examined by spiking 100 pg of each standard compound into 10 composite dietary samples before extraction. [d] $^{13}C_4$-labeled PFOA, $^{13}C_5$-labeled PFNA, $^{13}C_2$-labeled PFDA, $^{13}C_2$-labeled PFUnDA, and $^{13}C_2$-labeled PFDoDA were derivatized and were fortified at 100 pg to food extracts. Matrix effects are expressed as relative detector responses (%) to the signal area responses of corresponding solvent-based preparations. [e] Food sample of 1 g. The method detection limit is defined as the concentration that produces a signal three times that of the blank. The mean blank signal was subtracted from the calculated sample concentration.

chlorobiphenyl (CB-111) was obtained from Cambridge Isotope Laboratories (Andover, MA)

2.2. Sample Collection. Diet samples from the Kyoto University Human Specimen Bank[10,11] were used for the evaluation. At the time of collection, participants were requested to donate duplicate samples of all food and drink items that they consumed over a 24-h period. These samples are referred to as duplicate 24-h diet samples. Two hundred duplicate 24-h diet samples were collected from the following locations: 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] In addition, 100 duplicate 24-h diet samples (i.e., a typical day's worth of food and drink) were purchased by volunteers from markets in Kyoto, Okinawa, and Hokkaido in 2009. The study populations were the same as those in earlier studies.[13,14] This gave a total of 300 duplicate 24-h diet samples. All food and drink samples in each duplicate sample were mixed together and homogenized. Then the 300 homogenized diet samples were combined into 60 groups (150 g), referred to as the composite dietary samples, each containing five samples (30 g) from the same location and sampling year. This process is detailed in Figure S1 of the Supporting Information. The composite dietary samples were then stored in glass bottles at −30 °C. The study protocol was approved by the Ethics Committee of Kyoto University (Kyoto, Japan). Written informed consent was obtained from all study participants.

2.3. Extraction of the Composite Dietary Samples. Each of the composite dietary samples was subjected to an ion-pair extraction. Briefly, approximately 1 g of each composite dietary sample and an internal standard mixture (100 pg of each $^{13}C_4$-labeled PFOA, $^{13}C_5$-labeled PFNA, $^{13}C_2$-labeled PFDA, $^{13}C_2$-labeled PFUnDA, and $^{13}C_2$-labeled PFDoDA) were placed in a 15-mL polypropylene centrifugation tube. Next, 1 mL of 0.5 mol L^{-1} tetrabutylammonium/0.25 mol/L sodium carbonate buffer (pH adjusted to 10 using NaOH) and 1 mL of MTBE were added to the samples, and the tubes were vortex mixed for 60 m. The samples were then centrifuged at 9840g for 5 min. The organic layer was separated twice and placed in a clean glass tube, then dried under a gentle stream of nitrogen. The residue was redissolved in 100 μL of 0.1 mol L^{-1} benzyl bromide/acetone solution containing 1 ng of 11H-perfluoroundecanoic acid and 1 ng of $^{13}C_{12}$-labeled CB111 to monitor the derivatization efficiency. The solution was then derivatized at 60 °C for 1 h. No further cleanup was conducted. Derivatized samples were analyzed by GC/MS within 24 h.

2.4. Instruments and Quantification. Derivatized PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, and perfluorotetradecanoic acid (PFTeDA) were analyzed by GC/MS in scan mode with selected ion monitoring (Agilent 6890GC/5973MSD inert, Agilent Technologies Japan, Ltd., Tokyo, Japan). PFCAs were dissolved in 100 μL of 0.1 mol L^{-1} benzyl bromide/acetone solution and derivatized at 60 °C for 60 min with 1 ng of 11H-perfluoroundecanoic acid and 1 ng of $^{13}C_{12}$-labeled CB111 to monitor the derivatization efficiency. After benzylation, the stability was investigated by monitoring the peak area ratio over 24 h. PFCA benzyl esters were separated on a DB-5MS column (30 m length, 0.25 mm i.d., 1 μm film thickness; Agilent Technologies Japan, Ltd.) with a helium carrier gas (99.9999% purity; Air Liquide Japan Ltd., Tokyo, Japan). Splitless injections (1 μL) were performed with an injector temperature of 220 °C, and the split vent was opened after 1.5 min. The initial oven temperature was 70 °C for 2 min, after which it was increased to 100 °C at 20 °C min^{-1}, and then to 280 at 30 °C min^{-1}. To compare the limits of detection, both ECNI and EI were used to quantify the PFCA benzyl esters. In ECNI, methane (99.9999% purity; Air Liquide Japan Ltd.) was used as the reagent gas (2 mL min^{-1}). The ion source temperature was maintained at 150 °C. In EI, the ion source temperature was maintained at 250 °C. The target ions for determination of PFCAs in both ECNI and EI are summarized in Table 1.

Standard stock solutions (2 μg mL^{-1}) were diluted to seven working standard solutions (4, 2, 1, 0.8, 0.4, 0.2, and 0.1 ng mL^{-1}) by serial dilution with acetone. All the standard solutions were stored in a refrigerator at 4 ± 2 °C for a maximum period of 3 months from the date of preparation. Quantification was conducted using an internal standard dissolved in acetone. $^{13}C_4$-labeled PFOA, $^{13}C_5$-labeled PFNA, $^{13}C_2$-labeled PFDA, $^{13}C_2$-labeled PFUnDA, and $^{13}C_2$-labeled PFDoDA were used as internal standards for the PFCAs. These standards were diluted to 1 ng mL^{-1}. The instrumental detection limit (IDL) is defined as the mass of the analyte producing a peak with a signal-to-noise ratio of three. Because blank levels were larger for shorter-chain PFCAs than for longer-chain PFCAs, the final net IDLs of the shorter- and longer-chain PFCAs were nearly equivalent.

2.5. Method Detection Limits (MDLs), Blank Contamination, Total Extraction Recovery, and Possible Matrix

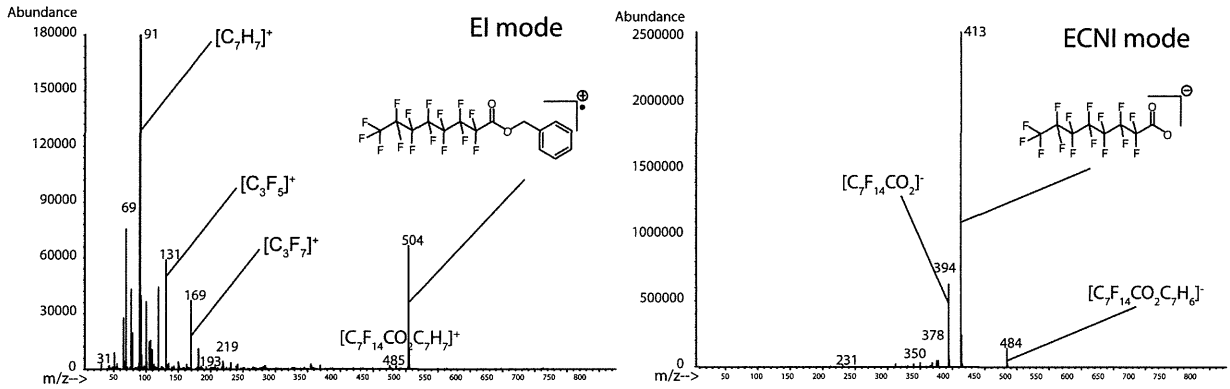

Figure 1. Mass spectra of PFOA benzyl ester in EI mode and ECNI mode (m/z 30–800).

Effect. Milli-Q water (Millipore, Billerica, MA) was used as the procedural blank control, and was analyzed after every 10 samples (n = 6). The procedural blank was extracted using the process described above, and six replicate procedural blanks were prepared independently. In this study, we observed blank contamination for all PFCAs (Table 1). The MDL is defined as the concentration that produces a signal three times that of the blank. The mean blank signal was subtracted from the calculated sample concentration (Table 1). The recoveries of the PFCAs were examined by spiking 100 pg of each standard compound into 10 composite dietary samples before extraction. Possible matrix effects on the GC-MS detector response were evaluated by comparing the response factors of PFCA benzyl esters in acetone and in food extracts prepared in acetone. The ^{13}C-labeled internal standards (100 pg) were derivatized in acetone before being used for spiking, and then added to the food extracts.

2.6. Statistical Analysis. All statistical analyses were conducted using SPSS (Version 16.0 for Windows 2007, IBM Corporation, Armonk, NY). Values of $p < 0.05$ were considered statistically significant. Concentrations lower than the detection limits were given a value of half the detection limit for statistical analyses. Statistical analyses were conducted after logarithmic transformation of the PFCAs concentrations. When the statistical tests by two-way ANOVA were significant, analysis of covariance was used to demonstrate the effect of time or location and their interactions influenced on the PFCA levels in the food composite samples.

3. RESULTS AND DISCUSSION

3.1. Quantification and Quality Assurance. *Derivatization, Mass Spectra, and IDL.* In the present study, we developed a very simple yet sensitive method for PFCAs analysis in food using GC-ECNI-MS with benzyl esterification. Benzyl esterification was used for PFCAs analysis because stabilization of the benzyl radical leaving group preferentially gives carboxylic anions of PFCAs in ECNI.[15] The PFCAs extracted from the composite dietary samples were dissolved in benzyl bromide/acetone solution and derivatized with 11H-perfluoroundecanoic acid and $^{13}C_{12}$–CB-111 (Section 2.3). Peak area ratios to $^{13}C_{12}$–CB-111 showed that the derivatization reaction time of 60 min at 60 °C was sufficient for benzylation to reach completion. After benzylation, the peak area ratios did not change significantly over 24 h (arithmetic mean ± relative standard deviation: 104 ± 5.6%, n = 10).

Mass spectra of the standard solutions were initially acquired in full-scan mode with EI or ECNI to determine the retention times and fragmentation patterns. In EI mode, the molecular ion $[M]^+$ of PFOA benzyl ester was observed at m/z 504 (Figure 1). In ECNI mode the PFOA benzyl ester showed an abundant fragment ion $[M - C_7H_7]^-$ at m/z 413 (Figure 1), corresponding to a carboxylate anion ($C_7F_{15}COO^-$). Similar fragmentation was observed among the PFCA benzyl esters with different chain lengths (ECNI mode, Figure S2; EI mode, Figure S3). As shown in Table 1, other PFCA benzyl esters also gave abundant fragment ions $[M - C_7H_7]^-$ in ECNI mode, and the IDLs ranged from 0.003 to 0.007 pg. Therefore, ENCI was used for quantification of PFCA benzyl esters in subsequent experiments.

Extraction. To avoid freeze-drying the samples and to simplify the method, the PFCAs from the composite dietary samples were extracted by ion-pair extraction[15] into an organic solvent (MTBE). This method prevented coextraction of water from the food sample.[7] The presence (or absence) of matrix effects on the GC-MS detector response was examined by spiking the food sample extracts with derivatized internal standards immediately before injection into the GC-MS (Section 2.5). The food extracts displayed only minor suppression of the internal standards (95–89% of solvent based standards) (Table 1). Because no large interferences were observed in the chromatogram in ECNI mode (Table of Contents Art), sample cleanup was not performed after ion-pair extraction.

Blank Contamination and MDLs. A widely reported problem in ultratrace analysis of PFCAs is contamination of procedural blanks.[7,16] To overcome this problem, all disposable laboratory equipment was sonicated in methanol, and the methanol was analyzed to determine potential sources of contamination. None of the laboratory equipment contributed to background levels of any analyte. The purity of each solvent was tested by evaporating 10 mL of the solvent to dryness. Artifacts originating from the evaporation procedure were investigated by comparing drying the solvent under nitrogen gas and vacuum. Comparable results were obtained with both methods. When the solvents were tested, HPLC-grade MTBE was found to contain low levels of target analytes. Therefore, the HPLC-grade MTBE was distilled by rotary evaporation to reduce the levels of contaminants. The final procedural blank levels were 5 pg g^{-1} for PFOA, 2 pg g^{-1} for PFNA, 1 pg g^{-1} for PFDA, 1.5 pg g^{-1} for PFUnDA, and 1 pg g^{-1} for PFDoDA, PFTrDA, and PFTeDA.

Table 2. Dietary Intake of PFCAs from Composite Food Samples (ng day^{-1})[a]

		year (no. of pooled diets)		ng day^{-1} PFOA (C8)	PFNA (C9)	PFDA (C10)	PFUnDA (C11)	PFDoDA (C12)	PFTrDA (C13)	PFTeDA (C14)	total (C8–C14)
China	Beijing	1993	n > MDL (%)	0 (0)	3 (60)	4 (80)	4 (80)	4 (80)	4 (80)	2 (40)	4 (80)
			median (range)	<22.5	9.4 (n.d.–12.3)	8.9 (n.d.–15.4)	9.6 (n.d.–13.9)	6.5 (n.d.–13.3)	15.0 (n.d.–16.0)	<4.5	66.6 (n.d.–80.2)
		(n = 5)	mean ± SD	–	<9.0	9.0 ± 4.9	9.7 ± 4.0	7.3 ± 4.1	12.6 ± 5.8	–	61.7 ± 20.0
			GM (GSD)	–	<9.0	7.6 (2.1)	8.8 (1.7)	6.3 (1.9)	10.3 (2.4)	–	58.1 (1.5)
		2009	n > MDL (%)	0 (0)	2 (40)	5 (100)	2 (40)	3 (60)	3 (60)	2 (40)	5(100)
			median (range)	<30.9	<12.4 (n.d.–15.8)	13.8 (6.9–19.1)	<9.3 (n.d.–32.4)	8.7 (n.d.–14.9)	8.0 (n.d.–29.6)	<6.2 (n.d.–19.4)	68.1 (35.1–141.8)
		(n = 5)	mean ± SD	–	9.4 ± 4.9	13.1 ± 4.9	–	8.0 ± 5.3	13.0 ± 12.1	–	78.7 ± 40.8
			GM (GSD)	–	8.4 (1.7)	12.2 (1.5)	–	6.4 (2.2)	8.4 (3.0)	–	70.7 (1.7)
Korea	Seoul	1994	n > MDL (%)	0 (0)	0 (0)	2 (40)	4 (80)	2 (40)	5 (100)	1 (20)	5 (100)
			median (range)	<17.8	<7.7	<3.6 (n.d.–6.8)	8.2 (n.d.–13.2)	<3.6 (n.d.–5.2)	9.3 (5.2–22.2)	<3.6 (n.d.–3.7)	40.4 (28.8–56.4)
		(n = 5)	mean ± SD	–	–	–	8.5 ± 4.4	–	10.5 ± 6.9	–	40.1 ± 11.6
			GM (GSD)	–	–	–	7.3 (1.9)	–	9.0 (1.8)	–	38.8 (1.3)
		2007	n > MDL (%)	0 (0)	2 (40)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)
			median (range)	<21.0	<8.4 (n.d.–16.1)	8.6 (6.8–13.4)	60.3 (46.9–80.2)	17.1 (12.6–25.3)	49.3 (41.4–67.8)	9.6 (5.6–11.4)	172.8 (132.3–225.2)
		(n = 5)	mean ± SD	–	–	9.4 ± 2.8	63.4 ± 12.4	17.4 ± 4.9	54.1 ± 11.1	9.4 ± 2.3	171.6 ± 34.8
			GM (GSD)	–	–	9.1 (1.3)	62.4 (1.2)	16.9 (1.3)	53.2 (1.2)	9.2 (1.3)	168.9 (1.2)
Japan	Hokkaido	1992, 1995	n > MDL (%)	3 (43)	1 (14)	2 (29)	7 (100)	2 (29)	7 (100)	0 (0)	7 (100)
			median (range)	<22.2 (n.d.–35.8)	<8.9 (n.d.–13.7)	<4.4 (n.d.–5.2)	14.5 (8.9–25.0)	<4.4 (n.d.–4.9)	13.1 (5.2–29.7)	<4.4	79.3 (33.8–88.5)
		(n = 7)	mean ± SD	–	–	–	15.3 ± 4.8	–	15.7 ± 9.8	–	64.6 ± 22.4
			GM (GSD)	–	–	–	14.7 (1.4)	–	13.2 (1.9)	–	60.8 (1.5)
		2009	n > MDL (%)	2 (29)	4 (57)	3 (43)	7 (100)	5 (71)	6 (86)	3 (43)	7 (100)
			median (range)	<18.1 (n.d.–25.4)	7.8 (n.d.–20.3)	<3.6 (n.d.–11.3)	20.6 (14.7–30.0)	4.9 (n.d.–16.1)	14.5(n.d.-40.0)	<3.6 (n.d.–9.4)	57.8 (50.7–146.8)
		(n = 7)	mean ± SD	–	8.6 ± 6.1	–	22.3 ± 5.4	6.0 ± 4.8	18.4 ± 12.8	–	76.5 ± 37.2
			GM (GSD)	–	<7.6	–	21.7 (1.3)	4.7 (2.0)	13.4 (2.7)	–	70.5 (1.5)

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Table 2. continued

	year (no. of pooled diets)		ng day^{-1} PFOA (C8)	PFNA (C9)	PFDA (C10)	PFUnDA (C11)	PFDoDA (C12)	PFTrDA (C13)	PFTeDA (C14)	total (C8–C14)
Kyoto	1996, 1997	n > MDL (%)	6 (100)	1 (17)	1 (17)	5 (83)	1 (17)	5 (83)	0 (0)	6 (100)
		median (range)	23.7 (19.7–30.6)	<7.5	2.0 (n.d.–4.5)	8.7 (n.d.–17.2)	<3.7 (n.d.–4.3)	5.6 (n.d.–13.6)	<3.7	46.7 (38.4–79.2)
	(n = 6)	mean ± SD	24.3 ± 3.6	–	–	9.1 ± 4.6	–	6.2 ± 4.0	–	49.8 ± 15.1
		GM (GSD)	24.1 (1.2)	–	–	8.1 (1.7)	–	5.3 (1.9)	–	48.3 (1.3)
	2009	n > MDL (%)	5 (83)	3 (50)	2 (33)	4 (67)	2 (33)	5 (83)	1 (17)	6 (100)
		median (range)	23.9 (n.d.–38.2)	<6.3 (n. d.–9.9)	<3.1 (n.d.–5.9)	8.4 (n.d.–33.0)	<3.1 (n.d.–9.3)	9.7 (n.d.–34.8)	<3.1 (n.d.–4.3)	56.6 (23.5–117.5)
	(n = 6)	mean ± SD	24.0 ± 9.9	–	–	11.4 ± 11.5	–	12.5 ± 11.8	–	61.6 ± 34.2
		GM (GSD)	21.8 (1.7)	–	–	7.4 (2.8)	–	8.4 (2.9)	–	53.8 (1.8)
Okinawa	1992, 1995	n > MDL (%)	3 (43)	0 (0)	1 (14)	6 (86)	0 (0)	6 (86)	0 (0)	6 (86)
		median (range)	<25.9 (n.d.–49.2)	<10.4	<5.2	14.4 (n.d.–21.0)	<5.2	10.6 (n.d.–16.2)	<5.2	55.5 (n.d.–93.9)
	(n = 7)	mean ± SD	–	–	–	13.5 ± 5.9	–	10.6 ± 4.9	–	62.3 ± 25.9
		GM (GSD)	–	–	–	11.9 (1.9)	–	9.1 (2.0)	–	57.4 (1.6)
	2009	n > MDL (%)	4 (57)	6 (86)	6 (86)	7 (100)	6 (86)	7 (100)	3 (43)	7 (100)
		M edian (range)	19.2 (n.d.–26.6)	9.0 (n.d.–11.9)	4.0 (n.d.–8.2)	20.7 (12.0–30.6)	5.1 (n.d.–10.1)	15.9 (8.0–26.0)	<3.7 (n.d.–8.1)	86.8 (52.1–92.2)
	(n = 7)	mean ± SD	<18.5	9.0 ± 2.6	4.8 ± 2.2	20.6 ± 6.9	5.7 ± 2.7	16.5 ± 5.4	–	78.0 ± 16.2
		GM (GSD)	<18.4	8.6 (1.4)	4.4 (1.6)	19.6 (1.4)	5.1 (1.8)	15.7 (1.4)	–	76.4 (1.3)

[a]n.d.: not detected; MDL: method detection limit; SD: standard deviation; GM: geometric mean; GSD: geometric standard deviation. Concentrations lower than the detection limits were given a value of half the detection limit for statistical analyses.

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Table 3. Statistical Tests for PFCAs in Food Composite Samples

two-way analysis of variance			
factor	locations	year	interaction (location*year)
p-value	0.292	**0.003**[b]	**0.002**[b]

analysis of covariance with PFCAs food intakes, historical, and demographic status in East Asia a model for PFCAs[a]

		location (Kyoto = 0)					interaction (location*year (2000s = 1))			
		Hokkaido	Okinawa	Beijing	Seoul	year (2000s = 1)	Hokkaido	Okinawa	Beijing G	Seoul
	intercept	α	β	γ	σ	ε	ζ	η	θ	ι
parameter	1.822	0.007	−0.072	−0.012	0.098	0.077	−0.020	−0.084	−0.027	0.223
t-value	–	0.150	−1.580	−0.230	1.910	3.170	−0.430	−1.850	−0.520	4.350
p-value	–	0.880	0.120	0.821	0.062	**0.003**[b]	0.668	0.070	0.606	**<.0001**[b]

[a]Regression models are described as ($\log_{10}$ PFCAs food intakes) = (intercept) + $\alpha \times$ [location:Hokkaido] + $\beta \times$ [location:Okinawa] + $\gamma \times$ [location:Beijing] + $\sigma \times$ [location:Seoul] + $\varepsilon \times$ [Year] + $\zeta \times$ [interaction:Hokkaido] + $\eta \times$ [interaction:Okinawa] + $\theta \times$ [interaction:Beijing] + $\iota \times$ [interaction:Seoul]. [b]Bold type indicates significant difference ($p < 0.05$).

Table 4. Comparison of Dietary Intake of PFCAs Observed in the Present Study (Japan, Korea, China) with Reported Data (Japan, Norway)[a]

				dietary intake (ng day^{-1})							
sampling site	year	study type		PFOA (C8)	PFNA (C9)	PFDA (C10)	PFUnDA (C11)	PFDoDA (C12)	PFTrDA (C13)	PFTeDA (C14)	reference
Japan											
overall Japan	1990s	daily duplicate diet	mean	22.8	<8.9	<4.4	12.8	<4.4	11.1	<4.4	this study
	2009	daily duplicate diet	mean	18.0	7.9	3.9	18.4	<3.6	16.0	<3.6	this study
Hokkaido	1992, 1995	daily duplicate diet	mean	<22.5	<9.0	<4.5	15.3	<4.5	15.7	<4.5	this study
	2009	daily duplicate diet	mean	<19.0	8.6	3.9	22.3	6.0	18.4	4.0	this study
Kyoto	1996, 1997	daily duplicate diet	mean	14.3	7.0	<3.5	9.1	<3.5	6.2	<3.5	this study
	2009	daily duplicate diet	mean	24.0	<6.3	<3.2	11.4	<3.2	12.5	<3.2	this study
Okinawa	1992, 1995	daily duplicate diet	mean	<26.1	<10.5	<5.2	13.5	<5.2	10.6	<5.2	this study
	2009	daily duplicate diet	mean	<18.5	9.0	4.8	20.6	5.7	16.5	3.8	this study
Osaka	2004	daily duplicate diet	mean	68.5	–	–	–	–	–	–	Kärman et al., 2009
Miyagi	2004	daily duplicate diet	mean	48.6	–	–	–	–	–	–	Kärman et al., 2009
Korea											
Seoul	1994	daily duplicate diet	mean	<17.8	<7.1	<3.6	8.5	<3.6	10.5	<3.6	this study
	2007	daily duplicate diet	mean	<20.6	<8.2	9.4	63.4	17.4	54.1	9.4	this study
China											
Beijing	1993	daily duplicate diet	mean	<22.5	<9.0	9.0	9.7	7.3	12.6	<4.5	this study
	2009	daily duplicate diet	mean	<30.5	<12.2	13.1	11.0	8.0	13.0	9.0	this study
Norway											
	2008–2009	estimated intakes	mean	31	9.5	13	6.7	6.7	–	–	Haug et al. 2010

[a]Calculated assuming a body weight of 70 kg.

The mean blank signal was subtracted from the calculated sample concentration only if the calculated sample concentration was three times higher than the blank concentration (Section 2.5). Using this method, we established that the MDLs for PFCAs ranged from 2 to 10 pg g^{-1} (Table 1). This was 1 or 2 orders of magnitude higher than the detection response (lower detection limit) for PFCAs in previous studies (MDLs = 100 pg g^{-1} for PFNA, and 500 pg g^{-1} for PFDA and PFUnDA).[6,16]

Total Method Recovery. The mean recoveries (n = 6, ± standard deviation) of the PFCAs obtained by spiking 100 pg of each standard compound into 10 of the composite dietary samples before extraction (Section 2.5) were as follows: 97 ± 16%, PFOA; 98 ± 19%, PFNA; 91 ± 17%, PFDA; 94 ± 18%,

PFUnDA; 90 ± 18%, PFDoDA; 93 ± 16%, PFTrDA; and 97 ± 17%, PFTeDA (Table 1).

Comparison with Other Methods. There have been fewer reports on analytical methods for PFCAs in composite dietary samples than in serum samples. The methods from two reports for PFCAs are summarized in Table S1.[6,7] In one of these methods (No. 2 in Table S1), the samples were freeze-dried for pretreatment, and weak anion exchange and dispersive carbon methods were used for subsequent cleanup.[6] Even after purification, the method had a high detection limit (>100 pg g^{-1}) because of the complex sample matrix. In the other method (No. 3 in Table S1), a solid-phase extraction column containing florisil and ECNI-carb was used for sample cleanup.[7] This method eliminated matrix effects, and the target compounds were detected at low parts per trillion levels. In our method (No. 1 in Table S1), ion-pair extraction and benzyl esterification were used for sample pretreatment. With ECNI, the detection limits with the present method were comparable to those with one of the earlier methods (No. 3).

3.2. Profile of PFCAs in Food Composite Samples. The method was applied to 24-h dietary samples from subjects in Japan (Hokkaido, Kyoto, and Okinawa; 1992 to 2007, and 2009), Korea (Seoul; 1994 and 2007), and China (Beijing; 1993 and 2009). The dietary intakes of PFCAs (nanograms per day and nanograms per wet weight of food) are summarized in Table 2 and Table S2. The levels of the PFCAs were between 39 and 169 ng day^{-1} in Korea, 58 and 71 ng day^{-1} in China, and 56 and 67 ng day^{-1} in Japan. (Table 2) Between the two sampling years, the total levels of PFCAs (C8–C14) increased significantly ($p < 0.05$). The interaction between the sampling location in Korea and year was significant ($p < 0.05$) (Table 3). The PFCAs with longer chains than PFOA comprised 68% (1990s) and 82% (2000s) of the average total PFCAs for the three countries. This finding suggests that the East Asian population has been exposed to both PFOA and long-chain PFCAs.

PFCA exposure from food is only one exposure pathway. Other pathways include exposure to aerosols and household dust. In this study, we assumed the contribution of PFOA from food composite samples in Kyoto. It has been estimated that the adult intake of indoor dust is 50 mg day^{-1} [17] and adult humans inspire 13.3 m^3 of air day^{-1}, with 69% of the particles in air respirable and PFOA completely absorbed into the body. The estimated exposure through food composite samples was dominant (86% of total intake), followed by ambient air (2.4%) and indoor dust (1.1%) (Table S3). This result suggests food and drinking water are the major sources of PFOA exposure for humans. However, one earlier study showed the contribution from ambient air was dominant (about 70% for serum PFOA levels) in Osaka, Japan where there was a strong point source of PFOA.[18]

Dietary intakes of PFCAs in the present study (Japan, Korea, and China) are compared with reported data (Japan, Norway) in Table 4. The dietary intakes observed in our study are compared with those from a report on PFCAs (C8–C12) in Norway.[19] In Norway, the dietary intakes (in ng day^{-1}) of long-chain PFCAs from food were 31 for PFOA, 9.5 for PFNA, 13 for PFDA, 6.7 for PFUnDA, and 6.7 for PFDoDA. In contrast to what was observed in the Japanese and Korean samples, odd-numbered PFCAs did not predominate in the Norwegian samples. This trend is consistent with previous biomonitoring of human serum samples[4,20] which also implies that intake of PFCAs via food may be an important exposure route.

The sources of long-chain PFCAs are not well characterized. A review indicated that odd-numbered PFCAs have been manufactured via oxidation of fluorotelomer olefins.[3] Industrial application of these odd-numbered PFCAs might contribute to the East Asian-specific pattern of PFCAs in daily duplicate diet samples and serum. The temporal increase in long-chain PFCAs, especially in Korea, warrants further investigation of the sources and exposure routes. This would assist in predicting future changes in the food, water, and serum levels of these contaminants.

Even though long-chain PFCAs are prevalent, comprising 82% of the total PFCAs in this study, their toxicokinetics and toxicities are not well characterized. In several in vitro studies, long-chain PFCAs have caused biological responses at lower doses than PFOA.[21−23] Because of these uncertainties, comprehensive toxicological studies on long-chain PFCAs are required.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Figure S1 sample collection and treatment; Figure S2 mass spectra of PFCA benzyl esters in ECNI mode (m/z 30−800); Figure S3 mass spectra of PFCA benzyl esters in EI mode (m/z 30−800); Table S1 comparison of analytical methods for PFCAs analysis in composite dietary samples; Table S2 concentrations of PFCAs in composite dietary samples; Table S3 estimates of adult exposures (ng day^{-1}) to PFOA in Kyoto, Japan. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

PFCAs	perfluorinated carboxylic acids
PFOA	perfluorooctanoic 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
GC/MS	gas chromatography and mass spectrometry
ECNI	electron-capture negative ionization

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Comparative survey of levels of chlorinated cyclodiene pesticides in breast milk from some cities of China, Korea and Japan

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HIGHLIGHTS

- Levels of chlorinated cyclodiene pesticides in Asian breast milk were measured.
- Heptachlor epoxide, dieldrin, endrin, toxaphenes and mirex detected in most samples.
- Levels significantly higher in Japanese, followed by Korean, then Chinese samples.
- α- and β-endosulfans detected at 0.9–1.5 ng g^{-1} lipid in the three countries.
- First report of endosulfan and toxaphene levels in human samples in Asia.

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ABSTRACT

Exposure of mothers to organochlorine pesticides (OCPs) was assessed by measuring the levels of 20 OCPs in 70 human breast milk samples pooled from 210 individuals from China, Korea and Japan. The OCPs were analyzed using gas chromatography/mass spectrometry (GC/MS) in electron capture negative ionization (ECNI) monitoring. The results showed that β-hexachlorocyclohexane and hexachlorobenzene were one order of magnitude higher in China than in the other nations, whereas chlordanes and polychlorinated biphenyl levels were highest in Japan. Heptachlor epoxide, dieldrin, endrin, toxaphenes and mirex were detected in most samples, and levels of these chemicals were significantly higher in Japan (0.8–4.5 ng g^{-1} lipid), followed by Korea (0.2–4.7 ng g^{-1} lipid), and lowest in China (less than 1.0 ng g^{-1} lipid). α- and β-endosulfans were detected at a range of 0.9–1.5 ng g^{-1} lipid levels in all samples analyzed, and their levels were higher in Korean than in Chinese samples.

1. Introduction

Widespread contamination and toxic effects of persistent organic pollutants (POPs) in humans and wildlife are of great concern and have received considerable attention during the past four decades. Despite the ban on use of organochlorine pesticides (OCPs) in most developed nations since the early 1970s, their use continued until very recently in many developing countries for agricultural and public health purposes (Wong et al., 2005). As OCPs are highly lipophilic and persistent, chronic exposure in humans via the food chain has led to the accumulation of both parent compounds and their metabolites in lipid-rich tissues such as adipose tissues and breast milk (Nakata et al., 2002; Sudaryanto et al., 2006).

The human body burden of POPs such as dichlorodiphenyltrichloroethanes (DDTs), hexachlorocyclohexanes (HCHs), chlordanes (CHLs), hexachlorobenzene (HCB) and polychlorinated biphenyls (PCBs) has been well documented worldwide. Although data have shown trends of decreasing contamination until the 1990s (Konishi et al., 2001; Kunisue et al., 2006), there is limited information on recent trends for a wide range of POPs, especially chlorinated cyclodiene congeners in Asian countries. Surveys of human milk contamination in Asia revealed that the levels of DDTs and HCHs are higher in China and Vietnam, whereas the levels of CHLs and PCBs are higher in Japan and Korea (Haraguchi et al., 2009; Hedley et al., 2010; Zhou et al., 2011). We recently detected dicofol in human milk samples from Asia at 1–10 ng g^{-1} lipid ranges, which accounted for about 1% of DDTs (Fujii et al., 2011). The source of the recent DDT body burden might be dicofol because the product is contaminated with ~20% DDT as an impurity in China (Qiu et al., 2005).

Endosulfan has been used as an insecticide around the world (Weber et al., 2010) and is classified by the World Health Organization as a priority pollutant. In some European countries (Denmark, Finland and Italy), exposure to endosulfan and its

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