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).

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).
* $p < 0.01$.
** $p < 0.001$.

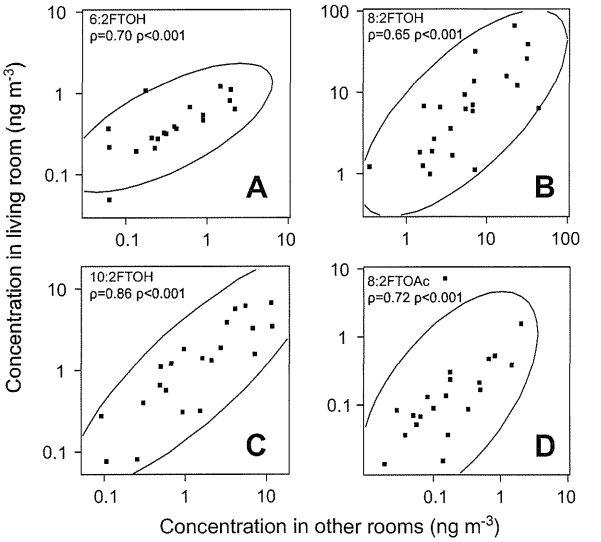


Fig. 1. Correlations of fluorotelomer concentration between living room (*Y* axis) and other rooms (*X* axis) for 6:2 (A), 8:2 (B), 10:2 (C) FTOHs, and 8:2 FTOAc (D), in the same households. Overall, 95% of values are within boundary circles.

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 ($p < 0.001$).

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.

Acknowledgements

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

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2012.09.062.

References

Barber, J.L., Berger, U., Chaemfa, C., Huber, S., Jahnke, A., Temme, C., Jones, K.C., 2007. Analysis of per- and polyfluorinated alkyl substances in air samples from Northwest Europe. J. Environ. Monit. 9, 530–541.

Dreyer, A., Matthias, V., Temme, C., Ebinghaus, R., 2009. Annual time series of air concentrations of polyfluorinated compounds. Environ. Sci. Technol. 43, 4029–4036.

Haug, L.S., Huber, S., Schlabach, M., Becher, G., Thomsen, C., 2011. Investigation on per- and polyfluorinated compounds in paired samples of house dust and indoor air from Norwegian homes. Environ. Sci. Technol. 45, 7991–7998.

Huber, S., Haug, L.S., Schlabach, M., 2011. Per- and polyfluorinated compounds in house dust and indoor air from northern Norway – a pilot study. Chemosphere 84, 1686–1693.

Ishibashi, H., Ishida, H., Matsuoka, M., Tominaga, N., Arizono, K., 2007. Estrogenic effects of fluorotelomer alcohols for human estrogen receptor isoforms alpha and beta *in vitro*. Biol. Pharm. Bull. 30, 1358–1359.

Ishibashi, H., Yamauchi, R., Matsuoka, M., Kim, J.W., Hirano, M., Yamaguchi, A., Tominaga, N., Arizono, K., 2008. Fluorotelomer alcohols induce hepatic vitellogenin through activation of the estrogen receptor in male medaka (*Oryzias latipes*). Chemosphere 71, 1853–1859.

Jahnke, A., Ahrens, L., Ebinghaus, R., Temme, C., 2007. Urban versus remote air concentrations of fluorotelomer alcohols and other polyfluorinated alkyl substances in Germany. Environ. Sci. Technol. 41, 745–752.

Jogsten, I.E., Nadal, M., van Bavel, B., Lindström, G., Domingo, J.L., 2012. Per- and polyfluorinated compounds (PFCs) in house dust and indoor air in Catalonia, Spain: implications for human exposure. Environ. Int. 39, 172–180.

Kissa, E., 2001. Fluorinated Surfactants and Repellents, second ed. Marcel Dekker, New York.

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

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

Kudo, N., Iwase, Y., Okayachi, H., Yamakawa, Y., Kawashima, Y., 2005. Induction of hepatic peroxisome proliferation by 8-2 telomer alcohol feeding in mice. formation of perfluorooctanoic acid in the liver. Toxicol. Sci. 86, 231–238.

Langer, V., Dreyer, A., Ebinghaus, R., 2010. Polyfluorinated compounds in residential and nonresidential indoor air. Environ. Sci. Technol. 44, 8075–8081.

Liu, C., Zhang, X., Chang, H., Jones, P., Wiseman, S., Naile, J., Hecker, M., Giesy, J.P., Zhou, B., 2010. Effects of fluorotelomer alcohol 8:2 FTOH on steroidogenesis in H295R cells: targeting the cAMP signalling cascade. Toxicol. Appl. Pharmacol. 247, 222–228.

Oda, Y., Nakayama, S., Harada, K.H., Koizumi, A., 2007. Negative results of umu genotoxicity test of fluorotelomer alcohols and perfluorinated alkyl acids. Environ. Health Prev. Med. 12, 217–219.

Oono, S., Harada, K.H., Mahmoud, M.A., Inoue, K., Koizumi, A., 2008a. Current levels of airborne polyfluorinated telomers in Japan. Chemosphere 73, 932–937.

Oono, S., Matsubara, E., Harada, K.H., Takagi, S., Hamada, S., Asakawa, A., Inoue, K., Watanabe, I., Koizumi, A., 2008b. Survey of airborne polyfluorinated telomers in Keihan area. Jpn. Bull. Environ. Contam. Toxicol. 80, 102–106.

Phillips, M.M., Dinglasan-Panlilio, M.J., Mabury, S.A., Solomon, K.R., Sibley, P.K., 2007. Fluorotelomer acids are more toxic than perfluorinated acids. Environ. Sci. Technol. 41, 7159–7163.

Shoeib, M., Harner, T., Ikonomou, M., Kannan, K., 2004. Indoor and outdoor air concentrations and phase partitioning of perfluoroalkyl sulfonamides and polybrominated diphenyl ethers. Environ. Sci. Technol. 38, 1313–1320.

Shoeib, M., Harner, T.M., Webster, G., Lee, S.C., 2011. Indoor sources of poly- and perfluorinated compounds (PFCS) in vancouver, canada: implications for human exposure. Environ. Sci. Technol. 45, 7999–8005.

Stock, N.L., Lau, F.K., Ellis, D.A., Martin, J.W., Muir, D.C., Mabury, S.A., 2004. Polyfluorinated telomer alcohols and sulfonamides in the North American troposphere. Environ. Sci. Technol. 38, 991–996.

Washburn, S.T., Bingman, T.S., Braithwaite, S.K., Buck, R.C., Buxton, L.W., Clewell, H.J., Haroun, L.A., Kester, J.E., Rickard, R.W., Shipp, A.M., 2005. Exposure assessment and risk characterization for perfluorooctanoate in selected consumer articles. Environ. Sci. Technol. 39, 3904–3910.

Environmental Science & Technology

Article

pubs.acs.org/est

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

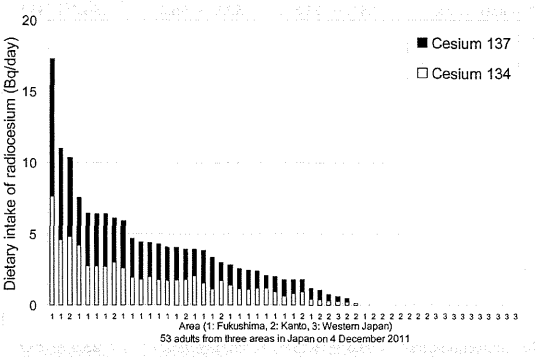
Kouji H. Harada,[†] Yukiko Fujii,[†] Ayumu Adachi,[†] Ayako Tsukidate,[‡] Fumikazu Asai,[‡] and Akio Koizumi*[,†]

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

[‡]Science and Medical News Section, The Asahi Shimbun Company, Tokyo, Japan

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

Received: April 5, 2012
Revised: December 19, 2012
Accepted: December 24, 2012
Published: December 24, 2012

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:

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

To confirm the results, persimmons and apples produced in Fukushima, which were frequently consumed by the participants, were analyzed ($n = 16$). The mean radioactivity ($\pm$SD) was 23 $\pm$ 28 and 30 $\pm$ 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 $\pm$ 52 and 24 $\pm$ 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 $\times$ 110 + 24/1000 $\times$ 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.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: 81-75-753-4456. Fax: 81-75-753-4458. E-mail: koizumi.akio.5v@kyoto-u.ac.jp.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This study was mainly supported by the Asahi Shimbun Company and Grants-in-Aid for Health Sciences Research from the Ministry of Health, Labour and Welfare (H21-Food-003 and H24-Food-002), the Overall Promotion of Environmental Research of the Ministry of the Environment (ZB-1202), and the special cooperation budget of Kyoto University (H24-3).

■ REFERENCES

(1) Ohnishi, T. The Disaster at Japan's Fukushima-Daiichi Nuclear Power Plant after the March 11, 2011 Earthquake and Tsunami, and the Resulting Spread of Radioisotope Contamination. *Radiat. Res.* **2011**, *177*, 1–14.

(2) Chino, M.; Nakayama, H.; Nagai, H.; Terada, H.; Kataya, G.; Yamazawa, H. Preliminary Estimation of Release Amounts of ^{131}I and ^{137}Cs Accidentally Discharged from the Fukushima Daiichi Nuclear Power Plant into the Atmosphere. *J. Nucl. Sci. Technol.* **2011**, *48*, 1129–1134.

(3) *Handling of food contaminated by radioactivity (Relating to the accident at the Fukushima Nuclear Power Plant)*, Department of Food Safety, Ministry of Health, Labour and Welfare, Labour and Welfare (Notice No. 0317, March 17, 2011). http://www.mhlw.go.jp/stf/houdou/2r9852000001558e-img/2r98520000015apy.pdf and http://www.mhlw.go.jp/stf/houdou/2r9852000001558e-img/2r98520000015av4.pdf. 2011.

(4) *Regarding the Evaluation of the Conditions on Reactor Cores of Unit 1, 2 and 3 related to the Accident at Fukushima Dai-ichi Nuclear Power Station, Tokyo Electric Power Co. Inc.*; Nuclear and Industrial Safety Agency (June 6, 2011). http://www.meti.go.jp/press/2011/06/20110606008/20110606008-2.pdf (in Japanese).

(5) United Nations Scientific Committee on the Effects of Atomic Radiation. Annex D. Health effects due to radiation from the

Chernobyl accident. Sources and Effects of Ionizing Radiation, UNSCEAR 2008 Report Vol. II: Effects, Report to the General Assembly Scientific Annexes C, D and E. United Nations: New York. 2011. ISBN 978-92-1-142280-1.

(6) *Results of Nuclide Analysis of Plutonium and Strontium by MEXT*; Ministry of Education, Culture, Sports, Science and Technology (30 September 2011). http://radioactivity.mext.go.jp/old/en/1750/2011/09/1750_093014.pdf.

(7) *Estimation of contribution of radiocesium to internal exposure from dietary intake*; Working group on radionuclides, Pharmaceutical Affairs and Food Sanitation Council (24 November 2011). http://www.mhlw.go.jp/stf/shingi/2r9852000001w5ek-att/2r9852000001w5je.pdf (in Japanese).

(8) Koizumi, A.; Harada, K.; Inoue, K.; Hitomi, T.; Yang, H.-R.; Moon, C.-S.; Wang, P.; Hung, N.; Watanabe, T.; Shimbo, S.; Ikeda, M. Past, Present, and Future of Environmental Specimen Banks. *Environ. Health Prev. Med.* **2009**, *14*, 307–318.

(9) Kaiser, H.; Specker, H. Bewertung und Vergleich von Analysenverfahren. *Fresenius' Z. Anal. Chem.* **1956**, *149*, 46–66.

(10) International Commission on Radiological ProtectionAge-dependent Doses to the Members of the Public from Intake of Radionuclides - Part 5 Compilation of Ingestion and Inhalation Coefficients, *ICRP Publication 72. Ann. ICRP 26 (1)*, 1995.

(11) United Nations Scientific Committee on the Effects of Atomic Radiation. Sources, effects and risks of ionization radiation, Report to The General Assembly, with Scientific Annexes B: Exposures from Natural Radiation Sources. 2000.

(12) Hayter, A. J. A Proof of the Conjecture that the Tukey-Kramer Multiple Comparisons Procedure is Conservative. *Ann. Statist.* **1984**, *12*, 61–75.

(13) Steel, R. D. G. A Rank Sum Test for Comparing All Pairs of Treatments. *Technometrics* **1960**, *2*, 197–207.

(14) Dwass, M. Some k-Sample Rank-Order Tests. In *Contributions to Probability and Statistics*; Olkin, I., et al. Eds.; Stanford University Press: Palo Alto, 1960; pp 198–202.

(15) *Preparation of Air Dose Rate Map based on the Readings of the Second Fukushima Prefecture Environmental Radiation Monitoring in Mesh Survey*; Disaster Provision Main Office of Fukushima Prefecture (December 2, 2011).http://radioactivity.mext.go.jp/en/contents/5000/4602/24/209_1203.pdf.

(16) National Institute of Population and Social Security Research. *Population Statistics Data Book 2012*. Health, Labour and Welfare Statistics Association: Tokyo, Japan, 2012; ISBN: 9784875115236.

(17) *Estimation of population of Fukushima Prefecture after Tohoku Earthquake*. Fukushima Prefecture (No. ToRo-93, March 2012). http://www.pref.fukushima.jp/toukei/data/02/jinkounen/23jinkounen/honpen.pdf (in Japanese).

(18) Ministry of Health, Labour and Welfare. The National Health and Nutrition Survey in Japan, 2009, 2011. Available on http://www.mhlw.go.jp/bunya/kenkou/eiyou/dl/h21-houkoku-01.pdf (in Japanese).

(19) Statistics Bureau. Japan Statistical Yearbook, Farm households by degree of engagement and farm household population, by prefecture (commercial farm households), 2010. www.stat.go.jp/data/nenkan/zuhyou/y0703000.xls.

(20) Statistics Bureau. Annual Report on the Family Income and Expenditure Survey 2010, released in June 2011. http://www.e-stat.go.jp/SG1/estat/GL38020103.do?_xlsDownload_&fileId=000004088929&releaseCount=1.

(21) Simon, V.; Avery, J. Fate of Caesium in the Environment: Distribution between the Abiotic and Biotic Components of Aquatic and Terrestrial Ecosystems. *J. Environ. Radioact.* **1996**, *30*, 139–171.

(22) Fukushima Prefecture. Monitoring results of agricultural products. 2012. http://www.new-fukushima.jp/monitoring.php (in Japanese) (accessed March 1, 2012).

(23) Yamamoto, M.; Sakaguchi, A.; Tomita, J.; Imanaka, T.; Shiraishi, K. Measurements of ^{210}Po and ^{210}Pb in Total Diet Samples: Estimate of Dietary Intakes of 210 Po and 210 Pb for Japanese. *J. Radioanal. Nucl. Chem.* **2009**, *279*, 93–103.

(24) Sugiyama, H.; Terada, H.; Isomura, K.; Iijima, I.; Kobayashi, J.; Kitamura, K. Internal Exposure to ^{210}Po and ^{40}K from Ingestion of Cooked Daily Foodstuffs for Adults in Japanese Cities. *J. Toxicol. Sci.* **2009**, *34*, 417–425.

(25) *Notification on amendment of Ordinance of the Ministry of Health, Labour and Welfare on ingredient standards of milk and dairy, specifying radionuclides provided by the Minister of Health, Labour and Welfare pursuant to the provision in item (1) of paragraph (1) of Table 2 of the Ordinance of the Ministry, and amendment of Standard limits for Radionuclides in Foods*; Director of the Food and Drug Administration Food Safety Ministry of Health, Labour and Welfare.(Food Safety 0315 No. 1, March 15, 2012). http://www.mhlw.go.jp/shinsai_jouhou/dl/tuuchi_120316.pdf (in Japanese).

(26) *New Standard limits for Radionuclides in Foods (provisional translation)*; Department of Food Safety, Pharmaceutical & Food Safety Bureau, Ministry of Health, Labour and Welfare (March 1, 2012).http://www.mhlw.go.jp/english/topics/2011eq/dl/new_standard.pdf. 2012.

(27) The Fukushima Health Management Survey, Estimation of external exposure; Review Committee for the Fukushima Prefecture Prefectural People's Health Management Survey (August 13, 2012). http://www.pref.fukushima.jp/imu/kenkoukanri/240813senryousuikei.pdf (in Japanese).

(28) Koizumi, A.; Harada, K. H.; Niisoe, T.; Adachi, A.; Fujii, Y.; Hitomi, T.; Kobayashi, H.; Wada, Y.; Watanabe, T.; Ishikawa, H. Preliminary Assessment of Ecological Exposure of Adult Residents in Fukushima Prefecture to Radioactive Cesium through Ingestion and Inhalation. *Environ. Health Prev. Med.* **2012**, *17*, 292–298, DOI: 10.1007/s12199-011-0251-9.

(29) Department of Food Safety, Ministry of Health, Labour and Welfare. Sum up of radionuclide test results carried out since 19 March 2011 (Up-to-date Report as of 19:00, 28 February 2012). 2012. http://www.mhlw.go.jp/english/topics/2011eq/dl/28Feb2012_Sum_up.pdf.

(30) Hamada, N.; Ogino, H.; Fujimichi, Y. Safety Regulations of Food and Water Implemented in the First Year Following the Fukushima Nuclear Accident. *J. Radiat. Res.* **2012**, *53*, 641–671.

(31) *Transition of radiocesium levels in monitoring of food products*; Department of Food Safety, Ministry of Health, Labour and Welfare (January 16, 2012). http://www.mhlw.go.jp/topics/bukyoku/iyaku/syoku-anzen/iken/dl/120420-2_3-2.pdf (in Japanese).

(32) Broadley, M.; White, P. Some Elements Are More Equal than Others: Soil-to-Plant Transfer of Radiocaesium and Radiostrontium, Revisited. *Plant Soil* **2012**, *355*, 23–27.

(33) Shutov, V. N.; Bruk, G. Y.; Basalaeva, L. N.; Vasilevitskiy, V. A.; Ivanova, N. P.; Kaplan, I. S. The Role of Mushrooms and Berries in the Formation of Internal Exposure Doses to the Population of Russia After the Chernobyl Accident. *Radiat. Prot. Dosim.* **1996**, *67*, 55–64.

(34) Hamada, N.; Ogino, H. Food Safety Regulations: What We Learned from the Fukushima Nuclear Accident. *J. Environ. Radioact.* **2012**, *111*, 83–99.

(35) Takano, K. Prevention of Internal Exposure to Cesium-137 (^{137}Cs) Radiation in Inhabitants of an Area Contaminated by the Chernobyl Accident. *Environ. Health Prev. Med.* **1996**, *1*, 28–32.

Marine Pollution Bulletin 69 (2013) 116–121

Contents lists available at SciVerse ScienceDirect

Marine Pollution Bulletin

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

Carbon and nitrogen stable isotope ratios and mercury concentration in the scalp hair of residents from Taiji, a whaling town

Tetsuya Endo [a,*], Moriaki Hayasaka [b], Yohsuke Hisamichi [a], Osamu Kimura [a], Koichi Haraguchi [c]

[a] School of Pharmaceutical Sciences, Health Sciences University of Hokkaido, 1757 Ishikari-Tobetsu, Hokkaido 061-0293, Japan
[b] Sapporo Higashi-Tokushukai Hospital, N33-E14 Higashi-Ku, Sapporo, Hokkaido 065-0033, Japan
[c] Daiichi College of Pharmaceutical Sciences, 22-1 Tamagawa-Cho, Minami-Ku, Fukuoka 815-8511, Japan

ARTICLE INFO

Keywords:
Scalp hair
Whale meat
Stable isotope ratio of carbon ($\delta^{13}C$)
Stable isotope ratio of nitrogen ($\delta^{15}N$)
Mercury

ABSTRACT

We analyzed stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) as well as mercury (Hg) concentration in the scalp hair of Japanese who consumed whale meat and those who did not, and investigated the relationships among the $\delta^{13}C$ and $\delta^{15}N$ values and Hg concentration. The average $\delta^{15}N$ and $\delta^{13}C$ values of whale meat-eaters (10.11‰ and −18.5‰) were significantly higher than those of non-eaters (9.28‰ and −18.9‰), respectively. The average Hg concentration of whale meat-eaters (20.6 μg/g) was significantly higher than that of non-eaters (2.20 μg/g). Significant positive correlations were found between the $\delta^{13}C$ and $\delta^{15}N$ values and between the $\delta^{15}N$ value and Hg concentration in the hair of whale meat-eaters, while the correlation between the $\delta^{15}N$ value and Hg concentration was not statistically significant in the non-eaters. The consumption of whale meat may increase Hg concentration as well as $\delta^{15}N$ and $\delta^{13}C$ values in scalp hair.

1. Introduction

Mercury (Hg) is distributed through the environment via both natural and anthropogenic processes. As Hg accumulation in marine biota increases with an increase in the trophic level, predators generally accumulate high levels of Hg. Among such predators, marine mammals are long-lived and occupy the top level of the marine food web, thus the accumulation of Hg in these animals is very high (Atwell et al., 1998; Wagemann et al., 1998; Endo et al., 2003, 2004, 2005, 2010). The Hg concentration in scalp hair is the preferred marker for evaluating Hg exposure over a period of several weeks or months (JECFA, 2003). High levels of Hg, exceeding 10 μg/g, have been found in the scalp hair of residents who consumed marine mammals in Greenland (Weihe et al., 2002), the Faroe Islands (Weihe and Joensen, 2008), Japan (Endo and Haraguchi, 2010) and Canada (Tian et al., 2011). The Food and Agriculture Organization (FAO)/WHO Joint Expert Committee on Food Additives (JECFAs) lowered its guideline value for the provisional tolerable weekly intake (PTWI) of methyl mercury from 3.3 μg/kg-bw/week to 1.6 μg/kg-bw/week (JECFA, 2003). This revised PTWI corresponds to a hair Hg level of 2.2 μg/g (Yasutake et al., 2004). Most consumers of marine mammals could exceed the revised PTWI of methyl mercury.

Stable isotope analysis has been used as a tool to obtain information on the feeding ecology of marine species. The $\delta^{15}N$ value is used to estimate the trophic level of a food chain, while the $\delta^{13}C$ value is used to estimate the relative contribution to the diet of potential primary sources (Kelly, 2000). A significant increase in $\delta^{15}N$ of 3.4 ± 1.1‰ has been shown to occur between consumer and prey (Minagawa and Wada, 1984), whereas only a small enrichment of about 1‰ is found in the $\delta^{13}C$ value (DeNiro and Epstein, 1981). High $\delta^{15}N$ values in the muscle of marine mammals, reflecting their high trophic positions, have been reported by Atwell et al. (1998), Hobson and Welch (1992), and Endo et al. (2010). Instead of muscle samples from the animal, stable isotope analysis of hair and fingernail samples from humans has been used for estimation of what in food items (Minagawa, 1992; Yoshinaga et al., 1996), the nutritional and metabolic status (Petzke et al., 2010) and life history with regard to geographical origin and/or typical food supply (O'Connell et al., 2001; Buchardt et al., 2007). In contrast to muscle samples from animals, increases in the $\delta^{15}N$ and $\delta^{13}C$ values in scalp hair samples were reported to be 4.0 and 2.5‰, respectively (Minagawa, 1992), and 5.1–6.9 and 1.8–4.8‰, respectively (Yoshinaga et al., 1996).

Positive correlations between the $\delta^{15}N$ value and the Hg concentration in the animal muscle or food product have been reported (Atwell et al., 1998; Campbell et al., 2005; Bisi et al., 2012; Harmelin-Vivien et al., 2012). However, the relationship between the $\delta^{15}N$ value and the Hg concentration in human hair or fingernail samples has not yet been investigated. Many research

* Corresponding author. Tel./fax: +81 133 23 3902.
E-mail address: endotty@hoku-iryo-u.ac.jp (T. Endo).

0025-326X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.marpolbul.2013.01.018

groups have reported very high levels of Hg in the scalp hair of people who ate marine mammals (Weihe et al., 2002; Weihe and Joensen, 2008; Endo and Haraguchi, 2010; Tian et al., 2011), but these groups did not determine the stable isotope ratio of $\delta^{15}N$. On the other hand, Buchardt et al. (2007) have reported very high levels of $\delta^{15}N$ in the fingernails of Inuit who ate marine mammals contaminated with Hg, but they did not analyze Hg concentration in the samples taken.

Taiji, is a small town in Wakayama Prefecture, Japan, which has suffered severely from emigration, with about one third of its residents now aged 65 or over. High levels of Hg have been found in the red meat products from short-finned pilot whales (*Globicephala macrorhynchus*) sold in and around Taiji, and the Hg level in the scalp hair of Taiji residents was found to be markedly higher than that in residents of other areas of Japan: the Hg level of scalp hair of Taiji residents who ate whale meat more than once each month was 24.6 ± 15.6 μg/g (n = 39) (Endo and Haraguchi, 2010), while the Hg level in the large-scale surveys in Japan was about 2.0 μg/g (Yasutake et al., 2004; Yasuda et al., 2005). Here, we report the stable isotope ratios of $\delta^{15}N$ and $\delta^{13}C$ as well as the Hg concentration in the scalp hair of whale meat-eaters and non-eaters in Taiji and other areas of Japan, and investigated the relationships among the $\delta^{15}N$ and $\delta^{13}C$ values and Hg concentration in hair samples.

2. Materials and methods

2.1. Sampling of scalp hair

Scalp hair samples from 57 residents living in Taiji (35 men and 22 women) were collected by local collaborators from their acquaintances between November and December 2007 and in July 2008. As reported previously (Endo and Haraguchi, 2010), at the time of collection, a simple questionnaire, detailing age, the species consumed and frequencies of consumption of toothed whales and dolphin products per month and tuna and other marine products per week, was completed, along with a declaration of informed consent. The average age of the 57 Taiji residents who cooperated in this survey was high (59, range 7–85) and 45 of the residents ate the whale products from toothed whales and dolphins. On the other hand, scalp hair samples from 72 donors living in Hokkaido and Aomori Prefectures and the Tokyo Metropolitan area (30 men and 42 women) were collected between November 2007 and January 2011, and no donor consumed products from either toothed whales or dolphins. The number and age of hair donors are summarized in Table 1. Taiji residents shown in Table 1 include the 50 Taiji residents for whom we previously reported scalp hair Hg levels (Endo and Haraguchi, 2010). The hair samples were packed in polyethylene bags and stored at room temperature until analysis.

2.2. Chemical analyses

Total mercury (Hg) concentration in the hair samples was determined using a flameless atomic absorption spectrophotometer (Hiranuma Sangyo Co. Ltd., HG-1, Ibaraki, Japan) after digestion by a mixture of HNO_3, HCO_4 and H_2SO_4 (Endo et al., 2002). Human hair No. 13, a certified reference material from NIES (Japan), was used as an analytical quality control for Hg, and the recovery of Hg was 96 ± 3% (n = 3).

The stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) in the hair samples after the removal of lipids using chloroform/methanol extraction were analyzed by a mass spectrometer (Delta S, Finnigan MAT, Bremen, Germany) coupled with an elemental analyzer (EA1108, Fisons, Roano, Milan, Italy) held in the Center for Ecological Research (CER), Kyoto University, as reported previously (Endo et al., 2012). CERKU-1, -2 and -5, certified by the Center for Ecology Research, Kyoto University (Kyoto, Japan), were used as the $\delta^{13}C$ and $\delta^{15}N$ reference materials.

The trophic magnification factor (TMF) of Hg was calculated from the slope of regression line generated from the $\delta^{15}N$ value and the logarithmic concentration of Hg in the scalp hair, according to the previous report (Harmelin-Vivien et al., 2012).

2.3. Statistical analyses

The data was expressed as the arithmetic mean (AM) ± SD. Some data for Hg were expressed not only as the AM but also the geometric mean (GM) for comparison with previous reports. The data were analyzed using Student's t test and Pearson's coefficient test, using the Statcel 2 program, and the level of significance was set at $p < 0.05$.

3. Results

Table 2 summarizes the analytical data for the $\delta^{13}C$ and $\delta^{15}N$ values and Hg concentration in the hair of both the donors who ate toothed whale meat and those who did not. All of the whale meat-eaters were Taiji residents (n = 45), and the non-eaters were residents who lived in Taiji (n = 12) as well as in Hokkaido and Aomori Prefectures and the Tokyo Metropolitan area (n = 72). The average age of whale meat-eaters (63 ± 17 year, n = 45) was significantly older than that of non-eaters (36 ± 21 year, n = 84). The average concentration of Hg in the hair of whale meat-eaters (20.6 ± 14.3 μg/g, n = 45) was about nine times higher than that of the non-eaters (2.20 ± 1.83 μg/g, n = 84). The average values of both $\delta^{13}C$ and $\delta^{15}N$ in the whale meat-eaters were slightly but significantly higher than those in the non-eaters. Irrespective of whale meat consumption, the average value of $\delta^{13}C$ was slightly but significantly higher in males than in females, and the average

Table 1
Numbers of hair donors who ate or did not eat whale meat.

	Whale meat eaters in Taiji			Non-eaters in Taiji			Non-eaters in other areas		
	Male	Female	Total	Male	Female	Total	Male	Female	Total
Age	60 ± 18	68 ± 13	63 ± 17	35 ± 26	50 ± 19	44 ± 23	33 ± 20	35 ± 21	34 ± 21
<10	0	0	0	2	0	2	5	5	10
10 ⩽ <20	1	0	1	0	1	1	2	4	6
20 ⩽ <30	1	0	1	0	0	0	6	12	18
30 ⩽ <40	2	0	2	1	0	1	6	6	12
40 ⩽ <50	1	1	2	0	2	2	4	4	8
50 ⩽ <60	12	5	17	1	2	3	3	6	9
60 ⩽ <70	3	0	3	1	1	2	3	2	5
70 ⩽ <80	5	6	11	0	1	1	1	2	3
80 ⩽ <90	5	3	8	0	0	0	0	1	1
Total number	30	15	45	5	7	12	30	42	72

Table 2
Analytical results of mercury, $\delta^{15}N$ and $\delta^{13}C$ in the hair of donors who ate the whale meat and those who did not in Japan.

	Age	Hg (μg/g)	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
Whale meat-eaters				
Male (n = 30)	60 ± 18[a]	23.4 ± 15.5[a]	10.15 ± 0.64[a]	−18.4 ± 0.5[a,b]
Female (n = 15)	68 ± 13[a]	15.1 ± 9.9[a]	10.04 ± 0.40[a]	−18.8 ± 0.6
Total (n = 45)	63 ± 17[a]	20.6 ± 14.3[a]	10.11 ± 0.57[a]	−18.5 ± 0.6[a]
Non-eaters				
Male (n = 35)	34 ± 21	2.62 ± 1.90	9.25 ± 0.42	−18.7 ± 0.7[b]
Female (n = 49)	37 ± 22	1.88 ± 1.74	9.30 ± 0.55	−19.1 ± 0.5
Total (n = 84)	36 ± 21	2.20 ± 1.83	9.28 ± 0.50	−18.9 ± 0.6

[a] Significantly different from non-eaters.
[b] Significantly different from females.

Hg concentration was slightly higher in males than in females ($p > 0.05$). As data not shown in Table 2, the $\delta^{13}C$ and $\delta^{15}N$ values and Hg concentration in the non-eaters from Taiji were all similar to those of non-eaters from other areas.

Fig. 1 shows the relationship between age and Hg concentration in the hair of donors who ate whale meat and those who did not. The Hg concentrations in the non-eaters increased with age up to 50–60 years and thereafter slightly decreased. On the other hand, the Hg concentrations in the hair of whale meat-eaters increased with age up to 50–60 years and did not decrease thereafter.

Significant correlations were found between the $\delta^{13}C$ and $\delta^{15}N$ value in the hair of whale meat-eater and non-eater groups (Fig. 2). The slopes of regression lines of the whale meat-eater group (0.4246) and the non-eater group (0.3626) were similar.

Fig. 3 shows the relationship between age and the $\delta^{15}N$ or $\delta^{13}C$ value in the scalp hair. No correlations were found between age and the $\delta^{15}N$ or $\delta^{13}C$ value in either group.

Fig. 4 shows the relationship between Hg concentration and the $\delta^{15}N$ or $\delta^{13}C$ values in the hair of the whale meat-eater and non-eater groups. The $\delta^{15}N$ values were significantly correlated with the Hg concentrations in the hair of whale meat-eater group ($\gamma = 0.549$, $p < 0.05$) and tended to correlate with the Hg concentration in the non-eater ($\gamma = 0.202$, $p > 0.05$). The $\delta^{15}N$ values were also significantly correlated with the logarithmic concentrations of Hg in the whale meat-eaters ($\gamma = 0.597$, $p < 0.05$) and tended to correlate in the non-eaters ($\gamma = 0.176$, $p > 0.05$). The TMFs of Hg calculated from the slopes of the regression lines (0.308 for the whale meat-eaters and 0.127 for the non-eaters) were 2.03 and 1.34, respectively. On the other hand, there was no correlation

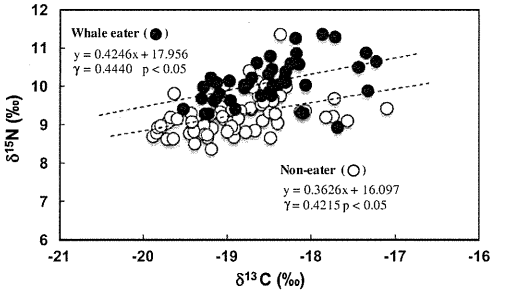


Fig. 2. The $\delta^{13}C$–$\delta^{15}N$ plots of the scalp hair from donors who ate whale meat (Taiji residents) and those who did not. See Tables 1 and 2.

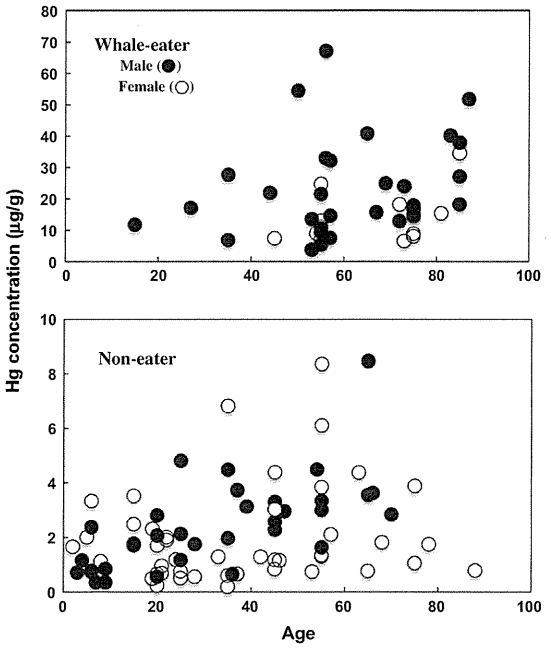


Fig. 1. Age-dependent accumulation of Hg in the scalp hair of donors who ate whale meat (Taiji residents) and those who did not (from Taiji and other areas). See Tables 1 and 2.

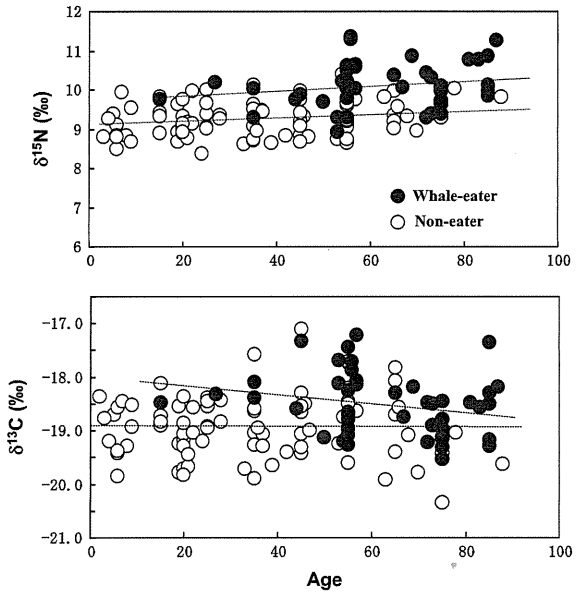


Fig. 3. Age-dependent changes in $\delta^{15}N$ and $\delta^{13}C$ in the scalp hair of donors who ate whale meat (Taiji residents) and those who did not (from Taiji and other areas). See Tables 1 and 2.

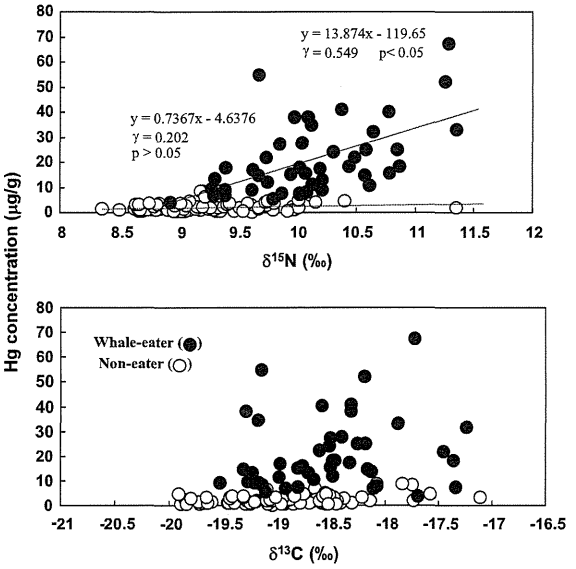


Fig. 4. Relationships between Hg concentration and $\delta^{15}N$ or $\delta^{13}C$ in the scalp hair of donors who ate whale meat (Taiji residents) and those who did not. See Tables 1 and 2.

between the Hg concentration and $\delta^{13}C$ value in the hair of whale meat-eater and the non-eater groups.

4. Discussion

4.1. A comparison of mercury level and stable isotope ratios in the hair of donors who ate whale meat and those who did not

The Hg concentrations in the hair of males and females who did not eat whale meat products (Table 2) were 2.62 ± 1.90 (n = 35) and 1.88 ± 1.74 (n = 49) μg/g, respectively (GMs were 2.08 and 1.34 μg/g, respectively), and those with the highest concentration were in their 50s (Fig. 1). Although the number of hair samples analyzed in this study was limited, these average Hg concentrations in males and females and the age-dependent changes in Hg concentration were in good agreement with the results of large-scale surveys of Hg in scalp hair: The GMs of Hg concentrations in male and female hair were 2.42 μg/g (n = 4274) and 1.37 μg/g (n = 4391), respectively (Yasutake et al., 2004), the Hg concentration tended to increase with age showing a peak of 5.9 μg/g at 50–60 years before decreasing with further aging (Yasuda et al., 2005), and the Hg concentration in the hair from males was higher than in that from females (Yasutake et al., 2004; Yasuda et al., 2005).

In the present survey, on the other hand, the average Hg concentrations in male and female whale meat-eaters were 23.4 ± 15.5 μg/g (n = 30) and 15.1 ± 9.9 μg/g (n = 15), respectively (GMs were 19.2 and 12.8 μg/g, respectively). These averages were about nine times higher than those of male and female non-eaters, respectively, although the average ages of the male and female whale meat-eater were markedly higher than those of the non-eaters (Table 2). The average Hg concentrations in the whale meat-eaters in their 60s and 70s were also several times higher than the corresponding concentrations in the non-eaters of similar ages (Fig. 1), suggesting that the consumption of whale meat increases the level of Hg in the scalp hair.

The Hg concentration in the hair from male whale meat-eaters was slightly higher than that in the hair from female whale meat-eaters, probably reflecting the higher consumption of whale meat by males (Endo and Haraguchi, 2010). However, the Hg concentration in the hair from male non-eaters was also slightly higher than that in the hair from female non-eaters (Table 2), and the Hg concentration in the hair from males was also higher than that in the hair from females in the large-scale surveys (Yasutake et al., 2004; Yasuda et al., 2005). Johnsson et al. (2004) reported a higher level of Hg in the scalp hair of males than females, in spite of the same level of fish consumption. The reason for the higher level of Hg in male scalp hair remains unclear.

The $\delta^{13}C$ and $\delta^{15}N$ values in the hair of whale meat-eaters were −18.5 ± 0.6‰ and 10.11 ± 0.57‰, respectively, and those in non-eaters were significantly lower (−18.9 ± 0.6‰ and 9.28 ± 0.50‰, respectively). The $\delta^{13}C$ value in the hair of whale meat-eaters was slightly but significantly higher in males than in females. Unfortunately, the current values of $\delta^{13}C$ and $\delta^{15}N$ in the scalp hair of Japanese are unavailable. Two decades ago, Minagawa (1992) reported the stable isotope ratios in the scalp hair of Japanese, with those $\delta^{13}C$ and $\delta^{15}N$ in males (n = 23) being −18.04 ± 0.45‰ and 10.36 ± 0.48‰, respectively, and those in females (n = 19) being −18.35 ± 0.33‰ and 10.31 ± 0.41‰, respectively. Minagawa implied that the increase in $\delta^{15}N$ value was due to fish consumption. These earlier averages of $\delta^{13}C$ and $\delta^{15}N$ in Japanese were similar to those in the whale meat-eaters in the present study, but not to those of the non-eaters (Table 2). The eating habits of Japanese have changed over the past two decades: the consumption of fish has decreased, while the consumption of livestock meat and lipids has increased. A large-scale of survey of $\delta^{13}C$ and $\delta^{15}N$ in scalp hair, with a concomitant analysis of Hg, is necessary to elucidate the latest dietary habits of Japanese.

4.2. Comparison of mercury level and stable isotope ratios in the hair of Inuit

In a traditional Inuit community in Greenland, they are eating the marine mammals contaminated with high level of Hg. The average Hg concentration (GM) in Inuit maternal hair was 15.5 μg/g (maximum was 32.9 μg/g, n = 31) and that in the hair of children was 5.5 μg/g (maximum was 18.4 μg/g, n = 43, median age was 8.4 years) (Weihe et al., 2002). The average Hg concentration in Inuit mothers is comparable with the average Hg concentration in the female whale meat-eaters in Taiji as shown in Table 2 (15.1 ± 9.9 μg/g, n = 15, GM was 12.8 μg/g), while the Hg average concentration in Inuit children is several times higher than that in Japanese children, as shown in Fig. 1 and reported by Yasutake et al. (2004) and Yasuda et al. (2005). To our knowledge, most Japanese children do not eat marine mammals.

Most of the whale meat-eaters in Taiji ate the red meat of pilot whales contaminated with high levels of Hg (12.4 ± 8.6 μg/g) (Endo and Haraguchi, 2010) at least once a month. In contrast, the Hg concentrations in the traditional food items daily consumed by Inuit (land animals, marine mammals, fish and birds) were below 1.0 μg/wet g (Wagemann et al., 1998; Tian et al., 2011). Some of the Hg concentrations in those animals are listed in Table 3. Thus the mode of Hg ingestion from food could differ markedly between Taiji residents and Inuit.

Buchardt et al. (2007) reported that the $\delta^{13}C$ and $\delta^{15}N$ values in the fingernails of Inuit were −18.2 ± 0.6‰ and 16.0 ± 1.5‰, respectively. The average $\delta^{15}N$ value in the scalp hair of Taiji residents was markedly lower than that in the fingernails of Inuit. The $\delta^{15}N$ values in cetaceans and tuna consumed by Taiji residents were markedly lower than those in the marine mammals consumed by Inuit (Table 3). Lower $\delta^{15}N$ values in the scalp hair of Taiji residents could be ascribed to the lower $\delta^{15}N$ values in the pilot whales and tuna consumed. We previously reported that the $\delta^{15}N$ values in cetacean samples from Taiji and vicinity (central Japan) were lower than those in samples from north Japan,

Table 3
Carbon and nitrogen stable isotope ratios and mercury concentrations in cetaceans and tuna consumed by Inuit in Greenland and Canada as well as in Taiji residents in Japan.

Species	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Hg (μg/wet g)
Greenland and Canada			
Walrus	−17.8 ± 0.3[a]	12.5 ± 0.6[a]	0.12[e]
Narwhal	−18.0 ± 0.4[a]	15.8 ± 0.7[a]	0.97 ± 0.33[d]
Beluga whale	−18.1 ± 0.5[a]	16.6 ± 0.6[a]	0.96 ± 0.39[d], 0.35[e]
Ringed seal	−17.3 ± 0.7[a]	17.3 ± 1.1[a]	0.43 ± 0.31[d], 0.13[e]
Bearded seal	−16.6 ± 0.5[a]	16.8 ± 0.2[a]	0.32[e]
Greenland seal	−19.0[a]	15.4[a]	
Fur seal	−16.6[a]	16.6[a]	
Japan			
Short-finned pilot whale	−16.9 ± 0.5[b]	12.2 ± 0.7[b]	12.4 ± 8.6[b]
Striped dolphin	−17.7 ± 0.6[b]	12.3 ± 0.9[b]	5.91 ± 4.04[b]
Risso's dolphin	−16.7 ± 0.3[b]	13.1 ± 0.5[b]	3.84 ± 1.52[b]
Yellowfin tuna	−16.4 ± 0.4[c]	10.4 ± 1.4[c]	0.31 ± 0.26[c]
Albacore tuna	−16.8 ± 1.0[c]	10.8 ± 1.2[c]	0.42 ± 0.14[c]

[a] Buchardt et al. (2007).
[b] Endo et al. (2010).
[c] Hisamichi et al. (2010).
[d] Wagemann et al. (1998).
[e] Tian et al. (2011).

reflecting differences in the marine environment around Japan (Endo et al., 2010).

4.3. Relationships among the $\delta^{13}C$ and $\delta^{15}N$ values and Hg concentration

Positive correlations ($p < 0.05$) were found between the $\delta^{13}C$ and $\delta^{15}N$ values of the whale meat-eater and non-eater groups, and the slopes of regression lines were 0.4246 and 0.3626, respectively. A significant correlation was also reported between the $\delta^{13}C$ and $\delta^{15}N$ values in the fingernails of Inuit (the slope of the regression line was 1.71: Buchardt et al., 2007). Difference in the $\delta^{13}C$ and $\delta^{15}N$ values in diet (Table 3) may be a reason for the differences in slopes between the Japanese and Inuit.

A significant positive correlation was found between the $\delta^{15}N$ value and Hg concentration in the hair of whale meat-eaters, while there was no significant correlation in the hair of non-eaters. There are many reports of positive correlation between the $\delta^{15}N$ value and Hg concentration in fish and marine mammals (Atwell et al., 1998; Endo et al., 2009, 2010, 2012, 2013; Hisamichi et al., 2010, 2012). To our knowledge, however, the present report is the first to present a relationship between the $\delta^{15}N$ value and Hg concentration in scalp hair. The TMF of Hg calculated from the whale meat-eaters was 2.03, which is higher than that from the non-eaters (1.34). Further study is necessary to investigate whether the difference in TMF is due to whale consumption. Interestingly, the TMF of Hg calculated from the hair of whale meat-eaters was higher than that calculated from the muscle of European hake (1.675: Harmelin-Vivien et al., 2012) and small shark species (1.63–1.82: Endo et al., in press).

Acknowledgments

We deeply appreciate the assistances provided by Mr. Jun-ichiro Yamashita in obtaining hair samples from Taiji residents.

This work was supported by Grants-in-Aid from Japan Society for the Promotion of Science (C21590135) and International Fund for Animal Welfare (IFAW).

Determinations of $\delta^{13}C$ and $\delta^{15}N$ were conducted using the Joint Usage/Research Grant from the Center for Ecological Research, Kyoto University.

References

Atwell, L., Hobson, K.A., Welch, H.E., 1998. Biomagnification and bioaccumulation of mercury in an arctic marine food web: insights from stable nitrogen isotope analysis. Can. J. Fish. Aquat. Sci. 55, 1114–1121.

Bisi, T.L., Lepont, G., Azevedo, A.D.F., Dorneles, P.R., Flach, L., Das, K., Malm, O., Lailson-Brito, J.L., 2012. Trophic relationships and mercury biomagnification in Brazilian tropical coastal food webs. Ecol. Indic. 18, 291–302.

Buchardt, B., Bunch, V., Helin, P., 2007. Fingernails and diet: stable isotope signatures of a marine hunting community from modern Uummannaq, north Greenland. Chem. Geol. 244, 316–329.

Campbell, L.M., Norstrom, R.J., Hobson, K.A., Muir, D.C.G., Backus, S., Fisk, A.T., 2005. Mercury and other trace elements in a pelagic Arctic marine food web (Northwater Polynya, Baffin Bay). Sci. Total Environ. 351–352, 247–263.

DeNiro, M.J., Epstein, S., 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochim. Cosmochim. Acta 45, 341–351.

Endo, T., Haraguchi, K., Sakata, M., 2002. Mercury and selenium concentrations in the internal organs of toothed whales and dolphins marketed for human consumption in Japan. Sci. Total Environ. 300, 15–22.

Endo, T., Hotta, Y., Haraguchi, K., Sakata, M., 2003. Mercury contamination in the red meat of whales and dolphins marketed for human consumption in Japan. Environ. Sci. Technol. 37, 2681–2685.

Endo, T., Haraguchi, K., Cipriano, F., Simmonds, M.P., Hotta, Y., Sakata, M., 2004. Contamination by mercury and cadmium in the cetacean products from Japanese market. Chemosphere 54, 1653–1662.

Endo, T., Haraguchi, K., Hotta, Y., Hisamichi, Y., Lavery, S., Dalebout, M.L., Baker, C.S., 2005. Total mercury, methyl mercury, and selenium levels in the red meat of small cetaceans sold for human consumption in Japan. Environ. Sci. Technol. 39, 5703–5708.

Endo, T., Hisamichi, Y., Kimura, O., Kotaki, Y., Kato, Y., Ohta, C., Koga, N., Haraguchi, K., 2009. Contamination levels of mercury in the muscle of female and male of spiny dogfish (*Squalus acanthias*) caught off the coast of Japan. Chemosphere 77, 1333–1337.

Endo, T., Hisamichi, Y., Kimura, O., Haraguchi, K., Lavery, S., Dalebout, M.L., Funahashi, N., Baker, C.S., 2010. Stable isotope ratios of carbon and nitrogen and mercury concentration in 13 toothed whale species from the western Pacific Ocean off Japan. Environ. Sci. Technol. 44, 2675–2681.

Endo, T., Haraguchi, K., 2010. High mercury levels in hair samples from residents of Taiji, a Japanese whaling town. Mar. Pollut. Bull. 60, 743–747.

Endo, T., Hotta, Y., Hisamichi, Y., Kimura, O., Sato, R., Haraguchi, K., Funahasi, N., Baker, C.S., 2012. Stable isotope ratios and mercury levels in red meat products from six baleen whales sold in Japanese markets. Ecotoxicol. Environ. Saf. 79, 35–41.

Endo, T., Hisamichi, Y., Kimura, O., Ogasawara, H., Ohta, C., Koga, N., Kato, Y., Haraguchi, K., in press. Levels of mercury in muscle and liver of Star-spotted dogfish (*Mustelus manazo*) from the northern region of Japan: a comparison with Spiny Dogfish (*Squalus acanthias*). Arch. Environ. Contam. Toxicol.

Harmelin-Vivien, M., Bodiguel, X., Charmasson, S., Loizeau, V., Mellon-Ducal, C., Tronczynski, J., Cossam, D., 2012. Differential biomagnification of PCB, PBDE, Hg and radiocesium in the food web of the European hake from the NW Mediterranean. Mar. Pollut. Bull. 64, 974–983.

Hisamichi, Y., Haraguchi, K., Endo, T., 2010. Contamination levels of mercury and organochlorine compounds, and stable isotope ratios in three tuna species taken from different regions of Japan. Environ. Sci. Technol. 44, 5971–5978.

Hisamichi, Y., Haraguchi, K., Endo, T., 2012. Levels of mercury and organohalogen compounds in Pacific bluefin tuna (*Thunnus orientallis*) cultured at different regions of Japan. Arch. Environ. Contam. Toxicol. 62, 296–305.

Hobson, K.A., Welch, H.E., 1992. Determination of trophic relationships within a high Arctic marine food web using $\delta^{13}C$ and $\delta^{15}N$ analysis. Mar. Ecol. Prog. Ser. 84, 9–18.

JECFA, 2003. Summary and Conclusions of the 61st Meeting of the Joint FAO/WHO Expert Committee of Food Additives (JECFA). JECFA/61/SC. Rome, Italy.

Johnsson, C., Sällsten, G., Schütz, A., Sjörs, A., Barregärd, L., 2004. Hair mercury levels versus freshwater fish consumption in household members of Swedish angling societies. Environ. Res. 96, 257–263.

Kelly, J.F., 2000. Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. Can. J. Zool. 78, 1–27.

Minagawa, M., 1992. Reconstruction of human diet from $\delta^{13}C$ and $\delta^{15}N$ in contemporary Japanese hair: s stochastic method for estimating multi-source contribution by double isotopic tracers. App. Geochem. 7, 145–158.

Minagawa, M., Wada, E., 1984. Stepwise enrichment of ^{15}N along food chains further evidence and relation between ^{15}N and animal age. Geochim. Cosmochim. Acta 48, 1135–1140.

O'Connell, T.C., Hedges, R.E.M., Healey, M.A., Simpson, A.H.R.W., 2001. Isotopic comparison of hair, nail and bone: modern analyses. J. Archaeol. Sci. 28, 1247–1255.

Petzke, K.J., Fuller, B.T., Metges, C.C., 2010. Advance in natural stable isotope ratio analysis of human hair to determine nutritional and metabolic status. Curr. Opin. Cli. Metab. Care 13, 532–540.

Tian, W., Egeland, G.M., Sobol, I., Chan, H.M., 2011. Mercury hair concentrations and dietary exposure among Inuit preschool children in Nunavut, Canada.. Environ. Int. 37, 42–48.

Wagemann, R., Trebacz, E., Boila, G., Lockhart, W.L., 1998. Methylmercury and total mercury in tissues of arctic marine mammals. Sci. Total Environ. 218, 19–31.

Weihe, P., Hansen, J.C., Murata, K., Debes, F., Jrgensen, P.J., Steuerwald, U., White, R.F., Grandjean, P., 2002. Neurobehavioral performance of Inuit children with increased prenatal exposure to methylmercury. Int. J. Circumpolar Health 61, 41–49.

Weihe, P., Joensen, H.D., 2008. Recommendations to the Government of the Faroe Islands concerning the pilot whale, English, translation. <www.chef-project.dk/PDF/Medical_Recom_Whale.pdf>.

Yasuda, H., Yoneshiro, T., Yoshida, K., Shibazaki, T., Ishii, T., Tsutsui, T., 2005. High toxic metal levels in scalp hair of infants and children. Biomed. Res. Trace Ele. 16, 39–45.

Yasutake, A., Matsumoto, M., Yamaguchi, M., Hachiya, N., 2004. Current hair mercury levels in Japanese for estimation of methylmercury exposure. J. Health Sci. 50, 120–125.

Yoshinaga, J., Minagawa, M., Suzuki, T., Ohtsuka, R., Kawabe, T., Inaoka, T., Akimichi, T., 1996. Stable carbon and nitrogen isotopic composition of diet and hair of Gidra-speaking Papuans. Am. J. Phys. Anthropol. 100, 23–34.

Arch Environ Contam Toxicol (2013) 64:467–474
DOI 10.1007/s00244-012-9858-0

Levels of Mercury in Muscle and Liver of Star-Spotted Dogfish (*Mustelus manazo*) from the Northern Region of Japan: A Comparison with Spiny Dogfish (*Squalus acanthias*)

Tetsuya Endo · Yohsuke Hisamichi · Osamu Kimura · Hideki Ogasawara · Chiho Ohta · Nobuyuki Koga · Yoshihisa Kato · Koichi Haraguchi

Received: 20 September 2012 / Accepted: 3 December 2012 / Published online: 28 December 2012

Abstract We analyzed mercury (Hg) concentrations in muscle and liver samples of star-spotted dogfish (*Mustelus manazo*) caught off the northern region of Japan and compared them with those of spiny dogfish (*Squalus acanthias*) caught in the same region. The average body length of male star-spotted dogfish specimens was significantly smaller than that of female specimens, reflecting the slower growth rate of male fish. Hg concentrations in liver and muscle increased with increases in body length and estimated age of both male and female star-spotted dogfish specimens. However, the relationships between Hg concentration in liver or muscle and body length or estimated age of male specimens differed markedly from those of female specimens, reflecting differences in growth rate and cessation of growth on reaching maturity. Marked increases in Hg concentration in liver of male and female star-spotted dogfish specimens were observed slightly later than increases in Hg concentration in muscle of those specimens due to growth cessation. These marked increases in Hg in liver may reflect increases in Hg due to the formation of mercury selenide. Similar results were previously reported in spiny dogfish specimens, except spiny dogfish showed only trace levels of Hg in liver (Endo et al., Chemosphere 77:1333–1337, 2009). The greater lipid content in liver and the larger liver size in spiny dogfish may explain the much lower levels of Hg observed in liver of spiny dogfish compared with those in the star-spotted dogfish.

T. Endo (✉) · Y. Hisamichi · O. Kimura
Faculty of Pharmaceutical Sciences, School of Pharmaceutical Sciences, Health Sciences University of Hokkaido, 1757, Ishikari-Tobetsu, Hokkaido 061-0293, Japan
e-mail: endotty@hoku-iryo-u.ac.jp

H. Ogasawara
Sapporo Higashi-Tokushukai Hospital, Sapporo, Hokkaido 065-0033, Japan

C. Ohta · N. Koga
Faculty of Nutritional Sciences, Nakamura Gakuen University, Fukuoka 814-0198, Japan

Y. Kato
Kagawa School of Pharmaceutical Sciences, Tokushima Bunri University, Sanuki, Kagawa 769-2193, Japan

K. Haraguchi
Daiichi College of Pharmaceutical Sciences, Minami-ku, Fukuoka 815-8511, Japan

The star-spotted dogfish (*Mustelus manazo*), also commonly known as the star-spotted smooth-hound, is a small shark that feeds on crustacea, crabs, and small fish (Taniuchi et al. 1983). This shark is fished in all regions of Japan, the East China Sea, and around Taiwan. Although the growth rate of this shark varies with habitat, the body length and life span of female fish are greater than those of male fish (Tanaka and Mizue 1979; Taniuchi et al. 1983). Star-spotted dogfish inhabiting waters off the central region of Japan reach maturity at 64-cm body length/4 years of age for male fish and 70-cm body length/4 or 5 years of age for female fish, and maximal body lengths of male and female dogfish ($n = 435$) are 100 and 124 cm, respectively (Taniuchi et al. 1983). In contrast, maximal body lengths of male and female dogfish collected from the southern region of Japan (East China Sea) ($n = 400$) were 78 and 87 cm, respectively (Tanaka and Mizue 1979). No dogfish older than 10 years were reported among samples collected from either the central (Taniuchi et al. 1983) or southern regions of Japan (Tanaka and Mizue 1979). No information on age-dependent body length or age of maturation of dogfish inhabiting the northern region of Japan is currently available.

The spiny dogfish (*Squalus acanthias*), another common shark species, is fished for food all over the world. This dogfish is also used as a source of liver oil because it has a large liver containing high-quality oil. The spiny dogfish is well known among shark species for its slow growth rate and long life span (Ketchen 1975). Like the star-spotted dogfish, greater body size and faster growth rate have been reported in female compared with male spiny dogfish. According to Ketchen (1975), male spiny dogfish are regarded as having reached maturity at 70-cm body length/14 years of age, whereas female fish reach maturity at 90-cm body length/23 years of age; the life span of female fish is older than 60 years. A greater body length has also been reported in female shortspine dogfish (*Squalus mitsukurii*) caught in the central region of Japan (Taguchi et al. 1979).

Mercury is typically accumulated in predators by way of the marine food web in an age-dependent manner (Ketchen 1975; Taguchi et al. 1979; Honda et al. 1983). The level of Hg contamination in spiny dogfish has been investigated in detail in Canada (Forrester et al. 1972; Ketchen 1975), the United States (Greig et al. 1977), and Japan (Endo et al. 2009), and that in shortspine dogfish has been investigated in Japan (Taguchi et al. 1979). As in other marine predators, Hg concentrations in muscle of spiny and shortspine dogfish increase with body length (age), but the relationship between Hg concentration in muscle and body length differs markedly between male and female fish of both species, reflecting the slower growth rate of male fish: The Hg concentration in male fish of both species is greater than that in female fish of similar body length (Forrester et al. 1972; Ketchen 1975; Taguchi et al. 1979; Endo et al. 2009). Although the commercial fishing of star-spotted and spiny dogfish is undertaken in Aomori and Hokkaido Prefectures in the northern region of Japan, no information on Hg contamination in the star-spotted dogfish in this or in other regions of Japan is currently available.

Stable isotope analysis has been used as a tool to obtain information on the feeding ecology of marine biota. The $\delta^{15}N$ value shows a stepwise increase in the trophic level of a food chain (Kelly 2000), and a positive correlation between the $\delta^{15}N$ value and Hg concentration has been reported (Campbell et al. 2005; Endo et al. 2009; Bisi et al. 2012; Harmelin-Vivien et al. 2012). However, $\delta^{15}N$ values in a given species are dependent not only on the trophic position but also the $\delta^{15}N$ values at the base of the food web, which vary widely with habitat. The trophic magnification factor (TMF) of Hg, which is calculated from the slope of the regression line generated from the $\delta^{15}N$ value or trophic level and the logarithmic concentration of Hg, is used to compare the magnification of Hg in animals inhabiting different areas (Campbell et al. 2005; Bisi et al. 2012; Harmelin-Vivien et al. 2012). In contrast, the $\delta^{13}C$ value is used to indicate the relative contribution to the diet of potential primary sources, slightly enriched by increases in trophic level, and can demonstrate differences between species consuming coastal versus offshore prey or between those consuming pelagic versus benthic prey (Kelly 2000). We previously compared $\delta^{13}C$ or $\delta^{15}N$ values and Hg concentration in muscle samples from male and female spiny dogfish of different body lengths: $\delta^{13}C$ and $\delta^{15}N$ values, as well as Hg concentration, in muscle increased with increased male and female fish body length, but no particular differences in the relationship between $\delta^{13}C$ and $\delta^{15}N$ values were observed between samples from male and female dogfish, despite the greater body length and age of female fish (Endo et al. 2009).Further study of $\delta^{13}C$ and $\delta^{15}N$ values, as well as Hg concentration, regarding sex differences in body length and life span are necessary to confirm the previous results.

We analyzed Hg concentrations in muscle and liver, and $\delta^{15}N$ and $\delta^{13}C$ values in muscle, from male and female star-spotted dogfish of different body lengths. Growth-related changes in Hg concentration, $\delta^{15}N$ and $\delta^{13}C$ values, and TMFs of Hg from male star-spotted dogfish were compared with those from female fish, respectively. Furthermore, we measured liver weight and liver oil content of star-spotted dogfish and spiny dogfish and compared present data from star-spotted dogfish with present and previous data obtained from spiny dogfish caught off the same region of Japan (Endo et al. 2009).

Materials and Methods

Sampling of Star-Spotted and Spiny Dogfish

Forty-seven star-spotted dogfish caught in the coastal waters off Aomori and Hokkaido Prefectures (northern region of Japan) were purchased from a shark wholesale specialty store in Aomori Prefecture, Japan, during August and September 2008. Total body length and total weight of the dogfish were measured, and muscle and liver samples were extracted and stored at −20 °C until analysis of total mercury (T-Hg). An additional 14 star-spotted dogfish and 14 spiny dogfish were purchased in December 2009, and total body length and total weight as well as liver weight were measured. The hexane-extractable lipid (HEL) content in extracted liver samples from both dogfish species was determined before freezing.

The ages of the star-spotted and spiny dogfish were estimated from the growth curves of male and female star-spotted dogfish collected from the central region of Japan (Taniuchi et al. 1983) and from the growth curves of male and female spiny dogfish caught off the Washington coast (Ketchen 1975), respectively.