Table 1
Characteristics of personal care product samples and compounding agents.

(a) Personal care products (PCP) samples

Sample No.	Type of PCP	Specific features	Application body site	Fluorinated ingredients (Ingredients listed in the labels on each product)	Sampling year	Country of origin	Manufacturing company
Cosmetics							
1	Manicure	–	Nail	PAPs[c]	2009	Japan	A
2	Manicure	–	Nail	PAPs[c]	2009	Japan	B
3	Foundation	–	Face	PAPs[c]	2009	France	C
4	Lip rouge	–	Lip	PAPs[c]	2009	Japan	D
5	Manicure (base coat)	–	Nail	PAPs[c]	2009	Japan	A
6	Powder fundation	–	Face	PAPs[c]	2011	Japan	E
7	Powder fundation	–	Face	PAPs[c]	2011	France	F
8	Powder fundation	–	Face	PAPs[c], polyfluorooctylmethyl trimethoxysilane	2011	Japan	G
9	Powder fundation	–	Face	PAPs[c], perfluorooctyl triethoxysilane	2011	Japan	B
10	Liquid foundation	–	Face	PAPs[c], perfluoroalkyl ethoxydimethicone	2011	Japan	E
11	Liquid makeup base	–	Face	PAPs[c], perfluoroalkyl ethoxydimethicone	2011	Japan	E
12	Foundation	–	Face	Polyfluoroalkyl silylated mica	2009	Korea	H
13	Foundation	–	Face	Polyfluorooctyl trimethoxysilane	2009	Japan	A
14	Lip rouge	–	Lip	Stearyl methacrylate/perfluorooctylethyl methacrylate copolymer	2009	Japan	E
15	Powder fundation	–	Face	Grapeseed fatty acid perfluorononyl octyldodecyl glycol	2011	United States	I
Sunscreen							
1-1[b]	Skin milk	Waterproofness	Face and body	PAPs[c]	2007	Japan	J
1-2[b]	Skin milk	Waterproofness	Face and body	PAPs[c]	2009	Japan	J
1-3[b]	Skin milk	Waterproofness	Face and body	PAPs[c]	2011	Japan	J
1-4[b]	Skin milk	Waterproofness	Face and body	PAPs[c]	2011	Japan	J
2	Skin milk	Waterproofness	Face and body	PAPs[c]	2011	Japan	J
3	Skin milk	–	Face	PAPs[c]	2011	Japan	J
4	Powder foundation	Waterproofness	Face	PAPs[c]	2011	Japan	B
5	Liquid foundation	Waterproofness	Face	PAPs[c], PEG-8 trifluoropropyl dimethicone copolymer	2011	Japan	B
6	Foundation	–	Face	Polyperfluoroethoxymethoxy difluoroethyl PEG phosphate	2011	Japan	J
Control samples (does not list any fluorinated ingredients in their labels)[a]							
1	Cosmetic (manicure)	–	Nail	–	2009	Japan	A
2	Sunscreen (skin milk)	–	Face and body	–	2011	Japan	J

(b) Compounding agents of PCPs

Sample No.	Component	Composition[d] (Weight%)	Sampling year	Country of origin
Compounding agents				
1	Mica treated with PAPs[c]	Mica: 95 (%), PAPs[c]: 5 (%)	2012	Japan
2	Talc treated with PAPs[c]	Talc: 95 (%), PAPs[c]: 5 (%)	2012	Japan

[a] The cosmetic and sunscreen samples that did not list any fluorinated ingredients in their labels were used as control samples.
[b] Sunscreen nos. 1-1, 1-2, 1-3 and 1-4 are the same product with different lot numbers.
[c] PAPs; polyfluoroalkyl phoshate esters.
[d] Composition listed in their raw material specification sheet.

2.5. Method detection limits (MDLs), method quantification limits (MQLs), blank contamination, extraction recovery and total analyte recovery

PFCAs were dissolved in 100 μL of a 0.1 M benzyl bromide/acetone solution and derivatized at 60 °C for 60 min with 1 ng of 11H-perfluoroundecanoic acid as external calibration standard. After benzylation, the stability was investigated by monitoring the peak area ratio over 24 h. Milli-Q water (Merck KGaA Millipore, Billerica, MA) was used as the procedural blank control, and was analyzed after every three samples ($n = 9$). The procedural blank was extracted using the process described above, and five replicate procedural blanks were prepared independently. In this study, we observed blank contamination for all PFCAs except PFTeDA (Table 2). The MDL and MQL were defined by the following equation; MDL = $\alpha + 3\beta$, MQL = $\alpha + 10\beta$, where α is the mean of the blank measurements and β is the standard deviation of the blank measurements (ACS Committee, 1980). For PFTeDA, because there was no blank signal, the MDL and the MQL were defined as the mass of the analyte producing a peak with a signal-to-noise ratio of three and ten, respectively. The MDL and MQL varied according to the sample volume being used in chemical analysis. Using the established MDL and MQL values, we calculated the sum of the PFCAs concentrations using the following upper and lower boundary rules. (1) Upper boundary: concentrations lower than the MQL (and higher than MDL) were assigned the MQL for the calculation.

Table 2
Recoveries, method detection limits and method quantification limits for PFCAs analysis.

Compound (carbon atoms)		Quantification ions (confirmation ions) m/z	Instrument detection limit[a] (pg) (S/N = 3)	Extraction recovery of PFCAs [b]% (SD%)		Total analyte recovery of PFCAs [c]% (SD%)		Procedural blank (SD) (pg, $n = 9$)	Method detection limit[d,e] (for 1 mg samples)[f] (ng g^{-1})	Method quantification limit[d,e]
				10 ng spiked ($n = 6$)	0.1 ng spiked ($n = 6$)	10 ng spiked ($n = 6$)	0.1 ng spiked ($n = 6$)			
PFHxA	(C6)	313 (294)	0.004	82(11)	79(6)	63(9)	60(5)	19(15.6)	66	176
PFHpA	(C7)	363 (344)	0.004	79(8)	94(26)	61(11)	73(18)	40(6.8)	60	108
PFOA	(C8)	413 (394)	0.003	106(10)	101(13)	82(20)	78(9)	26(10.5)	57	131
PFNA	(C9)	463 (444)	0.003	96(8)	102(10)	74(19)	79(7)	11(3.2)	21	43
PFDA	(C10)	513 (494)	0.004	85(5)	91(7)	65(17)	70(4)	13(4.1)	25	54
PFUnDA	(C11)	563 (544)	0.004	86(4)	78(6)	66(15)	60(8)	9(2.0)	15	29
PFDoDA	(C12)	613 (594)	0.005	97(7)	107(7)	75(15)	83(11)	10(4.8)	24	58
PFTrDA	(C13)	663 (644)	0.005	95(9)	92(12)	73(16)	71(14)	3(2.4)	10	27
PFTeDA	(C14)	713 (694)	0.007	94(6)	103(7)	72(16)	79(10)	<0.7	0.7[d]	2.3[d]
11H-PFUnDA	–	554	–	–	–	77(19)	75(8)	–	–	–

SD: relative standard deviation.
[a] 1 μL injection.
[b] Extraction recovery; all native PFCAs were spiked into control samples (see Table 1) before extraction. Extraction recovery is observed by comparing the response signal of native PFCAs and 11H-PFUnDA, which were added after extraction. This extraction recovery does not include derivatization efficiency.
[c] The total analyte recovery is obtained by multiplying extraction recovery rate by derivatization efficiency. We obtained the derivatization efficiency by comparing the response signals of 11H-PFUnDA and CB111.
[d] The values for MDL and MQL are given by MDL = $\alpha + 3\beta$ and MQL = $\alpha + 10\beta$ (α is the mean of the blank measures; β is the standard deviation of the blank measures).
[e] No blank response was observed for PFTeDA. Its MDL was calculated from the instrument detection limit (signal-to-noise ratio of 3) and its MQL was calculated from a signal-to-noise ratio of 10.
[f] The sample size was approximately 1–200 mg and varied according to sample concentration.

Concentrations lower than the MDL were assigned the MDL for the calculation. (2) Lower boundary: concentrations lower than the MQL (and higher than MDL) were given the MDL for the calculation. Concentrations lower than the MDL were given zero for the calculation.

For method validation, we have checked extraction recovery and total analyte recovery. First, native PFCAs were added in the control PCP samples before extraction. Then, after extraction, we added underivatized 11H-PFUnDA as the external calibration standard. In addition to that, we added $^{13}C_{12}$-labeled PCB (CB111) to check the derivatization efficiency of 11H-PFUnDA. We chose CB111 because it was not affected by the derivatization and was not a naturally-occurring chemical. Then, we derivatized the compound with benzyl bromide/acetone solution and directly injected the derivative into the GC–MS. Extraction recovery and total analyte recovery were calculated based on the following assumptions: (1) Extraction recovery was observed by comparing the response signal of native PFCAs and 11H-PFUnDA and (2) The total analyte recovery was obtained by multiplying extraction recovery rate by derivatization efficiency. We obtained the derivatization efficiency by comparing the response signal of 11H-PFUnDA and CB111. Derivatization efficiency of all PFCAs and 11H-PFUnDA was assumed to be the same. The recoveries of the PFCAs were examined by spiking 10 and 0.1 ng of each native PFCA standard into control sunscreen samples ($N = 3$, for each spiking levels) and control cosmetic samples ($N = 3$, for each spiking levels) before extraction. In total, six samples were used for each spiking level. The results are summarized in the Table 2.

3. Results and discussion

3.1. The levels of PFCAs in PCPs

In the present study, we successfully quantified PFCAs in consumer-ready PCPs with total analyte recoveries ranging from 60% to 83% (Table 2). The concentrations of the PFCAs in the PCP samples are presented in Table 3. Twenty-one of the 24 samples that listed fluorinated compounds in their INCI labels contained detectable levels of PFCAs (13 of 15 cosmetic samples, 8 of 9 sunscreen samples). The maximum concentrations of total PFCAs were 5.9 μg g^{-1} for cosmetics and 19 μg g^{-1} for sunscreens. There was a large variation in PFCAs concentrations. For example, the levels of PFCAs in cosmetics samples nos. 4 and 5 and sunscreen sample No. 3 were two orders of magnitude lower than those in the other samples. The concentrations of PFCAs were highest in sunscreen sample no. 1–1, which contained 6.5 μg g^{-1} PFHxA, 5.7 μg g^{-1} PFOA, 2.9 μg g^{-1} PFDA, and 1.4 μg g^{-1} PFDoDA. Although PFHxA and PFOA were present at the highest concentrations, many different PFCAs were detected. The levels of PFOA in the PCPs were higher than those previously reported for end consumer products (Washburn et al., 2005). The contribution of PCPs to human PFCAs exposure is still unclear. The relatively high concentration of PFCAs in consumer-ready PCPs suggests that they could represent an exposure route for humans. If so, PCPs may explain the exposure source to females who have higher PFCAs levels in serum. Although the concentrations of PFCAs in female serum are similar or lower than that in males in the National Health and Nutrition Examination Survey in the US (Calafat et al., 2007), a recent study in China revealed PFOA levels in female serum have been increasing (Jin et al., 2007). The contribution of the PCPs to the total human PFCA exposure could be wide range of variations due to individual preferences as well as market supplies. Further studies are needed to investigate the relationship between the use of PCPs and exposure to PFCAs.

3.2. The levels of PFCAs in compounding agents

We further searched sources of PFCAs in end consumer products. In this study, the PCPs that listed PAPs and the other fluorinated compounds in their INCI labels were collected (Table 1). All the samples that had PAPs in their labels contained detectable levels of PFCAs (11 cosmetic samples, five sunscreen samples), while some of PCPs that had other fluorinated compounds in their labels did not contain detectable concentrations of PFCAs (cosmetic sample nos. 14 and 15, and sunscreen sample No. 6) (Table 3). By contrast, cosmetic and sunscreen samples that were produced by the same manufacturers and did not list PAPs or other fluorinated

Table 3
Levels of PFCAs in personal care products.

Sample No.		Compound (carbon atoms), concentration (ng g^{-1})											
		PFHxA (C6)	PFHpA (C7)	PFOA (C8)	PFNA (C9)	PFDA (C10)	PFUnDA (C11)	PFDoDA (C12)	PFTrDA (C13)	PFTeDA (C14)	ΣPFCAs Actual[e]	Upper[f]	Lower[g]
(In a concentration order)													
Cosmetics													
8	Powder fundation	1100	170	1700	240	1000	150	940	67	590	5900	–	–
10	Liquid foundation	2100	290	1500	230	610	110	260	35	84	5100	–	–
3	Foundation	410	150	1400	250	880	130	430	56	210	3900	–	–
6	Powder fundation	800	200	1100	380	650	180	360	71	140	3900	–	–
9	Powder fundation	910	160	390	110	320	67	160	28	59	2200	–	–
1	Manicure	140	45	910	140	450	62	230	16	44	2000	–	–
12	Foundation	180	150	430	340	270	150	140	56	62	1800	–	–
2	Manicure	24	Trace (<29)[a]	204	42	108	21	58	8.2	12	–	506	492
7	Powder fundation	110	25	91	22	74	14	43	8.0	18	410	–	–
13	Foundation	360	25	7.9	Trace (<1.2)[a]	3.6	Trace (<0.80)[a]	Trace (<1.6)[a]	n.d. (<0.28)[b]	n.d. (<0.019)[b]	–	402	400
11	Liquid makeup base	55	13	43	3.4	12	2.1	5.9	0.91	1.8	140	–	–
5	Manicure (base coat)	4.7	Trace (<2.0)[a]	15	2.5	7.0	1.2	3.3	Trace (<0.50)[a]	1.5	–	38	37
4	Lip rouge	Trace (<3.8)[a]	Trace (<2.4)[a]	4.1	1.0	2.8	0.76	2.4	Trace (<0.59)[a]	0.75	–	19	15
14	Lip rouge	n.d. (<5.7)[b]	n.d. (<5.1)[b]	n.d. (<4.9)[b]	n.d. (<1.8)[b]	n.d. (<2.1)[b]	n.d. (<1.3)[b]	n.d. (<2.1)[b]	n.d. (<0.87)[b]	n.d. (<0.060)[b]	–	24	0
15	Powder fundation	n.d. (<1.9)[b]	n.d. (<1.7)[b]	n.d. (<1.7)[b]	n.d. (<0.60)[b]	n.d. (<0.73)[b]	n.d. (<0.43)[b]	n.d. (<0.71)[b]	n.d. (<0.30)[b]	n.d. (<0.020)[b]	–	8.1	0
Sunscreen													
1–1[d]	Skin milk	6500	670	5700	670	2900	330	1400	140	600	19,000	–	–
2	Skin milk	3300	530	1700	350	1100	210	640	100	300	8200	–	–
1–4[d]	Skin milk	2900	450	1500	300	950	190	570	89	260	7200	–	–
1–2[d]	Skin milk	2700	390	1400	280	970	170	590	88	280	6900	–	–
1–3[d]	Skin milk	1300	210	680	140	440	81	250	39	107	3200	–	–
4	Powder foundation	350	62	270	73	210	47	160	29	76	1300	–	–
5	Liquid foundation	180	53	380	170	170	76	78	28	28	1200	–	–
3	Skin milk	Trace (<4.6)[a]	n.d. (<1.6)[b]	3.7	Trace (<1.1)[a]	1.9	Trace (<0.77)[a]	Trace (<1.5)[a]	n.d. (<0.27)[b]	n.d. (<0.018)[b]	–	15	8.8
6	Foundation	n.d. (<2.3)[b]	n.d. (<2.1)[b]	n.d. (<2.0)[b]	n.d. (<0.72)[b]	Trace (<1.9)[a]	Trace (<1.0)[a]	n.d. (<0.90)[b]	n.d. (<0.36)[b]	n.d. (<0.025)[b]	–	11	1.4
Control samples (do not list any fluorinated ingredients in their labels) [c]													
1	Cosmetics (manicure)	n.d. (<0.61)[b]	n.d. (<0.56)[b]	n.d. (<0.53)[b]	n.d. (<0.19)[b]	n.d. (<0.23)[b]	n.d. (<0.14)[b]	n.d. (<0.23)[b]	n.d. (<0.094)[b]	n.d. (<0.0065)[b]	–	2.6	0
2	Sunscreen (skin milk)	n.d. (<0.32)[b]	n.d. (<0.29)[b]	n.d. (<0.28)[b]	n.d. (<0.10)[b]	n.d. (<0.12)[b]	n.d. (<0.071)[b]	n.d. (<0.12)[b]	n.d. (<0.049)[b]	n.d. (<0.0034)[b]	–	1.3	0

Each sample was quantified once.
MDL: method detection limit; MQL: method quantification limit.
[a] If the concentration levels are under MQL (and upper MDL), the levels are descrived as "Trace (<the value of MQL)". MQL varied according to the sample volume used in chemical analysis.
[b] If concentration levels are under MDL, the levels are descrived as "n.d. (<the value of MDL)". MDL varied according to the sample volume used in chemical analysis.
[c] The cosmetic and sunscreen samples that did not list any fluorinated ingredients in their labels were used as control samples.
[d] Sunscreen Nos. 1-1, 1-2, 1-3 and 1-4 are the same product with different lot numbers.
[e] Actual; If all PFCA compounds are quantified, actual sum of PFCAs were calculated.
[f] Upper boundary; concentrations lower than the MQL and higher than MDL were given the MQL for the calculation. Concentrations lower than the MDL were given the MDL for the calculation.
[g] Lower boundary; concentrations lower than the MQL and higher than MDL were given the MDL for the calculation. Concentrations lower than the MDL were given zero for the calculation.

Table 4
Levels of PFCAs in compounding agents of PCPs.

Sample No.	Component	Composition[b] (Wt%)	Compound (carbon atoms), concentration (ng g^{-1})										
			PFHxA (C6)	PFHpA (C7)	PFOA (C8)	PFNA (C9)	PFDA (C10)	PFUnDA (C11)	PFDoDA (C12)	PFTrDA (C13)	PFTeDA (C14)	ΣPFCAs	PFCAs/PAPs[b,c] (μg g^{-1}-PAPs)
Compounding agents													
1	Mica treated with PAPs[a]	Mica: 95, PAPs:5	8400	3100	6000	3200	5700	2300	3800	1100	1900	35,000	700
2	Talc treated with PAPs[a]	Talc: 95, PAPs:5	500	130	350	190	600	140	350	62	150	2500	50

Each sample was quantified once.
[a] PAPs: polyfluoroalkyl phoshate esters.
[b] Composition listed in their raw material specification sheet.
[c] On the basis that the specified amounts of PAPs are reliable, the levels of PFCAs in PAPs were estimated.

compounds in their INCI labels did not contain any detectable levels of PFCAs (control samples nos. 1 and 2). These results suggested that PAPs may be a major source of PFCAs in consumer-ready PCPs. To confirm this possibility, we analyzed commercially available compounding agents for PCPs, mica and talc, which were treated with PAPs. As anticipated, high concentrations of PFCAs were detected in these compounding agents (total PFCAs 2.5 μg g^{-1} for talc treated with PAPs, 35.0 μg g^{-1} for mica treated with PAPs) (Table 4).

3.3. The possible source of PFCAs to PCPs and compounding agents

The contamination pathway of PFCAs in PCPs and compounding agents is still unknown. As mentioned above, FTOHs are important intermediates for the synthesis of PAPs. The starting material, perfluoroalkyl iodide ($CF_3(CF_2)_nI$, Telomer A) is manufactured by telomerization with a telogen such as pentafluoroethyl iodide (CF_3CF_2I) and a taxogen such as tetrafluoroethylene ($CF_2{=}CF_2$) (Lehmler, 2005). Telomer A is then coupled with ethylene ($CH_2{=}CH_2$) and converted to perfluoroalkyl ethyl iodide ($CF_3(CF_2)_n CH_2CH_2I$, Telomer B). Hydrolysis of Telomer B forms FTOH ($CF_3(CF_2) CH_2CH_2OH$: Telomer BA). FTOHs unintentionally contain perfluoroalkylate ester ($CF_3(CF_2)_nCOO(CF_2)_nCF_3$) and unreacted Telomer A (DuPont Company, 2006). Hydrolysis and oxidation of these impurities could result in the formation of PFCAs (DuPont Company, 2006). Thus we speculate that the origin of PFCAs in PCPs and compounding agents are impurities of PAPs. In addition to that, biotransformation of PAP to PFCA has been observed in rats (D'eon and Mabury, 2007, 2011) and in a microbial system (Lee et al., 2010). However, degradation of PAPs in finished consumer products is still not well-characterized. Future studies are needed to reveal the exact origin and pathway of PFCAs in PCPs.

3.4. Possible health concerns from direct application of PCPs

Cosmetics and sunscreens are applied directly to human skin, which is of concern because of dermal exposure to PFCAs. Recent data have indicated that statistically significant and dose-responsive increases in serum PFOA concentrations follow topical dermal application in rodent models, which suggests that PFOA is dermally absorbed (Franko et al., 2012). PFOA was also found to be immunotoxic following dermal exposure, with an enhancement of a hypersensitivity response to ovalbumin, which implies that PFOA exposure may augment the immunoglobulin E response to environmental allergens (Fairley et al., 2007). Because PFCAs are likely to cause similar effects in humans, health risk from PAP usage in PCPs needs to be assessed. In addition, measures need to be taken to reduce impurities in PCPs or promote use of alternative materials.

3.5. Possible effect of PCPs to the indoor environment

In addition to the effect of direct application to human skin, residual PFCAs in the PCPs may be an important emission source in indoor spaces. The indoor environment is thought to be an important source of human exposure for PFCAs. Several studies have reported detection of PFCAs in indoor dust (Strynar and Lindstrom, 2008; Zhang et al., 2010; Liu et al., 2011; Shoeib et al., 2011) and associations between levels of fluorinated chemicals in indoor dust and the levels in human serum (Haug et al., 2011; Fraser et al., 2012). However, the exact source has not been identified. Previous studies have revealed that organic chemicals used in PCPs, like polycyclic musks, are detected in house dust (Fromme et al., 2004; Lu et al., 2011). Residual PFCAs in PCPs could also be a source of PFCAs in house dust. Indeed, PAPs were detected in house dust and positively correlated with the PFCAs level in a recent study (De Silva et al., 2012). Clearly detailed emission studies and exposure assessments are required.

3.6. Limitation of this study

A major limitation of this study is that the concentrations of PAPs in PCP samples and the compounding agents were not determined. Therefore, the amounts of PAPs in PCPs are unclear. Thus our discussion at the present time remains speculative. For compounding agents, the composition is listed in their raw material specification sheet (95% of Mica or Talc and 5% of PAPs). On the basis that the specified amounts of PAPs are reliable, the levels of PFCAs in PAPs were estimated to 700 μg g^{-1}-PAPs and 50 μg g^{-1}-PAPs (Table 4). However compositional information such as chain length of the PAPs was not specified. Thus rigorous analyses of those PAPs and PFCAs are not possible. To investigate further, chemical analysis of PAPs in PCP and the compounding agents is strongly recommended. In terms of the sample size, we analyzed 21 samples for PCPs, but only 2 samples for compounding agents. Clearly a more extensive study is needed to properly define the relationship between PAPs and PFCAs.

4. Conclusion

In conclusion, we have quantified PFCA concentrations in PCPs and identified compounding agents as a major source of PFCAs. The limitations of this study are the small sample size and the fact that the concentrations of PAPs in the PCP samples and the compounding agents were not determined. To establish rational management policies for PAPs in PCPs, further studies are needed to ascertain the relationship between PAPs and PFCAs.

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Dietary Intake of Radiocesium in Adult Residents in Fukushima Prefecture and Neighboring Regions after the Fukushima Nuclear Power Plant Accident: 24-h Food-Duplicate Survey in December 2011

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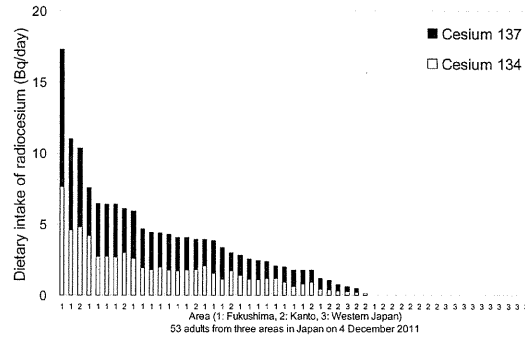
Supporting Information

ABSTRACT: Since the nuclear power plant accident in Fukushima in March 2011, the Japanese government has conducted screening and removal of contaminated foods from the market that exceed provisional regulation limits for radionuclides. This study aimed to provide an urgent estimate of the dietary exposure of adult residents recruited from three areas in Japan to cesium 134 (^{134}Cs), cesium 137 (^{137}Cs), and, for comparison, natural potassium 40 (^{40}K) on December 4, 2011. Fifty-three sets of 24-h food-duplicate samples were collected in Fukushima Prefecture and neighboring regions. The ^{134}Cs, ^{137}Cs, and ^{40}K levels in the samples were measured using a germanium detector. Items in the food-duplicate samples were recorded and analyzed for radiocesium intake. Radiocesium was detected in 25 of 26 samples from Fukushima. The median dietary intake of radiocesium was 4.0 Bq/day (range <0.26−17 Bq/day). The estimated annual dose from radiocesium was calculated assuming that the daily intake of radiocesium was constant throughout the year. The median estimated dose level was 23 μSv/year (range <2.6−99 μSv/year). The estimated dose level of radiocesium was significantly higher in Fukushima than in the Kanto region and western Japan. Stepwise multiple linear regression analyses demonstrated that the intake of fruits and mushrooms produced in Fukushima were significant factors for the dietary intake of ^{137}Cs in the 26 participants from Fukushima. The average radioactivity (±SD) of locally produced persimmons and apples (n = 16) were 23 ± 28 and 30 ± 35 Bq/kg for ^{134}Cs and ^{137}Cs, respectively. The preliminary estimated dietary dose levels among Fukushima residents were much lower than the maximum permissible dose 1 mSv/year, based on new Japanese standard limits for radiocesium in foods (100 Bq/kg for general foods). In future studies, the exposure estimates should be refined by probability sampling to eliminate biases.



INTRODUCTION

Following the Tohoku earthquake and tsunami on March 11, 2011 off the Pacific coast, the Fukushima Daiichi nuclear power plant suffered explosions between March 12 and 15.[1] Radionuclides, including iodine, cesium, strontium, and plutonium, were released into the northern part of Japan and the Pacific Ocean. Although the direct threat from the radioactive plume of the crippled plant has passed, there are serious concerns about the deposition of and soil contamination by emitted radionuclides with long half-lives.[2]

Exposure doses from the deposited radioactivity demand continuous assessment. In particular, residents have serious concerns about their levels of internal exposure to radionuclides through the ingestion of contaminated food and drink. The local food supply has been monitored for the presence of radioactive elements by the authorities, and restrictions were set up to prevent contaminated foods being distributed to market.[3] When sampled food products were found to contain radiocesium levels that exceeded safety limits, their distribution was prohibited. However, the radioactive content of a particular food item (in becquerels per kilogram) does not necessarily reflect the daily dose for a resident, owing to the dilution of contaminated material in the market and the variety of available food items. Therefore, the ingested dose needs to be evaluated on the basis of the level of radioactivity contained in complete meals consumed (becquerels per day per person). In our previous study in Fukushima, the dietary exposure to radiocesium from food products available in local grocery stores was estimated to be 0.5 (range, <0.2−7.2) and 0.6

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Table 1. Demographic Characteristics and Average Food Intake of the Study Participants[b]

area	*n*	sex male/female	age (yr)	HSD test[a]	height (cm)	weight (kg)	BMI	food consumption (g/day)	HSD test[a]
total	53	1/52	48.3 ± 14.3		157.4 ± 5.3	53.4 ± 7.4	21.5 ± 2.6	2787 ± 641	
Fukushima	26	1/25	55.2 ± 13.1	A	156.3 ± 5.8	55.4 ± 9.1	22.6 ± 2.9	3044 ± 567	A
Kanto	16	0/16	41.4 ± 12.2	B	158.1 ± 4.6	52.2 ± 4.2	20.9 ± 1.9	2645 ± 715	AB
Western Japan	11	0/11	42.2 ± 13.1	B	159.4 ± 4.9	50.0 ± 5.1	19.6 ± 1.2	2387 ± 433	B

[a]Means with different letters differ significantly ($p < 0.05$, 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. [b]BMI: body mass index; HSD test: Tukey–Kramer honestly significant difference test. Data are presented as means ± SD.

Table 2. Composition of the Food-Duplicate Samples and Their Origins[c]

	daily consumption (g/day)[a]				daily consumption of products of Fukushima origin in the Fukushima group (g/day)	
food group	Fukushima	Kanto	Western Japan	National Health and Nutrition Survey 2009 females (40–49 yr old)	mean	median
1. rice	310.7 ± 153.4	313.5 ± 238.7	200.8 ± 116.8	274.3 ± 143.7	157.6 ± 187.1	114.6
2. other cereals	144.2 ± 128.6A	243.5 ± 215.7AB	310.8 ± 177.2B	109.6 ± 108.1	38.1 ± 72.0	0
3. potatoes, etc.	84.7 ± 75.8	50.5 ± 56.0	56.7 ± 55.5	49.1 ± 57.1	57.6 ± 78.4	14.9
4. beans	101.3 ± 98.5	55.9 ± 54.8	38.5 ± 32.4	52.0 ± 66.7	36.0 ± 61.9	0
5. nuts and seeds	0.02 ± 0.10	4.0 ± 6.8	0.6 ± 1.5	1.7 ± 4.9	0	0
6. vegetables	383.5 ± 196.4A	237.6 ± 98.8B	191.6 ± 94.3B	263.3 ± 144.0	241.8 ± 194.9	205.8
7. vegetable juice	15.2 ± 53.9	40.4 ± 76.9	5.5 ± 18.3	–	0	0
8. fruits	216.9 ± 124.0A	110.9 ± 70.4B	110.9 ± 88.5B	88.3 ± 111.3	109.5 ± 84.2	101.3
9. fruit juice	5.4 ± 27.7	12.8 ± 51.3	14.0 ± 46.4	–	0	0
10. mushrooms	20.9 ± 31.3	13.6 ± 21.4	17.1 ± 16.0	15.1 ± 24.9	3.8 ± 11.0	0
11. seaweeds	18.8 ± 27.0	6.2 ± 13.5	3.2 ± 7.3	7.2 ± 13.3	–	–
12. fish and shellfish	64.6 ± 58.9	46.0 ± 62.9	35.8 ± 31.7	57.3 ± 61.5	–	–
13. meats	52.0 ± 27.2	64.7 ± 53.5	59.0 ± 45.7	90.1 ± 70.0	10.5 ± 19.1	0
14. eggs	34.6 ± 40.0	32.2 ± 36.2	18.9 ± 20.9	35.5 ± 33.3	20.2 ± 25.9	0
15. milks	142.4 ± 126.3	114.9 ± 119.7	101.5 ± 119.2	99.0 ± 136.3	62.0 ± 106.0	0
16. confectioneries[b]	33.6 ± 37.5	57.1 ± 65.0	23.6 ± 23.3	26.8 ± 43.7	11.6 ± 24.8	0
17. beverages	650.1 ± 336.4	833.1 ± 408.4	762.8 ± 386.6	671.1 ± 444.2	63.1 ± 143.1	0
18. liquids	704.1 ± 414.2A	391.1 ± 295.6B	427.8 ± 261.6AB	–	435.7 ± 367.3	389.4
water content (%)	83.1 ± 2.9A	85.3 ± 2.7AB	86.5 ± 3.7B			

[a]Means with different letters differ significantly ($p < 0.05$, 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. [b]Confectioneries include baked goods, rice crackers, fried confectionery, unbaked cake, dry confectionery, candy, chocolate, chewing gum, confiture, snacks, and frozen desserts. [c]Liquids include tap water, well water, soup, seasonings, and oils. Data are presented as means ± SD.

(<0.2–7.0) Bq/day for ^{134}Cs and ^{137}Cs, respectively, in July 2011.[4] However, those doses may differ from the actual exposure to residents depending on various dietary habits. The estimated release of radionuclides from the Fukushima Daiichi nuclear power plant (reactors nos. 1, 2, and 3) into the atmosphere was 1.8×10^{16}, 1.5×10^{16}, and 1.4×10^{14} Bq for ^{134}Cs, ^{137}Cs, and ^{90}Sr, respectively.[4] In the case of the Chernobyl accident, emission was estimated to be 4.7×10^{16}, 8.5×10^{16}, and 1.0×10^{16} Bq for ^{134}Cs, ^{137}Cs, and ^{90}Sr, respectively.[5] Indeed, the proportion of ^{90}Sr to ^{137}Cs in soil samples in Fukushima was 2.6×10^{-3}.[6] The estimated contribution of ^{90}Sr, plutonium, and ^{106}Ru in the dietary exposure among local residents was 12%–16% of doses from ^{134}Cs and ^{137}Cs 1 year after the accident.[7]

In the present study, we investigated the dietary exposure to ^{134}Cs and ^{137}Cs using the food-duplicate method among adult residents of Fukushima Prefecture outside the evacuation zone and neighboring regions in December 2011. In this study, participants were recruited by nonprobability sampling to obtain an urgent estimate of exposure to obtain the scales of risk at an early stage after the accident. Such nonprobability sampling can be regarded as justifiable because urgency outweighs rigorousness in the emergency situation. The composition of the items in the food-duplicate samples was also analyzed to elucidate the possible sources of exposure to radiocesium. ^{40}K was analyzed to compare the dietary intake of natural ^{40}K with radiocesium intake.

METHODS

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.

Recruitment of participants was conducted at the Fukuoka, Osaka, Nagoya, Tokyo, and Fukushima offices of the Asahi Shimbun Company. News writers were asked to recruit adult females living in the vicinity of their offices by means of the address book they used for newsgathering activities; the subjects had to cook their own meals, not live in a single-person household, and not be either family members of the writers or employees of the Asahi Shimbun Company. Individuals who intentionally avoided food products from eastern Japan were excluded. This sampling relied on available subjects. Although there were reports of certain food items in

some areas being contaminated with radiocesium, the levels of contamination were largely unknown to the participants and recruiters. Thus, the sampling design can be assumed to be blind. Possible biases might may have occurred be inherent to the selection of residents in Fukushima.

Each participant was instructed to cook ordinary everyday meals. The 24-h food-duplicate portion samples consisted of whole-day meals and beverages using the menus provided, as previously described.[8] Meals were collected on 4 December 2011 from 26 people in Fukushima Prefecture living outside the evacuation zone, 16 people in the Kanto (Tokyo and its surrounding area) region, and 11 people in western Japan, including the Chubu, Kansai, and Kyushu regions (Table 1). Residential area, sex, age, and occupation of the individuals are listed in Supporting Information, Table S1. All food items were transported daily to Kyoto University at 4 °C for processing and analysis.

The food-duplicate portion samples were homogenized together with the beverages, desserts, and snacks. The final volumes were recorded, and the samples were processed for freeze-drying. The water content was determined based on the sample weights after freeze-drying.

Each food item in the food-duplicate samples was separated and weighed taking advantage of the menu record and then coded by veteran nutritionists in accordance with food composition tables. The foods consumed by the subjects were divided into 18 different groups (Table 2). The places of origin of the food items were also recorded in the menu record, and the consumption of food originating from Fukushima Prefecture was calculated.

In addition to the food-duplicate samples, fruits (persimmons and apples) grown through summer to autumn in three cities (Fukushima, Nihonmatsu, and Minamisoma) in Fukushima Prefecture were purchased at markets in December 2011. The edible portions of the fruits were separated and homogenized.

Determination of ^{137}Cs, ^{134}Cs, and ^{40}K. Aliquots (200–300 g) of the processed food-duplicate samples or fruit homogenates were weighed and sealed in cylindrical plastic containers. Radiometric determinations were performed using a high-purity, low-background, high-resolution germanium detector at the Radioisotope Research Center of Kyoto University, which was protected by a lead shield (10 cm thick internally) and covered with 0.5 mm of electrolytic copper. A multichannel analyzer (4,096 channels; range 0–3000 keV; model MCA8000; Princeton Gamma Technologies, Princeton, NJ, USA) was used for gamma spectrum acquisition and processing. Characteristic gamma ray energies were monitored to identify and quantify the radionuclides (^{134}Cs, 604.7 and 795.9 keV; ^{137}Cs, 661.7 keV; ^{40}K, 1461 keV). The detector was calibrated using a gamma ray reference source from the Japan Radioisotope Association (Tokyo, Japan). The gamma spectrum of each sample was measured for >20,000 s for food-duplicate samples and >2,000 s for fruit homogenates. The limits of detection (LODs) were calculated according to Kaiser's method with $K = 3$.[9] ^{137}Cs was assumed to be in radioactive equilibrium with its daughter product, ^{137m}Ba. All activities were corrected to December 4, 2011 using the physical half-lives (^{134}Cs, 2.06 yr; ^{137}Cs: 30.1 yr).

Procedural blanks were processed in parallel with every batch of 18 samples to check for interference or contamination by solvents and glassware. There were no detectable residues in any of the procedural blanks ($n = 3$).

The radioactivity was converted into effective doses using effective dose coefficients of 0.019 μSv/Bq for ^{134}Cs and 0.013 μSv/Bq for ^{137}Cs by ingestion.[10] The estimated annual dose was calculated assuming that the daily intake of radiocesium was constant throughout the year. The dose from ^{40}K in the body was estimated from the potassium content in reference man because the body burden of ^{40}K is metabolically maintained irrespective of dietary intake. In this study, the annual dose from ^{40}K was assumed to be 165 μSv for adults.[11]

Statistical Analysis. To calculate the summary statistics and performing statistical comparisons, data values below the LODs were assumed to have concentrations equal to one-half of the LODs. All statistical analyses were conducted using JMP (version 4; SAS Institute Inc., Cary, NC, USA). The mean, range, and geometric mean (GM) were calculated for the dietary intake of radionuclides. Since there were large variations in the concentrations among the study areas, log-transformed values were tested using the Tukey-Kramer honestly significant difference (HSD) test after analysis of variance.[12] The 95th percentile estimate of the dietary intake was calculated by multiplying the GM by the geometric standard deviation (GSD) to the power of 1.64. Correlations were tested using Pearson's product moment correlation coefficient. To select variables for the multivariate analyses, we used stepwise multiple linear regression based on monovariables with an entry p value of 0.20 and a stay p value of 0.10. Probability values of less than 0.05 were considered to indicate statistically significant differences. We also conducted nonparametric analyses, the Steel-Dwass test,[13,14] and Spearman's rank correlation to confirm the results by parametric analysis when deviation from normal distribution may have affected the analysis even after log-transformation. The significance level was set at 0.05 as in parametric analysis.

RESULTS

Characteristics of the Study Participants. A total of 53 samples were collected from the three study areas, as indicated in Table 1 and Supporting Information, Table S1. Participants in Fukushima ($n = 26$) were recruited from six of seven geographic divisions in Fukushima Prefecture (Supporting Information, Table S1 and Figure S1). Most of them ($n = 22$) were from the Kenpoku, Kenchu, and Sousou divisions, where the radiation dose rate was relatively high.[15] One male subject in Fukushima Prefecture was recruited owing to a misunderstanding of the sampling protocol; he was not excluded from the data set because his food menu for the day was the same as his wife's. The mean (±SD) age of the participants was 48.3 ± 14.3 years. There was a significant difference in the mean ages among the study areas ($p < 0.05$, Tukey-Kramer HSD test). Except for Fukushima Prefecture, recruitment was conducted in urban areas (Fukuoka, Osaka, Nagoya, and Tokyo), where the average ages of residents (44.5 yr, 44.3 yr, 43.0 yr, and 43.8 yr, respectively) were lower than in Fukushima (46.2 yr).[16] Many residents in Fukushima Prefecture, especially those aged 0–14 and 25–44 yr, moved out following the disaster,[17] which may have caused differences in the average age among the study areas. By occupation, most of the participants were homemakers (49%), followed by farmers (9.4%), office workers (9.4%), and part-time workers (9.4%).

The food consumption by individuals on the day of the survey varied between Fukushima and western Japan ($p < 0.05$, Tukey–Kramer HSD test), which was a reflection of differences in the age, weight, and food habits among the subjects. The

Table 3. Dietary Intake of Radionuclides[b]

		dietary intake (Bq/day)			estimated dose (μSv/year)		
		^{134}Cs	^{137}Cs	^{40}K	^{134}Cs+^{137}Cs	HSD test[a]	Steel-Dwass test[a]
Fukushima	n > LOD (%)	25 (96.2%)	25 (96.2%)	26 (100%)			
n = 26	range (median)	<0.20–7.7 (1.8)	<0.26–9.7 (2.3)	25.1–120 (74)	<2.6–99 (23)	A	A
	mean ± SD	2.0 ± 1.6	2.5 ± 2.0	77 ± 31	26 ± 20		
	GM (GSD)	1.5(2.3)	1.9(2.3)	70(1.6)	20(2.3)		
	P95	5.9	7.4	150	76		
Kanto	n > LOD (%)	8 (50%)	7 (43.8%)	16 (100%)			
n = 16	range (median)	<0.10–4.8 (0.20)	<0.12–5.6 (0.23)	7.3–78 (40)	<1.3–60 (2.5)	B	B
	mean ± SD	0.88 ± 1.4	0.94 ± 1.5	34 ± 21	11 ± 17		
	GM (GSD)	0.31(4.3)	0.35(4.1)	27(2.1)	3.9(4.2)		
	P95	3.5	3.6	94	41		
Western Japan	n > LOD (%)	1 (9.1%)	1 (9.1%)	11 (100%)			
n = 11	range (median)	<0.16–0.28	<0.21–0.34	16.4–73 (37)	<2.1–3.6	B	B
	mean ± SD	–	–	40 ± 17	–		
	GM (GSD)	–	–	36(1.6)	–		
	P95	–	–	76	–		

[a]The Tukey–Kramer HSD test and Steel-Dwass test were conducted to compare the GMs and medians of the estimated doses of radiocesium among the sampling sites, respectively. [b]LOD: detection limit; GM: geometric mean; GSD: geometric standard deviation; P95, 95th percentile; estimated dose, total for doses attributable to exposure to ^{134}Cs and ^{137}Cs; HSD test, Tukey–Kramer honestly significant difference text. The effective dose coefficients for ^{134}Cs and ^{137}Cs by the oral route were 0.019 and 0.013 μSv/Bq, respectively. The P95 estimates were calculated by multiplying the GM by the GSD to the power of 1.64.

composition of the food-duplicate samples showed differences in some categories (Table 2). Although the participants in Fukushima consumed more vegetables, fruits, and liquids than the other two areas (p < 0.05, Tukey-Kramer HSD test), the deviations from the averages in the National Health and Nutrition Survey were within 130 g for daily vegetable and fruit consumption.[18] Consumption of vegetables by adult females in Fukushima Prefecture was reported to be 318 g/day in 2010, which was higher than the national average of 285 g/day.[18] As shown in Table S1, the participants in Fukushima consumed homegrown vegetables and shared vegetables among neighbors. The number of farmers in Fukushima Prefecture was the second highest in Japan in 2010.[19] In addition, purchases of apples and persimmons in Fukushima were reported to be 37.9 kg/year and 5.2 kg/year, respectively, in 2010, which was higher than the national average (12.4 kg/year and 2.3 kg/year for apples and persimmons, respectively).[20] These characteristics in food habits may differ from the national average, but they are not related to sampling bias. Agricultural products of Fukushima Prefecture, such as rice, potatoes, other vegetables, fruits, and eggs, were consumed by the study participants in Fukushima and accounted for more than half of their consumed total amounts (Table 2). On the other hand, the participants in the Kanto region consumed only 3.1 ± 8.8 g/day of vegetables and 4.9 ± 19.7 g/day of fruits from Fukushima Prefecture, and participants in western Japan consumed no products from Fukushima Prefecture.

Radiocesium in the Daily Consumed Food Samples. A total of 53 sets of food-duplicate samples were analyzed. ^{134}Cs and ^{137}Cs were frequently detected in 25 of the 26 food-duplicate samples from Fukushima Prefecture compared with eight of sixteen from the Kanto region and only one of eleven from western Japan (Table 3). The median intake of radiocesium was 1.8 and 2.3 Bq/day for ^{134}Cs and ^{137}Cs, respectively, in Fukushima Prefecture, and the estimated dose level was 23 μSv/year (range <2.6–99 μSv/year). The estimated dose level of radiocesium was significantly higher in Fukushima than in the Kanto region and western Japan (p < 0.05, Tukey-Kramer HSD test; p < 0.05, Steel-Dwass test). Though samples from the Kanto region indicated a median intake of radiocesium of 0.20 and 0.23 Bq/day for ^{134}Cs and ^{137}Cs, respectively, they showed large variations, with GSDs of more than 4, and the maximum dose level of radiocesium was 60 μSv/year. The dietary intake of ^{40}K among participants from Fukushima was high compared with that of the other areas (p < 0.05, Tukey-Kramer HSD test; p < 0.05, Steel–Dwass test), which may have resulted from the high consumption of vegetables and fruits. Compared with the assumed dose of ^{40}K, 165 μSv/year for adults,[11] the annual dose of radiocesium was low, even in the participants from Fukushima Prefecture.

Correlations between Radiocesium ^{137}Cs Intake and Consumption of Different Food Items. The correlations between radionuclides and food items were examined for the 53 food-duplicate samples (Supporting Information, Table S2). ^{137}Cs was used for this analysis because it has a long half-life and reflects dietary exposure to radiocesium. ^{40}K was also included in this analysis because cesium is a chemically similar alkali metal to potassium and may accumulate in food products.[21] The intake of ^{137}Cs was positively correlated with the intake of ^{40}K and fruits (Pearson's r = 0.52 and 0.29, respectively; p < 0.05). The correlation with intake of ^{137}Cs was also confirmed in nonparametric analysis (Spearman's ρ = 0.61 and 0.49 for ^{40}K and fruits, respectively; p < 0.05). The intake of ^{40}K was positively correlated with the intake of potatoes, vegetables, fruits, and seaweed (Pearson's r = 0.44, 0.58, 0.46, and 0.29, respectively; p < 0.05. Spearman's ρ = 0.46, 0.58, 0.54, and 0.33, respectively; p < 0.05) and negatively correlated with intake of cereals (r = −0.38, p < 0.05; ρ = −0.35, p < 0.05).

As shown in Table 2, the daily consumption of potatoes, vegetables, fruits, and seaweed was found to be higher in Fukushima than in Kanto and western Japan in this study. The association among these items may be confounded. Therefore, the correlations between radionuclides and food items produced in Fukushima Prefecture were investigated among the 26 participants from Fukushima (Supporting Information, Table S3). The intake of ^{137}Cs was significantly associated with

the intake of fruits, mushrooms, and confectionery produced in Fukushima (Pearson's $r = 0.45$, 0.42, and 0.44, respectively; $p < 0.05$). An association between ^{137}Cs and ^{40}K was not observed in this analysis (Pearson's $r = 0.23$; $p > 0.05$). In Spearman's correlation, the intake of ^{137}Cs was not correlated with any items produced in Fukushima ($p > 0.05$).

To determine the relationship between the intake of ^{137}Cs and three food items produced in Fukushima Prefecture (fruits, mushrooms, and confectionery), stepwise multiple linear regression analyses were conducted for the correlation with the daily uptake of radionuclides. The intake of fruits and mushrooms produced in Fukushima was found to be a significant factor for the dietary intake of ^{137}Cs in the 26 participants from Fukushima (F statistics, $p = 0.022$ and 0.030, respectively). Finally, we obtained the following:

$$\begin{aligned} &^{137}\text{Cs intake (Bq/day)} = 1.2 + 0.0089\ \text{(Bq/g)} \\ &\quad \times \text{fruit intake (g/day)} + 0.069\ \text{(Bq/g)} \\ &\quad \times \text{mushroom intake (g/day)}\ (R^2 = 0.35, \quad F = 6.2, \\ &\quad p = 0.007) \end{aligned} \tag{1}$$

To confirm the results, persimmons and apples produced in Fukushima, which were frequently consumed by the participants, were analyzed ($n = 16$). The mean radioactivity (±SD) was 23 ± 28 and 30 ± 35 Bq/kg for ^{134}Cs and ^{137}Cs, respectively. The monitoring results for shiitake mushrooms (*Lentinula edodes*) in Fukushima Prefecture were averaged ($n = 45$; November to December 2011),[22] and the mean radioactivity was 19 ± 52 and 24 ± 61 Bq/kg for ^{134}Cs and ^{137}Cs, respectively.

For subjects who consume the average amounts of fruits (110 g/day) and mushrooms (3.8 g/day), eq 1 gives an estimate of the daily intake as 2.5 Bq/day, which amounts to 74% of the values estimated from the measurements: (30/1000 × 110 + 24/1000 × 3.8 = 3.4 Bq/day). The estimates thus agreed well with the average daily intake.

DISCUSSION

We examined the dietary exposure to ^{134}Cs and ^{137}Cs among study participants in Fukushima Prefecture and neighboring regions in December 2011 based on the food-duplicate method. The maximum dose through ingestion was estimated to be 99 μSv/year in the participants from Fukushima, which was still less than the dose of natural ^{40}K—165 μSv/year for adults.[11] In addition, the Japanese consume marine food, which contributed to ^{210}Pb and ^{210}Po exposures of 0.050 and 0.053 mSv/y, respectively.[23] In another study, ^{210}Po exposure was estimated to be 0.29 mSv/yr.[24] The amount of radioactivity from radiocesium in the daily meals consumed by the residents in the study population was well below the provisional regulation limit of 5 mSv/year[3] and also within the proposed new standard limit of 1 mSv/year.[25,26]

In the current study, the participants were recruited by nonprobability sampling, which facilitates an urgent study: urgency outweighs rigorousness. Thus, we need to consider whether selection bias and other biases could have occurred. Exposure levels in the study population did not properly represent those in the entire population. This is an important limitation. However, the food items consumed in the study population were consistent with those indicated in statistics; they included a substantial amount of products from Fukushima, which potentially contain radiocesium. Potential selection bias would not be great unless the food consumption patterns deviated from the standard ones indicated in national surveys. There may have been a preference among the participants to certain food products, but this effect is likely to have been small. Nevertheless, in future studies, exposure estimates should be refined among the general population by eliminating study biases.

The external exposure to radiation among Fukushima residents was estimated based on behavior records from March 11 to July 11, 2011.[27] The dose of the external exposure ranged from under 1 mSv to 5 mSv, and the proportion of residents subject to a dose of under 1 mSv and 1–2 mSv was 58.6% and 36.1%, respectively. The effective dose levels by inhalation of radiocesium were estimated in several locations in Fukushima in a previous study: they were <3 μSv/year in nine locations; samples from three other locations in the planned evacuation zone showed doses of 14.7 in Iitate, 76.9 in Namie, and 27.7 μSv/year in Katsurao.[28] These estimates were not from identical populations, and therefore comprehensive evaluation of the exposure is required.

The study participants from Fukushima Prefecture consumed rice, vegetables, and fruits produced there. Their food consumption was higher than the national average in the National Health and Nutrition Survey in Japan. This regional difference may have resulted from the food habits of residents in Fukushima Prefecture. The maximum intake of radiocesium was 7.7 and 9.7 Bq/day for ^{134}Cs and ^{137}Cs, respectively. Many food items were screened by local authorities, and the distribution of foods exceeding provisional regulation limits was restricted.[29] Under the provisional regulation limits and restrictions legislated by the Ministry of Health, Labour and Welfare of Japan, the median annual dose was deterministically estimated to be 0.051 mSv/year,[30] which is comparable to the estimates in this study. Immediately after the accident, radiocesium was detected at levels over 500 Bq/kg in various food samples (especially vegetables); this was probably caused by direct deposition of released radioactive material. Monitoring data in December 2011 showed significantly decreased levels of radiocesium in food products compared with those in April.[31] In December 2011, the study participants consumed food products cultivated in winter. Therefore, the radiocesium in the current study samples would appear to have been translocated from the soil to the roots. Even though it is likely that the levels of exposure to radiocesium were not high, the current exposure levels could last for several years. Nevertheless, a high proportion of the foodstuffs in this study came from various areas within Fukushima Prefecture. The food-duplicate samples in this study were collected in six of seven geographic divisions in Fukushima Prefecture (Supporting Information Table S1 and Figure S1), and there was a large variation in the radiocesium content (^{134}Cs <0.20–7.7 Bq/day and ^{137}Cs <0.26–9.7 Bq/day). It is possible that even if relatively contaminated foodstuffs are distributed, they could be diluted by other foods.

^{40}K was not associated with radiocesium intake among the participants in Fukushima (Supporting Information, Table S3). The lack of association may be explained by the finding that plants (i.e., fruits and vegetable) have several very specific channels of K intake and radiocesium uptake that are anticorrelated with the cation exchange capacity of soil.[32] On the other hand, strontium correlated strongly with calcium because there are no specific channels for Ca uptake.[32]

Therefore, ^{40}K did not simply correlate with ^{137}Cs in the food-duplicate samples.

The intake of radiocesium was statistically correlated with the intake of several food items produced in Fukushima Prefecture. In multiple linear regression analyses, confectionery did not correlate with radiocesium intake. The intake of confectionery was marginally correlated with the intake of fruits ($r = 0.32$ and $p = 0.10$; Supporting Information, Table S3), which might confound radiocesium intake with confectionery. The coefficients in the multiple linear regression analyses were 8.9 Bq/kg and 69 Bq/kg for ^{137}Cs in fruits and mushrooms, respectively. These estimates for the measured values in this study are roughly comparable to the values obtained in screenings by authorities.[22] A study in the Bryansk region of Russia also showed the contributions of fruits and mushrooms to internal exposure after the Chernobyl accident.[33] However, in the current study, the annual dose was considered to be low compared with the regulation limit.

In our previous study in July 2011,[28] the average dietary intake of radiocesium in Fukushima was estimated to be 0.5 and 0.6 Bq/day for ^{134}Cs and ^{137}Cs, respectively. The difference in the dietary intake between the present and previous studies may have resulted from seasonal variation or the survey methods employed. Seasonal variation is a possible factor because food products in the market and their production areas vary by season. For example, the average content of fruits and mushrooms in the previous study samples was 24.1 ± 40.1 g/day (median 0 g/day) and 0.69 ± 2.3 g/day (median 0 g/day);[28] this was less than in the current study samples (Table 2). In the study, researchers visited local grocery stores in each city or town and purchased several sets of whole-day meals, which may have affected the composition of food products. Therefore, longitudinal follow-up of dietary exposure in a fixed population is needed to evaluate the annual dietary intake of radiocesium and dose. In addition, most of the participants in the current study were adult females. Age and sex influence dietary habits and preferences, and this may have affected the dietary intake of radiocesium. Further study extended to a wide population is needed to elucidate the effects of age and sex. Another limitation was that the food-duplicate survey was conducted during a single day. The intake that day did not necessarily represent the actual body burden of radiocesium. Biological monitoring of human excretions or the use of a whole body counter will promote a comprehensive understanding of internal exposure to radiocesium. Additionally, in this study, only radiocesium was analyzed, based on the assumption that the contribution of ^{90}Sr, plutonium, and ^{106}Ru in dietary exposure was ~16% and radiocesium represented most of the dose from the released radioactive material from the Fukushima nuclear power plant.[7] The provisional regulation limit also assumed that the content of ^{90}Sr was 10% that of ^{137}Cs, following the Chernobyl accident and analysis of ^{90}Sr.[34] However, the assumption needs to be properly determined in future investigations.

Following the Fukushima accident, residents were evacuated from a 20-km radius of the power plant and the planned emergency evacuation zone. Since April 2012, the government of Japan has re-examined the evacuation zones in Fukushima and categorize them into three zones. Residents from several municipalities plan to return. As a result, people may consume foods supplied locally within the contaminated areas, which would be similar to what happened after the Chernobyl accident.[28,35]

The estimated dietary dose levels in current study participants in Fukushima Prefecture were much lower than 1 mSv/year, which indicates that the health risk posed by the radiocesium is probably small. However, the results of this study may be confounded by biases attributable to flaws inherent to the study design. Thus, further studies are needed to provide a cautious estimate of exposure levels of the general population so as to evaluate the long-term health risks under various scenarios. Although the levels are low, the contribution of fruit and mushrooms produced in Fukushima to the total intake of radiocesium is discernible. In addition, evacuated residents will return to relatively contaminated areas. Therefore, it is highly recommended that levels of daily intake of radiocesium be monitored in such populations, where potentially contaminated local food products are consumed rather than items bought at markets.

■ ASSOCIATED CONTENT

Supporting Information

Information regarding the study participants, parametric and nonparametric correlation coefficients between food items and dietary intake of radionuclides (Supporting Information, Tables S1, S2, and S3). 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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Polyfluorinated telomers in indoor air of Japanese houses

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HIGHLIGHTS

- 6:2 FTOH, 8:2 FTOH, 10:2 FTOH and 8:2 FTOAc were detected in most samples.
- 8:2 FTOH is the predominant component among fluorotelomers in indoor air.
- 8:2 FTOH level is associated with sampled housing location and sampling season.
- There are fluorotelomer sources in indoor environments in the Keihan area, Japan.

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ABSTRACT

The fluorotelomer alcohols (FTOHs) have been detected in various environmental compartments, including indoor and outdoor air, in North America and Europe. In our previous studies, FTOHs were detected at a relative higher concentration in outdoor air in the Keihan (Kyoto–Osaka, one of the major industrial zones) area, Japan compared to reported data. The exposure level of FTOHs in indoor air in the Keihan area remains unclear. In the present study, indoor air FTOH concentrations were investigated using a passive air sampler containing activated carbon felts. The indoor air sampling was conducted in 49 households of the Keihan area, during winter and summer 2008. Most samples contained 6:2 FTOH, 8:2 FTOH, 10:2 FTOH and 8:2 FTOAc. The median concentration of 8:2 FTOH (5.84 ng m^{-3}) was highest among fluorotelomers, followed by those of 10:2 FTOH (1.12 ng m^{-3}), 6:2 FTOH (0.29 ng m^{-3}), and others. Significant correlations among fluorotelomers were observed in collected samples. The association between housing conditions and 8:2 FTOH concentrations showed that samples collected from bed rooms have higher 8:2 FTOH concentrations than those collected from other locations. In addition, samples collected in winter showed lower levels of 8:2 FTOH than those collected in summer. These findings suggest that 8:2 FTOH is the predominant component among fluorotelomers in indoor air, and that there are emission sources of fluorotelomers in indoor environments of the Keihan area. Further investigations into the origins of fluorotelomers are needed to evaluate indoor contamination with fluorotelomers.

1. Introduction

Fluorotelomer alcohols (FTOHs) belong to a class of perfluoroalkyl and polyfluoroalkyl substances (PFASs), that find use in a diverse range of commercial and industrial applications including coatings, polymers, and paints, and so on (Kissa, 2001). 6:2, 8:2, and 10:2 FTOH are now widespread in indoor and outdoor air in the North America and Europe as a result of their widespread use (Stock et al., 2004; Barber et al., 2007; Jahnke et al., 2007; Shoeib et al., 2011). Several lines of experimental evidence have demonstrated the toxic effects of FTOHs on the laboratory animals (Kudo et al., 2005; Ishibashi et al., 2007, 2008; Oda et al., 2007; Phillips et al., 2007; Liu et al., 2010). Therefore, further consideration of the potential biological effects and the risks of FTOHs on human health are warranted. Although there is lack of

Abbreviations: PFASs, perfluoroalkyl and polyfluoroalkyl substances; PFOA, perfluorooctanoic acid; FTOHs, fluorotelomer alcohols; PAS, passive air sampler; ACFs, activated carbon fiber felts; 6:2 FTOH, 1H,1H,2H,2H-perfluorooctanol; 8:2 FTOH, 1H,1H,2H,2H-perfluorodecanol; 10:2 FTOH, 1H,1H,2H,2H-perfluoro-1-dodecanol; 8:2 FTOAc, 1H,1H,2H,2H-perfluorodecyl acrylate; 8:2 FTOMac, 1H,1H,2H,2H-heptadecafluorodecyl methacrylate; 8:1 FA, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-heptadecafluoro-1-nonanol; IDL, instrument detection limits; MDL, method detection limits; HSD test, Tukey–Kramer's honestly significant difference test; ANOVA, analysis of variance; GM, geometric mean; GSD, geometric standard deviation; CV, coefficient of variation.

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epidemiological evidence, the potential adverse effects of FTOHs on human health might include endocrine toxicity, liver toxicity and reproductive toxicity, and so on (Kudo et al., 2005; Ishibashi et al., 2007, 2008; Liu et al., 2010) based on experimental evidence.

In our previous study (Oono et al., 2008a, 2008b), we identified 8:2 FTOH as the major component among investigated FTOHs in outdoor air of the Keihan (Kyoto–Osaka) area, which is one of the major industrial zones in Japan; it was detected at significantly higher concentrations than in North America and Europe. The varying patterns of FTOHs in Japan, North America and Europe strongly suggest a possible outdoor emission source in the Keihan area (Oono et al., 2008a, 2008b).

The aim of the current study is to clarify whether the higher concentrations of FTOHs in outdoor air in the Keihan area contribute to FTOH levels in indoor air in this area, or whether there are additional emission sources in indoor environments. In the current study, we investigated indoor air concentrations of FTOHs and their esters using a passive air sampler (PAS) in Keihan area houses. To evaluate potential factors influencing FTOH levels, questionnaires regarding housing conditions and household composition were also collected and analyzed.

2. Materials and methods

2.1. Sample collection

We recruited 49 households from the Keihan area, Japan, during February to March (winter) and June to September (summer) of 2008. Activated carbon fiber felts (ACFs) were used for passive sampling media. ACFs were suspended in chambers as previously reported (Shoeib et al., 2004; Oono et al., 2008a, 2008b) (Supplemental Fig. 1). The ACFs were pre-cleaned by soaking in ethyl acetate, followed by vacuum drying overnight. All sampling media were wrapped in polyethylene bags for transport to the sampling site. To test the linear relation between the concentrations and exposure time, samplers were deployed for 7–21 d at several locations in the 49 households. Additional ACF samplers were deployed in different rooms at 26 homes. PAS and high-volume active air samplers were set up simultaneously for 14 d, with six repetitions. The latter samplers consisted of a quartz fiber membrane, polyurethane foam plug and ACFs with a flow rate of 72 $m^3 d^{-1}$, which were analyzed as in Oono et al. (2008a, 2008b). The sampling rate of the PAS was calibrated to approximately 5 $m^3 d^{-1}$. Clean ACFs were transported as travel (field) blanks and stored with the environmental samples. Samples were shipped via an overnight delivery service to Kyoto University and stored at −30 °C until analysis at the Kyoto University Human Specimen Bank (Koizumi et al., 2005, 2009). Information on housing condition and household composition were then collected by questionnaire. The research protocol for the present study was reviewed and approved by the Ethics Committee of the Kyoto University Graduate School of Medicine (E25). Written informed consent was obtained from all participants.

2.2. Reagents

1H,1H,2H,2H-perfluorooctanol (6:2 FTOH), 1H,1H,2H,2H-perfluorodecanol (8:2 FTOH), 1H,1H,2H,2H-perfluoro-1-dodecanol (10:2 FTOH) and their $^{13}C_2$-substituted compounds were purchased from Wellington Laboratories (Guelph, Ontario, Canada). 1H,1H,2H,2H-perfluorodecyl acrylate (8:2 FTOAc; >96%) was purchased from Lancaster Synthesis (Lancashire, UK). 1H,1H,2H,2H-heptadecafluorodecyl methacrylate (8:2 FTOMac; >98%) was purchased from Fluorochem (Derbyshire, UK). 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-heptadecafluoro-1-nonanol (8:1 FA; >98%) and ethyl acetate (pesticide analysis grade 5000) were purchased from Wako Pure Chemicals (Osaka, Japan). ACFs (5 mm thick, 165 mm × 165 mm, 4 g) were purchased from Toyobo (KF-1700F, Toyobo Co. Ltd., Osaka, Japan).

2.3. Sample extraction and analysis

Before extraction, isotope-labeled FTOHs (2-perfluorohexyl-[1,1-$2H_2$]-[1,2-$^{13}C_2$]-ethanol, 2-perfluorooctyl-[1,1-$2H_2$]-[1,2-$^{13}C_2$]-ethanol and 2-perfluorodecyl-[1,1-$2H_2$]-[1,2-$^{13}C_2$]-ethanol) were added to all ACFs at concentration of 10 ng per sample to determine surrogate recoveries. ACF samples were soaked for 10 min in 100 mL of ethyl acetate, this was then repeated three more times (400 mL total). The aliquots were combined and dried with sodium sulfate. Extracts were rotoevaporated up to ca. 5 mL and concentrated to 1 mL under a nitrogen blow. 8:1 FA was added as an internal standard just prior to analysis, to correct for volume differences.

Each single extract was analysed by gas chromatography-mass spectrometry (Agilent 6890GC/5973MSD, Agilent Technologies Japan, Ltd., Tokyo, Japan) in electron impact ionization mode, using single ion monitoring. Analytes were separated on a DB-5MS column (30 m length, 0.25 mm i.d., 0.25 μm film thickness, Agilent Technologies Japan, Ltd.) with a helium carrier gas. Pulsed splitless injections (2 μL) were performed at an initial pressure of 30 psi for 1.5 min, with the injector set at 200 °C, and the split was opened after 1.5 min. Initial oven temperature was 50 °C for 4 min, ramped at 20 °C min^{-1} to 140 °C, and then at 40 °C min^{-1} to 280 °C, followed by a 2 min hold. The ion source and quadrupole were 230 °C and 150 °C, respectively.

Quantification was performed using standard curve analysis (range: 0.1 ng mL^{-1}–1000 ng mL^{-1}) and internal standards. 8:2 FTOAc and 8:2 FTOMac were quantitated by $^{13}C_2$-8:2 FTOH. Instrument detection limits (IDLs) were defined as the mass of analyte producing a peak with a signal-to-noise ratio of three using the ChemStation software (G1701 CA, Agilent), and ranged from 1 pg (8:2 FTOAc and 8:2 FTOMac) to 5 pg (6:2 FTOH) (Supplemental Table 1). Method detection limits (MDL) were calculated as the mean blank concentration within three standard deviations (SD), with six repetitions. MDLs for 8:2 FTOH and 10:2 FTOH were 0.12 ng m^{-3} (mean + 3SD: 0.052 + 3 × 0.021) and 0.07 ng m^{-3} (mean + 3SD: 0.026 + 3 × 0.012) (Supplemental Table 1). When no blank concentration was detected in travel blanks, the MDL value was calculated by dividing the IDL by air volume. The MDL equivalent air concentrations ranged from 32 pg m^{-3} for 8:2 FTOH to 3 pg m^{-3} for 8:2 FTOAc, assuming an air volume of 70 m^3 (Supplemental Table 1). Mean extraction recoveries were greater than 87% with the exception of 6:2 FTOH, for which recoveries were lower, at 71% ($n = 10$) (Supplemental Table 1).

2.4. Statistics

Because the levels of target compounds displayed skewed patterns, statistical analyses were conducted after logarithmic transformation of concentrations. Log-transformed data were distributed normally (Shapiro–Wilk's test: $p > 0.05$). Means were compared using Student's t test or Tukey–Kramer's honestly significant difference test (HSD test) when statistical tests by ANOVA were significant. Correlation was tested by Spearman's rank correlation coefficient. A value of $p < 0.05$ was considered significant. All statistical analyses were carried out with JMP software (Version 4; SAS Institute Inc., Cary, NC). For statistical analyses, data below the MDL were converted to half these values.

3. Results

3.1. Indoor air concentrations of fluorotelomers in the Keihan area

6:2 FTOH, 8:2 FTOAc and 8:2 FTOMac were not detected in the travel (field) blanks ($n = 12$). Surrogate recoveries were 87 ± 15%, 91 ± 7% and 94 ± 9% using isotope-labeled 6:2 FTOH, 8:2 FTOH and 10:2 FTOH, respectively.

Descriptive statistics for fluorotelomers in indoor air collected from 84 households are presented in Table 1. Most samples contained 6:2 FTOH, 8:2 FTOH, 10:2 FTOH and 8:2 FTOAc (detection rate 79%, 100%, 92% and 87%, respectively). 8:2 FTOMac was less frequently observed at concentrations above MDL (detection rate 40%). The median concentration of 8:2 FTOH (5.84 ng m^{-3}) was highest among fluorotelomers, followed by 10:2 FTOH (1.12 ng m^{-3}), 6:2 FTOH (0.29 ng m^{-3}) and others. The proportion of 8:2 FTOH in total investigated fluorotelomers was also high, ranging from 46–100% (mean ± SD: 76 ± 9%, Table 1). The 90th percentile concentrations of 6:2 FTOH, 8:2 FTOH and 10:2 FTOH were 1.38, 29.45 and 6.69 ng m^{-3}, respectively. Fluorotelomers in outdoor air were evaluated in two houses; levels of 8:2 FTOH were less than MDL (0.12 ng m^{-3}, Supplemental Table 1), whereas indoor air samples showed 0.68 and 5.80 ng m^{-3}.

3.2. Correlations among fluorotelomers

Nonparametric correlation coefficients among fluorotelomers for 84 samples are listed in Table 2. Both 8:2 FTOAc and 8:2 FTOMac showed significant correlation coefficients, with 6:2 FTOH, 8:2 FTOH, 10:2 FTOH (for 8:2 FTOAc, $\rho = 0.740$, 0.803 and 0.785, respectively; for 8:2 FTOMac, $\rho = 0.432$, 0.298 and 0.282, respectively).

Samples collected within the same households also showed significant correlation between the living room and other rooms for 6:2, 8:2, 10:2 FTOHs, and 8:2 FTOAc ($\rho = 0.70$, 0.65, 0.86 and 0.72, respectively; Fig. 1).

3.3. Effects of housing conditions on indoor concentration of 8:2 FTOH

8:2 FTOH was detected in all collected samples and observed in highest proportions (76%) among investigated fluorotelomers (Table 1). In addition, 8:2 FTOH was highly correlated with 6:2 FTOH, 8:2 FTOAc, 8:2 FTOMac and 10:2 FTOH (Table 2), which suggested 8:2 FTOH can be representative for other fluorotelomers. Therefore, we investigated the influence of housing conditions on the concentration of 8:2 FTOH in the indoor air samples by ANOVA (Table 3). The type of room was found to have a profound effect on 8:2 FTOH levels in indoor air ($p < 0.05$). Samples collected from bedrooms showed higher 8:2 FTOH concentrations than those collected from other locations ($p < 0.05$). Sampling season also showed a profound effect on the level of 8:2 FTOH. Samples collected in winter showed lower levels of 8:2 FTOH than those collected in summer ($p < 0.001$).

Table 2
Correlation among fluorotelomers.

	6:2 FTOH	8:2 FTOH	8:2 FTOAc	8:2 FTOMac	10:2 FTOH
6:2 FTOH	–				
8:2 FTOH	0.823**	–			
8:2 FTOAc	0.740**	0.803**	–		
8:2 FTOMac	0.432**	0.298*	0.307*	–	
10:2 FTOH	0.690**	0.849**	0.785**	0.282*	–

Numbers indicate Spearman's rank correlation coefficients (ρ).
* $p < 0.01$.
** $p < 0.001$.

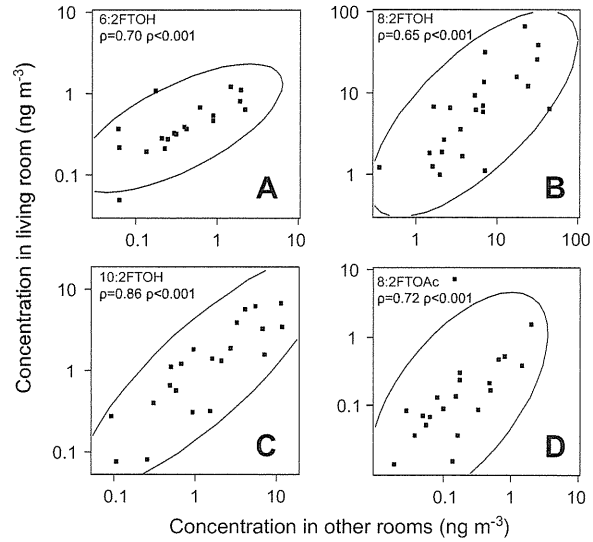

Fig. 1. Correlations of fluorotelomer concentration between living room (Y axis) and other rooms (X axis) for 6:2 (A), 8:2 (B), 10:2 (C) FTOHs, and 8:2 FTOAc (D), in the same households. Overall, 95% of values are within boundary circles.

4. Discussion

In this study, we identified fluorotelomers in the indoor environment of the Keihan area of Japan, finding intense contamination compared with outdoor air in the same area (Oono et al., 2008a, 2008b). 6:2 FTOH, 8:2 FTOH, 10:2 FTOH and 8:2 FTOAc were

Table 1
Fluorotelomer concentrations in indoor air ($n = 84$).

	Concentration (ng m^{-3})					
	6:2 FTOH	8:2 FTOH	10:2 FTOH	8:2 FTOAc	8:2 FTOMac	Total
Detection rate (%)	79	100	92	87	40	100
Range	<0.06–12.09	0.36–63.02	<0.07–13.00	<0.02–6.95	<0.02–0.74	0.49–76.30
Mean ± SD	0.59 ± 1.37	10.16 + 12.45	2.29 ± 2.89	0.34 ± 0.83	0.05 ± 0.10	13.43 ± 16.05
Mean ± SD (%)	4 ± 4	76 ± 9	17 ± 7	3 ± 3	0.4 ± 2	100 ± 0
Range (%)	0–24	46–100	0–36	0–25	0–11	100–100
GM (GSD)	0.26 (3.53)	5.16 (3.34)	0.92 (4.77)	0.11 (4.38)	<0.02	6.82 (3.38)
P25	0.09	1.82	0.30	0.03	<0.02	2.36
Median	0.29	5.84	1.12	0.09	<0.02	7.43
P75	0.64	13.88	3.32	0.29	0.05	18.54
P90	1.38	29.45	6.69	0.77	0.12	39.95

SD: standard deviation; GM: geometric mean; GSD: geometric standard deviation; P25: 25th percentile value; P75: 75th percentile value; P90: 90th percentile value.

Table 3
Effects of house conditions on indoor concentrations of 8:2 FTOH (ng m^{-3}).

Items		mean ± SD	ρ	p^a
Household composition	Male	1.3 ± 0.8	−0.06	0.61
	Female	0.9 ± 0.84	0.11	0.37
		n	GM (GSD)	p^b
Building age	<1 yr	11	5.7(2.6)	0.85
	1 yr–5 yr	7	9.1(3.7)	
	5 yr–10 yr	24	4.6(3.0)	
	10 yr<	42	5.7(3.6)	
House type	Houses	46	6.0(1.2)	0.81
	Apartments	33	5.6(1.2)	
Building construction	Timber	30	4.6(2.8)	0.33
	Concrete	54	5.7(3.5)	
Sampling place	Dining room	11	$6.2(3.4)^{AB}$	**0.04**
	Living room	31	$3.4(2.7)^{B}$	
	Bed room	15	$13.1(2.5)^{A}$	
	Entrance	21	$5.8(3.6)^{AB}$	
	Rest room	6	$4.3(1.7)^{AB}$	
Floor cover	Carpet	19	4.8(2.8)	0.14
	Wooden floor	51	7.1(3.4)	
	Rush mat	14	5.2(2.7)	
Ambient surrounding	Urban location	15	4.5(2.7)	0.42
	Residential zone	50	6.4(3.1)	
	Rural	19	4.1(4.5)	
Cleaning	More than once a week	43	4.3(3.9)	0.39
	Less frequent	41	5.6(3.1)	
Ventilation	Every day	40	4.6(3.0)	0.34
	Less frequent	44	6.0(3.8)	
Sampling season	Winter	13	2.0(2.0)	**<0.001**
	Summer	66	6.6(3.3)	

SD: standard deviation; GM: geometric mean; GSD: geometric standard deviation.
The geometric means without bearing the same superscripts differ significantly ($p < 0.05$). The geometric means bearing the same superscripts or without superscripts do not differ significantly ($p > 0.05$). Bold type indicates a significant difference.
[a] *p* value of Spearman's correlation.
[b] *p* value of ANOVA.

detected at higher levels in most collected samples. The median concentration of 8:2 FTOH was highest among fluorotelomers, followed by 10:2 FTOH and 6:2 FTOH. The levels of all investigated fluorotelomers from indoor air were significantly higher than those from outdoor air. The types of rooms and sampling season were demonstrated to have profound effects on FTOH levels in indoor air, which suggest specific emission sources of fluorotelomers in these rooms (Washburn et al., 2005).

Among investigated fluorotelomers, 8:2 FTOH was the major component in indoor air, accounting for 79.6% of total fluorotelomers (Table 4). Our previous studies showed that 8:2 FTOH was detected at high levels, and was the major component in outdoor air of the same area (Oono et al., 2008a, 2008b). However, indoor air concentrations of fluorotelomers were 1–2 orders of magnitude higher than in outdoor air of the same area and other areas of Japan (Oono et al., 2008a, 2008b). This suggests that these FTOHs are released from emission sources in houses and not from outdoor air. Such emission sources need further study. Compared to reported data from North America (Vancouver, 2.90 ng m^{-3}) and Europe (Catalonia, 0.0075–0.17 ng m^{-3}) (Table 4), 8:2 FTOH levels in Keihan (5.84 ng m^{-3}) were significantly higher (except for data from Oslo, 5.17 ng m^{-3}; Tromsø, 10.01 ng m^{-3}; Hamburg, 1.1–209 ng m^{-3}), whereas 6:2 FTOH (0.29 ng m^{-3} in Keihan; 0.98, 0.1–37, 0.93 and 2.99 ng m^{-3} in Vancouver, Hamburg, Oslo and Tromsø, respectively) and 10:2 FTOH levels (1.12 ng m^{-3} in Keihan; 0.95, 0.1–54, 2.82 and 3.56 ng m^{-3} in Vancouver, Hamburg, Oslo and Tromsø, respectively) were comparable or lower. This predominance of 8:2 FTOH in Keihan might result from varying formulations prepared for industrial applications. Concentrations of fluorotelomers were significantly correlated with each other (Table 2), which implies that variations in formulation were small. 8:2 FTOAc was also detected in most samples (Table 1). Fluorotelomer alcohol acrylate is an unpolymerized intermediate used in the manufacture of telomer-based polymers. Polymerized 8:2 FTOAc and other fluorotelomers are likely used for surface coatings, and residual 8:2 FTOAc and other fluorotelomers may be released from their application.

Effects of housing conditions on 8:2 FTOH levels were investigated using a questionnaire. Samples collected in winter contained lower 8:2 FTOH levels than those collected in summer. Temperature dependence of fluorotelomer alcohol concentration has been observed in outdoor air (Dreyer et al., 2009). Semi-volatility of fluorotelomers might cause these observations. Samples collected from bedrooms showed higher 8:2 FTOH concentrations than those collected from other rooms, suggesting that products (such as wood furniture) in bedrooms contain higher levels of fluorotelomers. High contamination of FTOH in indoor air, especially in the bedrooms was very likely to be an important exposure source to human because one third of our lives are spent sleeping in the bedrooms. On the other hand, fluorotelomer concentrations in the same house showed high correlation. There is a possibility that fluorotelomer-based application for household items might be uniform. In order to clarify the specific emission sources for fluorotelomers in indoor air, the content of fluorotelomers in specific products needs clarification in future study.

In summary, compared to the levels of fluorotelomers in outdoor air, significantly higher levels of fluorotelomers in indoor air suggest possible indoor emission sources in the Keihan area

Table 4
Comparison of fluorotelomer concentrations with literature data.

Sampling site	Year	*n*		Concentration (ng m^{-3})				References
				6:2 FTOH	8:2 FTOH	10:2 FTOH	8:2 FTOAc	
Indoor air								
Japan								
Keihan area	2008	84	Median	0.29	5.84	1.12	0.09	This study
			%	4.0	79.6	15.3	1.2	
Norway								
Oslo	2008	41	Median	0.93	5.17	2.82	–	Haug et al. (2011)
			%	10.5	57.9	31.6		
Tromsø	2005	4	Mean	2.99	3.42	3.56	–	Barber et al. (2007)
			%	30.0	34.3	35.7		
Tromsø	2007–2008	6	Mean	0.04	10.01	3.41	–	Huber et al. (2011)
			%	0.3	74.4	25.3		
Canada								
Vancouver	2007–2008	59	Geometric mean	0.98	2.90	0.95	–	Shoeib et al. (2011)
			%	20.3	60.0	19.7		
Germany								
Hamburg	2009–2010	16	Range	0.1–37	1.1–209	0.1–54	0.1–132	Langer et al. (2010)
Spain								
Catalonia	2009	10	Range	0.003–0.047	0.0075–0.17	<0.0006–0.047	–	Jogsten et al. (2012)
Outdoor air								
Japan								
Keihan area	2006	24	Median	0.022	0.447	0.056	–	Oono et al. (2008b)
			%	4.2	85.1	10.7		
Across Japan	2007	33	Mean	0.222	0.441	0.053	–	Oono et al. (2008a)
			%	31.0	61.6	7.4		

of Japan. Although the toxicity of FTOHs remains unclear (Kudo et al., 2005; Ishibashi et al., 2007, 2008; Oda et al., 2007; Phillips et al., 2007; Liu et al., 2010), exposure to high levels of indoor air FTOH contamination raises many health concerns, including endocrine toxicity, liver toxicity and reproductive toxicity (Kudo et al., 2005; Ishibashi et al., 2007, 2008; Liu et al., 2010). Further study is warranted for identification of specific indoor sources and measurement of human body burdens of fluorotelomers.

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Appendix A. Supplementary material

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Temporal trend and age-dependent serum concentration of phenolic organohalogen contaminants in Japanese men during 1989–2010

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Human exposure

ABSTRACT

The temporal trend in serum concentrations of phenolic organohalogen contaminants (POCs) were investigated in two age groups of men from Kyoto, Japan, from 1989 to 2010. These concentrations and trends were compared with neutral contaminants including polychlorinated biphenyls (PCBs) and pesticides. Serum concentrations of pentachlorophenol (PenCP) and 4-hydroxy-PCB187 were age-dependent and decreased to approximately one-half during the two decades, whereas no contamination trends were observed for 2,4,6-tribromophenol (TriBP), tetrabromobisphenol A (TBBPA) and 6-hydroxy-2,2′,4,4′-tetrabromodiphenyl ether (6-OH-BDE47). 6-OH-BDE47 was found in all samples (up to 3000 pg/g wet weight), whereas TBBPA was detected in 17 of 60 serum samples (up to 950 pg/g wet weight). The concentrations of TriBP, TBBPA and 6-OH-BDE47 were not correlated to those of PenCP or 4-OH-PCB187 in either age group, suggesting the different kinetics on exposure routes and fate between these brominated and chlorinated POCs.

1. Introduction

Despite the use of persistent organic pollutants, such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs), having either been phased-out or restricted, they are still found in the environment, in both neutral and phenolic forms. Phenolic organohalogen contaminants (POCs) of concern may include pentachlorophenol (PenCP), hydroxylated PCBs (OH-PCBs) and hydroxylated PBDEs (OH-PBDEs) and brominated flame retardants (BFRs), such as 2,4,6-tribromophenol (TriBP) and tetrabromobisphenol A (TBBPA). These POCs have endocrine-disrupting effects and may influence neurological, reproductive and sexual development (Lans et al., 1993; Meerts et al., 2000; Montaño et al., 2013; Suzuki et al., 2008). However, limited information is available on human exposure to these POCs in contrast to traditional neutral organohalogens.

PenCP, which is the predominant POC in human serum, has similar concentrations to persistent pesticide i.e., 4,4′-DDE (Meijer et al., 2008; Rylander et al., 2012). PenCP has been used as a herbicide in agricultural chemicals and preservatives in Japan until 1990 (Suzuki et al., 2008). The usage of PenCP in the past might have led to its ubiquitous presence because of its persistence and, consequently, to contamination of the indoor environments (Ge et al., 2007; Inoue et al., 2006; Suzuki et al., 2008). OH-PCBs are another group of POCs that have been commonly detected in human tissues. PenCP and certain OH-PCBs have shown the capability of interacting in human blood with thyroid hormones (Meerts et al., 2002; Otake et al., 2007), which play an important role in fetal growth and development (Zheng et al., 2012). Previous studies have suggested that PenCP and OH-PCBs are responsible for adverse effects on thyroid or sex hormone homeostasis (Meerts et al., 2002) and are transferred from mother to fetus via the placenta during pregnancy (Meijer et al., 2008).

Of brominated POCs, TriBP and TBBPA are now used as flame retardants in many industrial and computer products. In Asian countries, the total production of both BFRs accounted for 85,900 tons in 1999 of which 36% are produced in Japan (Watanabe and Sakai, 2003). TriBP is not used directly as a BFR but as an intermediate for brominated epoxy resins made from TBBPA (9500 tons/year in 2001) (Suzuki et al., 2008; Watanabe and Sakai, 2003). TriBP is also naturally produced by marine bacteria or plants and has been detected in fish (Haraguchi et al., 2010; Whitfield et al., 1999). Household materials containing TriBP may exist as indoor sources, whereas seafood may be a dietary source of TriBP. TBBPA, in contrast, is a reactive BFR that is released into the environment via

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many household electrical products and their disposal. Because of the short half-life of these phenolic BFRs in air, water, sediment and in humans (Covaci et al., 2009), their environmental distribution has been limited. However, phenolic BFRs have been reported to be thyroid hormone agonists and estrogenic *in vitro* (Kitamura et al., 2002; Meerts et al., 2001), and thus have potential for posing a threat to human health even at a low-dose exposure.

Exposure to hydroxylated PBDEs (OH-PBDEs) may be a more recent concern. Two congeners, e.g., 2′-OH-BDE68 and 6-OH-BDE47, have been frequently detected in blood of fish (Marsh et al., 2004), marine mammals (Marsh et al., 2005) and humans (Eguchi et al., 2012; Fujii et al., 2012a,b). The ortho-substituted OH-PBDEs are proposed to be of natural origin, whereas meta- or para-substituted OH-PBDE congeners detected in other studies may be possible metabolites of PBDEs (Athanasiadou et al., 2008). OH-PBDEs have been shown to have a higher affinity for human transthyretin (TTR) than PBDEs in liver (Meerts et al., 2000). However, the formation process and exposure routes of OH-PBDEs remain unclear.

Previous studies have suggested that the body burden of legacy neutral contaminants, such as PCBs or chlorinated pesticides, has decreased from 1973 to 1996 (Masuda et al., 2005), but there is much less information available on the temporal trends of POCs in human tissues. Therefore, retrospective monitoring of POCs is required to judge the development of pollution and to assess the fate of contaminants in humans.

The aim of the present study was to investigate the temporal trends of serum POC concentration in Japanese populations from 1989 to 2010. Target chemicals consisted of representative congeners, 1) PenCP and 4-OH-PCB187; 2) TriBP and TBBPA; and 3) 6-OH-BDE47. To avoid possible variation of body burdens by age and gender, this study was restricted to male subjects in two groups, aged in their 20s (21–29 years) and >50 years of age, at different sampling periods. Furthermore, the temporal trends in serum POC concentrations were compared with those of legacy neutral contaminants including α-endosulfan.

2. Materials and methods

2.1. Sample collection

Human serum samples were obtained from the Kyoto University Human Specimen Bank using a standardized protocol (Koizumi et al., 2005). Individual serum samples (1–2 mL each, $n = 60$) were collected from healthy volunteers in the Kyoto area that consisted of two groups of males, aged in their 20s and >50 years of age, in 1989, 1999 and 2010 (Table 1). The Ethics Committee of Kyoto University approved the protocol of the present study (E25), and appropriate written informed consent was obtained from all of the participants. Samples were stored in clean screw-capped plastic containers at −20 °C until the time of analysis.

2.2. Chemicals

Three internal standards, $^{13}C_9$-labeled α-endosulfan, $^{13}C_6$-labeled PenCP, and $^{13}C_{12}$-labeled 4-hydroxy-2,2′,4,4′,5,5′-hexachlorobiphenyl (4-OH-[$^{13}C_{12}$]PCB187), were used for the determination of target POCs. Pesticide standard solution (unlabeled pesticide mix #1037; 2 μg/mL) was purchased from Kanto Chemical Co., Ltd., (Tokyo, Japan). The standards of two PCB congeners (PCB-153 and PCB-187) and α-endosulfan were purchased from Cambridge Isotope Laboratories (Andover, MA, USA). PenCP, TriBP, 2,2-bis(3,5-dibromo-4-hydroxyphenyl)propane (TBBPA), 4-hydroxy-PCB187, 6-hydroxy-BDE47 and their O-methylated analogs were purchased from Wellington Laboratories Japan Inc. (Tokyo, Japan). These standards were used for the calibration, recovery, and quantification of the target compounds. Silica-gel (Wako gel S-1) used for purification was obtained from Wako Pure Industries Ltd. (Osaka, Japan) and was heated at 130 °C for 3 h prior to use. All solvents used (i.e., ethanol, diethylether, *n*-hexane and dichloromethane) were of pesticide-grade quality, and purchased from Kanto Chemical Co. Inc. Japan. Diazomethane was prepared by 1-methyl-3-nitro-1-nitrosoguanidine (Kanto Chemical Co., Inc. Japan).

Table 1
Information of serum samples collected from volunteers in Kyoto during 1989–2010.

Sample group	Region	M/F	*n*	Year of sampling	Mean age (range)
Serum89A	Kyoto	M	10	1989	20.6 (20–22)
Serum89B	Kyoto	M	10	1989	51.0 (50–52)
Serum99A	Kyoto	M	10	1999	24.1 (21–28)
Serum99B	Kyoto	M	10	1999	54.2 (50–59)
Serum10A	Kyoto	M	10	2010	21.5 (21–23)
Serum10B	Kyoto	M	10	2010	68.0 (65–69)

2.3. Extraction, clean-up and fractionation procedures

The methodology used to analyze neutral and phenolic contaminants in serum samples was based on lipid extraction, gel permeation chromatography (GPC) and silica-gel column cleanup, and gas chromatography-mass spectrometry with electron capture negative ionization (GC/MS/ECNI) analysis as previously described (Fujii et al., 2012a). Briefly, each 1-mL serum sample was spiked with three internal standards, namely [$^{13}C_6$]PenCP and 4-OH-[$^{13}C_{12}$]PCB187 for phenolic analytes (0.2 ng of each) and α-[$^{13}C_9$]endosulfan for neutral OCs (2 ng). The sample was extracted with *n*-hexane, after adding formic acid (0.1% v/v), ethanol, and diethylether. A combined extract was dissolved in dichloromethane (DCM):*n*-hexane (1:1), and then subjected to GPC with a Bio-Beads S-X3 column (Bio-Rad Laboratories, Hercules, CA, USA). The gel material (35 g) was packed in a glass column (55 cm × 27 m i.d.) with DCM:*n*-hexane (1:1) as the eluting solvent at a flow rate of 4 mL/min. The first 96-mL fraction of the eluate contained lipids and was discarded. Subsequently, the next 68 mL fraction was collected. The eluate was concentrated and partitioned between 1 M KOH:ethanol (7:3) and *n*-hexane. After acidification, the phenolic contaminants in the KOH solution were back-extracted twice with 20% diethylether in *n*-hexane. The phenolic fraction was derivatized to O-methylated analogs by diazomethane in diethylether. The neutral and methylated phenolic fractions were purified with a silica-gel column (0.2 g, Wako gel S-1) by an elution of 15 mL DCM:*n*-hexane (12:88, v/v). Each fraction was concentrated to 200 μL and spiked with ^{13}C-labeled PCB-153 to determine the recoveries of internal standards and to correct the volume of samples prior to GC/MS analysis.

2.4. Instruments and quantification

Fifteen analytes were measured by GC/MS/ECNI using an Agilent GC/MSD 5973i (Agilent Technologies, Santa Clara, CA, USA) coupled with a 6890N gas chromatograph. The GC/MS conditions and target ions for the determination of analytes are summarized in Supplementary Table S1. Quantification of the compounds was based on signals in the mass chromatograms and on comparison with ^{13}C-PCB153 that was used as a syringe standard. The concentrations of all analytes were reported as picogram per gram wet weight (pg/g ww).

2.5. Quality control and quality assurance

The extraction, cleanup, and fractionation steps were evaluated by measuring the absolute recoveries of the compounds ($^{13}C_{12}$-labeled internal and native surrogate standards). For recovery tests, two concentrations (2.0 and 10.0 ng/mL) of 11 analytes were spiked into cow milk, passed through the entire analytical procedure and determined based on GC/MS-selected ion monitoring. Procedural blanks were analyzed simultaneously with every batch of 10 samples to check for interference or contamination from solvents and glassware. The recoveries were between 81% and 94% with relative standard deviations of <10% ($n = 5$). The limits of quantification (LOQ), defined as 10-fold that of the noise level, ranged from 1 to 50 pg/g ww (Supplementary Table S1). When the concentrations of the target chemicals were less than their LOQs, we allocated half of the LOQ as the value for analysis. The calibration (2–100 ng/mL of each analyte) was linear and characterized by good correlation coefficients (>0.99). The quality of the method under validation was verified by Standard Reference Materials (non-fortified human serum, SRM1974, NIST) for PCBs and selected pesticides. Data from the current study were within 15% of the certified values of SRM1974.

2.6. Statistical analysis

The data were analyzed using SPSS version 16.0 for Windows 2007 (SPSS Inc., Chicago, IL, USA). Steel–Dwass test and Mann–Whitney *U*-test were used to examine differences in the target chemical concentrations in three sampling years and between two age groups. Spearman's rank correlation coefficients were used to evaluate the relationship between neutral and phenolic OCs. Probability values of less than 0.05 were considered to indicate statistical significance.

3. Results and discussion

3.1. Legacy persistent organohalogen pollutants (POPs)

Within the current study group of legacy organohalogens, serum concentrations of the representative congeners, 4,4′-DDE, PCB-153, *trans*-nonachlor, hexachlorobenzene (HCB) and α-