endosulfan are summarized in Table 2. During the two decades from 1989 to 2010, the mean serum concentrations were significantly decreased from 5620 to 1620 pg/g ww for 4,4′-DDE, from 1070 to 680 pg/g ww for PCB-153, and from 180 to 86 pg/g ww for *trans*-nonachlor. These contaminants were significantly higher in the >50 years of age group than in the 20 years of age group ($p < 0.01$) in each sampling year. For legacy POPs, older people in 2010 has been exposed to these contaminants for more decades, and when the contamination was decreasing, age-difference may influence the trend. Such age-dependency in the concentrations of legacy POPs has also been observed in blood samples in 1999 ($n = 151$) where the concentrations of pesticides were positively correlated with age (20s–50s) (Masuda et al., 2005).

α-Endosulfan was detected in all serum samples (mean, 160 pg/g ww in 1989) at comparable concentrations to *trans*-nonachlor, and then were gradually reduced to 85 pg/g ww in 2011. The concentrations of α-endosulfan were positively correlated to those of PCBs, *trans*-nonachlor and HCB ($p < 0.01$) but not to those of 4,4′-DDE (Table 3). Unlike 4,4′-DDE, no decreasing trend was observed for α-endosulfan between 1999 and 2010. This trend is most likely due to continuous local usage of endosulfans until its agricultural registration had expired in 2010. A recent dietary survey has reported an exponential increase in α- and β-endosulfans in diets from China and Korea (Desalegn et al., 2011). However, no such increasing trend appeared in foods from Japan in that study. The present concentrations of endosulfans and residue profiles of pesticides in serum of Japanese men may be correlated to those in Japanese breast milk which has been contaminated with endosulfans at similar concentrations to other pesticides (Fujii et al., 2012b).

3.2. PenCP and OH-PCBs

Serum PenCP concentrations ranged from 89 to 1670 pg/g ww with a mean of 650 pg/g ww in 1989 and then decreased to 140 pg/g ww in 2010 (Table 2). No age-dependency in these concentrations was observed. The mean serum PenCP concentrations were at comparable concentrations to *trans*-nonachlor but not significantly correlated to each other (Table 3). Based on previous studies, the current concentrations are lower than those of European women (Glynn et al., 2011; Meijer et al., 2008; Rylander et al., 2012).

Unlike other pesticides, such as 4,4′-DDE and *trans*-nonachlor, PenCP concentrations in the 20 years of age group in 1989 ranged from 37 to 1670 pg/g ww, which were an order of magnitude greater than those of the >50 years of age group (Fig. 1). Such large variation of PenCP in young ages has also been observed in Norwegian children (Thomsen et al., 2002). This may be attributed to the combined exposure routes, dietary ingestion of contaminated seafood (Ge et al., 2007; Guvenius et al., 2003; Sjödin et al., 2001), and dust ingestion via inhalation (Inoue et al., 2006; Suzuki et al., 2008). It should not be excluded that PenCP may exist as a demethylated metabolite of pentachloroanisole (Ikeda and Sapienza, 1995) or as a hydroxylated metabolite of hexachlorobenzene in serum (To-Figueras et al., 1997).

For the monitoring of hydroxylated PCBs, we measured 4-OH-PCB187 that has been detected as one of major components in human blood (Rylander et al., 2012; Sandau et al., 2002). Serum concentrations of 4-OH-PCB187 in both age groups ranged from <LOQ to 800 pg/g ww (mean 120 pg/g ww), which are comparable to previous results from Japanese women (15–43 pg/g ww, Kawashiro et al., 2008; 12–370 pg/g ww, Nomiyama et al., 2010), and those from eastern Slovakia (273 pg/g ww, Park et al., 2009), Latvia (50 pg/g ww), and Sweden (120 pg/g ww, Sjödin et al., 2001).

The concentration of 4-OH-PCB187 was positively correlated to that of the parent compound PCB-187 ($r = 0.747$, $p < 0.01$, Fig. 2) but not significantly to that of PenCP ($r = 0.178$, $p > 0.05$, Table 3). This finding was also observed in other studies (Rylander et al., 2012). No significant differences in the ratio were observed between the 20 and >50 years of age groups. The mean concentration ratio of OH-PCB187 to PCB-187 was 1.4 in 1989, but it declined to 0.6 in 1999 and 2010. These findings suggest that OH-PCBs may be eliminated faster than the parent PCBs in individuals with lower concentrations of PCBs. This association indicates that the variation of 4-OH-PCB187 would depend on the extent of dietary exposure to the parent PCBs. This same phenomenon has been observed by Dirtu et al. (2009).

3.3. TriBP and TBBPA

Serum TriBP concentrations ranged from 46 to 960 pg/g ww (mean 248 pg/g ww) and showed slightly increasing trends in both age groups during the two decades (Fig. 1). Unlike the chlorinated

Table 2
Serum concentrations in Japanese men (20 and>50 age groups) from Kyoto during 1989–2010.

Contaminants	LOQ(pg/g ww)	Freq (%)	AM concentration (pg/g wet weight)						
			Sampling year			p value	Age		p Value
			1989 ($n = 20$)	1999 ($n = 20$)	2010 ($n = 20$)		20s ($n = 30$)	>50s ($n = 30$)	
Neutral OCs									
4,4′-DDE	50	100	5620[a] (330–12000)	3260 (390–8890)	1620 (470–6000)	<0.001	1970 (330–10900)	5030[a] (620–13000)	<0.001
HCB	2.0	100	490[a] (130–1230)	230 (120–450)	260 (43–660)	<0.001	250 (43–710)	400[a] (130–1230)	0.009
PCB-153	10	100	1070 (100–2830)	640 (200–1390)	680 (100–2140)	0.051	390 (100–1050)	1200[a] (330–2830)	<0.001
PCB-187	10	100	150 (13–560)	104 (21–240)	135 (20–480)	0.383	60 (13–200)	200[a] (49–560)	<0.001
trans-nonachlor	10	100	180 (10–714)	90 (12–190)	86 (4.1–530)	0.051	41 (4.1–190)	200[a] (42–710)	<0.001
α-endosulfan	10	100	160 (28–600)	59 (14–110)	85 (39–150)	0.004	61 (42–710)	140[a] (14–600)	0.002
Phenolic OCs									
PenCP	1.0	100	650[a] (89–1670)	300 (150–1060)	140 (37–850)	<0.001	430 (37–1670)	290 (50–1060))	0.917
4-OH-PCB187	20	92	220 (23–800)	67 (<LOQ -300)	76 (<LOQ -340)	0.792	42 (<LOQ -140)	200 (6.6–800)	0.241
TriBP	2.0	100	150 (46–370)	240 (110–380)	350[a] (62–960)	<0.001	250 (46–960)	250 (67–500)	0.149
TBBPA	50	28	NR (5%) (<LOQ-940)	NR (45%) (<LOQ-950)	NR (35%) (<LOQ-420)	NR	NR (33%) (<LOQ-950)	NR (23%) (<LOQ-420)	NR
6-OH-BDE47	20	100	200 (52–680)	550 (25–1590)	610 (34–3075)	0.032	360 (25–1580)	550 (52–3080)	0.182
Ratio									
4-OH-CB187/ PCB-187			1.4	0.6	0.6		0.7	1.0	

AM, arithmetic mean; LOQ, limit of quantification; *n*, number of samples; Freq%, % proportion of numbers that were >LOQ.
Concentrations below LOQ were treated as 1/2 LOQ for arithmetic mean. NR: not reported due to low detection frequency (<50%).
[a] Significantly higher than the other group ($p < 0.05$, Steel–Dwass test and Mann–Whitney *U*-test). *p* values, Spearman's rank correlation.

Table 3
Spearman's rank correlation coefficients between concentrations of analytes in human serum (n = 60).

	HCB	PCB-153	PCB-187	*trans*-nonachlor	α-Endosulfan	TriBP	PenCP	4-OH-PCB187	TBBPA	6-OH-BDE47
4,4′-DDE	0.519*	0.778**	0.651**	0.683**	0.329	−0.241	0.388	0.646*	−0.260	−0.204
HCB		0.588*	0.516*	0.585*	0.367	−0.089	0.226	0.518*	0.069	−0.186
PCB-153			0.951**	0.770**	0.585*	0.053	0.091	0.793**	−0.115	−0.069
PCB-187				0.736**	0.538*	0.191	−0.067	0.747**	−0.020	−0.026
trans-nonachlor					0.455*	0.132	0.160	0.629**	0.011	0.014
α-endosulfan						0.162	−0.007	0.474*	−0.140	−0.144
TriBP							−0.411	−0.105	0.412	0.340
PenCP								0.178	−0.159	−0.245
4-OH-PCB187									−0.258	−0.082
TBBPA										0.131

*$p < 0.05$, **$p < 0.01$.

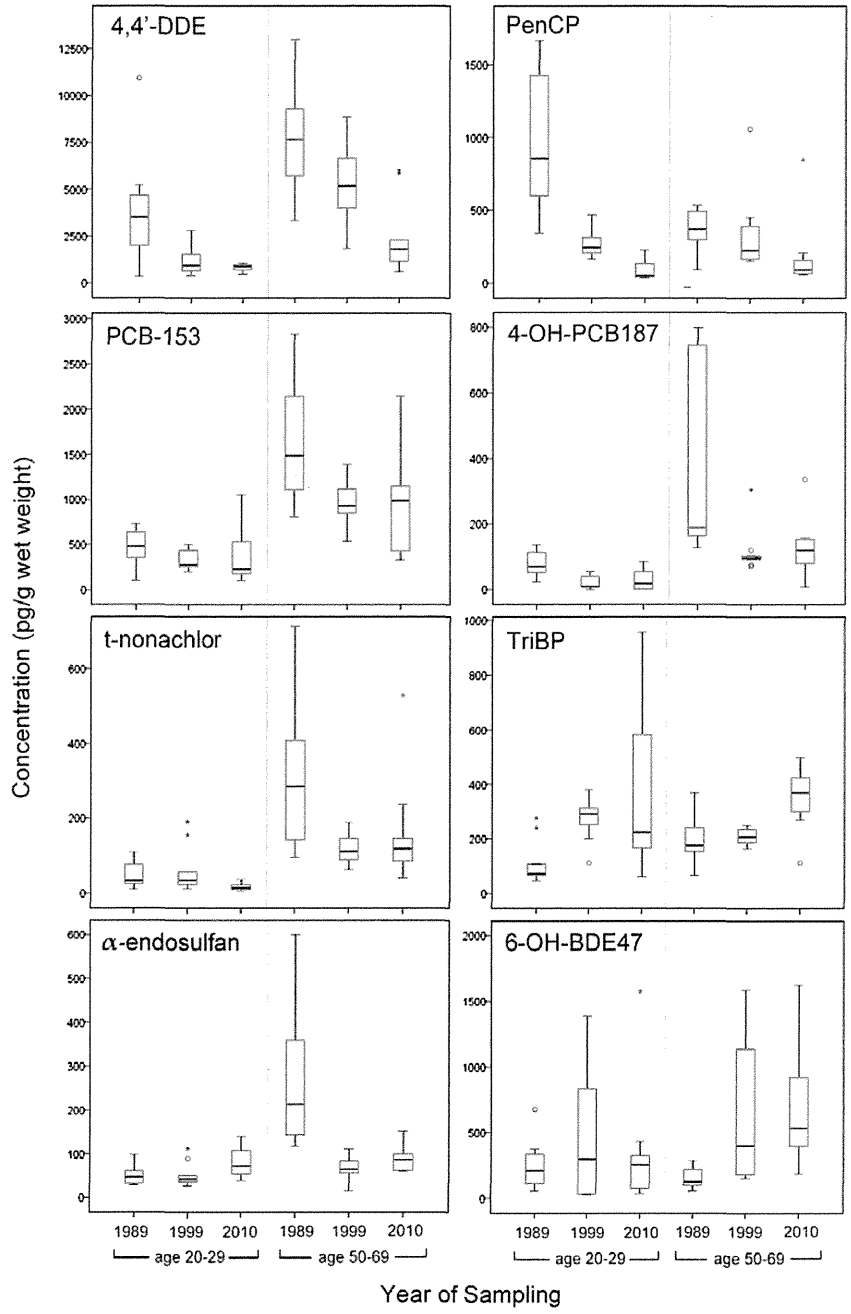

Fig. 1. Temporal trend in serum concentration (pg/g wet weight) of organohalogens in two age groups during 1989–2010 from Kyoto. Box plot: box, 25th–75th percentiles; center line, median value; whiskers, min–max values; dots, outliers; circle, extremes.

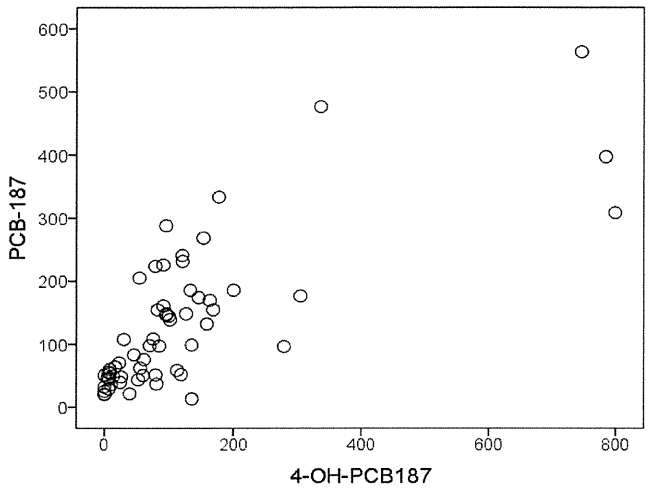

Fig. 2. Correlation between PCB-187 and 4-OH-PCB187 in serum concentration (pg/g wet weight) from Kyoto. Spearman's rank correlation coefficient, $r = 0.747$, $p < 0.01$.

pesticide, no age-dependency was observed for TriBP. Compared with other studies, the current TriBP concentrations were one order of magnitude greater than those of pregnant women (mean 22 pg/g ww; Kawashiro et al., 2008) or those of Norwegians during 1977–1999 (0.077–26 pg/g ww; Thomsen et al., 2002), whereas concentrations were comparable to those of Indians (72–1200 pg/g ww, mean 360 pg/g ww, Eguchi et al., 2012).

Serum TriBP concentrations were not correlated to those of the other POCs (Table 3). This may be explained by the combined exposure to TriBP from different sources. One possible exposure source would be via dietary seafood because TriBP is a naturally produced flavor in marine algae and fish (Haraguchi et al., 2010; Whitfield et al., 1999), which can be bioconcentrated in higher trophic organisms (Whitfield et al., 1999). Another possible source may be house dust contaminated with TriBP, which was used as a BFR intermediate and released into the air by leakage (Watanabe and Sakai, 2003) and indoor dust (Suzuki et al., 2008). Because of the lack of data on other TriBP exposure routes, we have no conclusion regarding the relative importance of diet or house dust.

TBBPA was detected in 28% of the studied samples (17 out of 60) with concentrations ranging from <LOQ (50 pg/g ww) to 950 pg/g ww (Table 2). The highest concentration was observed in the 20 years of age group in 1999. None of volunteers were occupationally exposed to TBBPA. The mean concentration of TBBPA in the detected samples ($n = 17$) was 98 pg/g ww, which is higher than that of the Fukuoka residents investigated in 2000 (7 pg/g ww, Nagayama et al., 2000) and that of pregnant women from Japan (26 pg/g ww, Kawashiro et al., 2008). As the present mean values of TBBPA were based on only 17 samples (28% detection frequency), the present TBBPA concentrations may be overestimated. However, our results in 1999 and 2010 are comparable to the serum concentrations from Belgium (80 pg/g ww; Dirtu et al., 2008) or France (154 pg/g ww; Cariou et al., 2008). This is the first study to report the temporal trend of TBBPA using human blood samples in Japan. This study could not clarify the relationship between concentrations of TriBP and TBBPA because of the small sample size utilized in this study (Table 3).

The exposure routes of TBBPA are possibly via diet and house dust, which is similar to that of TriBP. The Norwegian population was exposed to TBBPA via diet as a major source (Thomsen et al., 2001). However, plasma concentrations of TBBPA were higher in the workers at the dismantling plant (Thomsen et al., 2002) than that of the non-occupationally-exposed workers. Consistent with its phenolic structure, TBBPA can be rapidly conjugated in human liver and subsequently excreted in bile (Schauer et al., 2006). No age-dependency in serum concentrations of TBBPA may be because of its short half-life (two days) in human plasma (Hagmar et al., 2000). Therefore, the occurrence of TBBPA in human serum is likely to reflect recent exposure rather than past exposure (Covaci et al., 2009).

3.4. OH-PBDEs

Of phenolic PBDE congeners, 6-OH-BDE47 was predominantly detected in all serum samples investigated. The concentrations ranged from 25 to 3075 pg/g ww (mean 451 pg/g ww), which was 20-fold greater than those of BDE-47 (Table 2). The 6-OH-BDE47 concentrations were comparable to those of PenCP or 4-OH-PCB187. It was not significantly correlated to those of BDE-47 or to the other chlorinated OCs (data not shown). No age- or time-related differences were observed from 1988 to 2010 (Fig. 1). Other isomeric hydroxy- and methoxy-PBDEs have been found in human blood from India and Nicaragua (Athanasiadou et al., 2008; Eguchi et al., 2012), but we could not detect any metabolites in our samples. The *ortho*-substituted 6-OH-BDE47 detected in this study is considered to be of natural origin, although it can be also formed by human P460 *in vitro* (Erratico et al., 2013). The *meta*- and *para*-substituted OH-tetraBDEs detected in the other studies are most likely metabolites of parent PBDEs (Athanasiadou et al., 2008). The occurrence of 6-OH-BDE47 in serum in this study may be a result of dietary exposure to 6-OH-BDE47 itself via seafood (Marsh et al., 2004; Wang et al., 2012) or demethylation of 6-MeO-BDE47 by microsomes of mammals (Wan et al., 2009). The toxicological implication of human exposure to OH-PBDEs remains unknown, but some studies have shown that 6-OH-BDE47 exhibits thyroid-disrupting effects and a higher affinity to human TTR than BDE-47 *in vitro* (Liu et al., 2011; Meerts et al., 2001).

4. Conclusions

Serum concentrations of legacy neutral contaminants in Japanese men showed a decreasing trend in two age groups from 1989 to 2010. Although the concentrations of PenCP and 4-OH-PCB187 showed a decreasing trend, TriBP and 6-OH-BDE47 appeared to be slightly increasing. TBBPA was detected in 28% of serum samples, whereas 6-OH-BDE47 was present at the highest concentrations of POCs investigated in all samples. No age-dependency was observed for all POCs tested. The concentrations of TriBP and 6-OH-BDE47 were not correlated with those of PenCP, OH-PCBs and chlorinated pesticides. These findings suggest that the kinetics on exposure routes and fate of the studied brominated POCs are different from chlorinated POCs and legacy POPs.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2013.11.002.

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Dietary exposure to phenolic and methoxylated organohalogen contaminants in relation to their concentrations in breast milk and serum in Japan

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ABSTRACT

This study investigated human exposure to neutral, phenolic, and methoxylated organohalogen contaminants (OHCs) in a duplicate diet study to evaluate their concentrations in breast milk and serum of Okinawan people from Japan during 2004–2009. Dietary intakes of phenolic OHCs were predominantly 2,4,6-tribromophenol (TriBP), followed by tetrabromobisphenol A (TBBPA), and 6-hydroxy-2,2′,4,4′-tetrabromodiphenyl ether (6-OH-BDE47). After exposure, TriBP and TBBPA were transferred to breast milk, whereas 6-OH-BDE47 was selectively retained in serum. Despite a lower dietary exposure to pentachlorophenol and 4-hydroxy-CB187, both were retained in serum. For the methoxylated OHCs, 2,4,6-tribromoanisole (TriBA) and 6-methoxy-BDE47 were the predominant dietary contaminants, of which TriBA was present in both breast milk and serum, whereas 6-methoxy-BDE47 was selectively transferred to breast milk. These findings suggest that dietary exposure to phenolic and methoxylated OHCs may result in differential partitioning between breast milk and serum with different pharmacokinetic or exposure routes.

1. Introduction

Organohalogen contaminants (OHCs) include a diverse group of phenolic chemicals, such as pentachlorophenol (PenCP), 2,4,6-tribromophenol (TriBP), tetrabromobisphenol A (TBBPA) and hydroxylated analogs of polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs). These compounds are persistent, and bioaccumulative, and have been distributed in wildlife and humans (Fujii et al., 2012; Marsh et al., 2004). Recent studies have shown that these phenolic OHCs can cause carcinogenic, thyrotoxic, estrogenic, and neurotoxic effects in experimental animals and humans (Meerts et al., 2001; Otake et al., 2007; Saegusa et al., 2009). Of the phenolic pesticides, PenCP is a ubiquitous thyroid-disrupting compound (Zheng et al., 2011) and contributes to the human transthyretin (TTR) binding potency of OHCs in indoor dust (Suzuki et al., 2008), but data regarding its body burden are limited (Hong et al., 2005). PCBs and PBDEs are metabolized to hydroxy-CBs and hydroxy-BDEs, respectively, by cytochrome P450 in mammalian liver (Erratico et al., 2013). Some of these metabolites are selectively retained in blood (Sandau et al., 2002), but there are no data regarding their accumulation in adipose tissues (Nomiyama et al., 2010). TriBP and TBBPA are phenolic brominated flame retardants (BFRs) and have been detected separately from PBDEs in humans (Thomsen et al., 2002). However, accumulation and exposure kinetics of these phenolic BFRs are not largely understood.

Phenolic OHCs may be in part methylated to their anisoles by marine bacteria or fungi in the marine food web (Allard et al., 1987; Whitfield et al., 1997). For example, TriBP and PenCP are known to be biotransformed to 2,4,6-tribromoanisole (TriBA) and pentachloroanisole (PenCA), respectively, both of which have been distributed in marine biota (Watanabe et al., 1983a). Microbial O-methylation may also be observed for TBBPA, which leads to its mono- or dimethylether derivatization in the marine environment (George and Häggblom, 2008; Watanabe et al., 1983b). Furthermore, naturally occurring hydroxy-BDEs have been produced in marine algae or sponges, together with their methoxylated PBDEs (Haraguchi et al., 2011), suggesting that the possible microbial methylation of phenolic OHCs occurs in marine biota. Such methoxylated analogs may increase the probability of bioaccumulation in the food chain because of the addition of a hydrophobic methyl group (Glickman et al., 1977), whereas the phenolic OHCs have short half-lives owing to their rapid elimination (Covaci et al., 2009; Hagmar et al., 2000).

Although the relative importance of the various potential routes of exposure to phenolic and methoxylated OHCs remains unknown, it has been suggested that food, water, house dust, and airborne sources may all be significant (Sjödin et al., 2001). Chronic human exposure to phenolic OHCs is most likely the result of the long-term intake of contaminated foods, including drinking water (Shi et al., 2009).

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Therefore, it is important to survey the dietary intake of phenolic and methoxylated OHCs as it relates to their contamination status in breast milk or blood, and to investigate the dietary health risk for the general population and infants.

The aim of the present study was to investigate the association between the levels of phenolic and methoxylated OHCs in diet, breast milk, and serum. We selected five representative phenolic OHCs and their methoxylated analogs for comparison, together with legacy persistent organohalogen pollutants, such as PCBs, PBDEs or chlorinated pesticides, which were investigated by duplicate diet sampling during 2004 and 2009 in Okinawa, Japan.

2. Materials and methods

2.1. Sample collection

Diet samples from the Kyoto University Human Specimen Bank (Koizumi et al., 2005) were used for the chemical analysis. At the time of collection, participants were requested to donate the same duplicate samples as all food and drink items that they consumed over a 24-h period. Ten duplicate 24-h diet samples were collected in Okinawa in 2004. An additional 10 duplicate 24-h diet samples (i.e., a typical day's worth of food and drink for consumers) were purchased from markets in Okinawa in 2009. This study provided 20 duplicate 24-h diet samples. All food and drink samples in each duplicate sample were combined, homogenized, and stored as a dietary homogenate.

Okinawan breast milk and serum samples were obtained from the Kyoto University Human Specimen Bank using a standardized protocol (Koizumi et al., 2005). Human breast milk samples (5–10 mL each, n = 9) were obtained from healthy women in Okinawa between 2005 and 2006 (average age 31 years old). Individual serum samples (1–2 mL each, n = 10) were collected from healthy volunteers in the Okinawa area in 2006 (average age 44 years old). The Ethics Committee of Kyoto University approved the protocol of the present study (E25), and appropriate written informed consent was obtained from all of the participants. Samples were stored in clean screw-capped plastic containers at −20 °C until the time of analysis. The study populations are provided in Table 1.

2.2. Chemical reagents

Four ^{13}C-labeled standards, PenCP [$^{13}C_6$], 4-hydroxy-2,2′,3,4′,5,5′,6-heptachlorobiphenyl (4-OH-CB187[$^{13}C_{12}$]), 2,2′,4,4′,5,5′-hexachlorobiphenyl (CB-153[$^{13}C_{12}$]) and α-endosulfan[$^{13}C_9$] were purchased from Wellington Laboratories Inc. (Guelph, Canada). Another internal standard, 4′-methoxy-2,3′,4,5′,6-pentabromodiphenyl ether (4′-MeO-BDE121) was kindly provided by Göran Marsh (Stockholm University, Sweden). Target standards were purchased from AccuStandard Inc. (New Haven, CT, USA) for calibration, recovery, and quantification (Supplementary Table S1). Silica-gel (Wako gel S-1), which was used for purification, was obtained from Wako Pure Industries (Osaka, Japan) and heated at 130 °C for 3 h prior to use. All solvents used were of pesticide-grade quality.

Table 1
Information on dietary homogenate, human breast milk and serum samples in Okinawa, Japan.

Sample	City	M/F	n	Sampling year	Mean age
Diet 2004 (duplicate diet study)	Okinawa	Male	4	2004	32.2 (20–50)
		Female	6		
Diet 2009 (duplicate diet study)	Okinawa	Male	10	2009	36
Breast milk	Okinawa	Female	9	2005–2006	31.4 (26–39)
Serum	Okinawa	Female	10	2006	43.7 (40–47)

2.3. Extraction procedure

The methodology used to analyze phenolic and methoxylated contaminants in samples was based on lipid extraction, gel permeation chromatography (GPC), fractionation, derivatization of phenolic compounds, silica-gel column cleanup, and gas chromatography/mass spectrometry with electron capture negative ionization (GC/MS/ECNI) as previously described (Fujii et al., 2012). Briefly, 10 g dietary homogenate, 10 mL breast milk and 1 mL serum sample were spiked with the four internal standards, PenCP[$^{13}C_6$] and 4-OH-CB187[$^{13}C_{12}$] for phenolic analytes (0.2 ng of each), and α-endosulfan[$^{13}C_9$] (2 ng) and 4′-MeO-BDE121 (0.5 ng) for neutral analytes. The sample was extracted with *n*-hexane, after adding formic acid (0.1% v/v), ethanol, and diethyl ether. A combined extract was dissolved in dichloromethane (DCM):*n*-hexane (1:1), and then subjected to GPC with a Bio-Beads S-X3 column (Bio-Rad Laboratories, Hercules, CA, USA). The gel material (35 g) was packed in a glass column (55 cm × 27 m i.d.) with DCM:*n*-hexane (1:1) as the eluting solvent at a flow rate of 4 mL min^{-1}. The first 96-mL fraction of the eluate contained lipids and was discarded. Subsequently, the next 68-mL fraction was collected. The eluate was concentrated and partitioned between 1 M KOH:ethanol (7:3) and *n*-hexane. After acidification, the phenolic contaminants in the KOH solution were back-extracted twice with 20% diethylether in *n*-hexane. The phenolic fraction was derivatized to O-methylated analogs by diazomethane in diethylether. The residues in both neutral and methylated phenolic fractions were purified with a silica-gel column (0.2 g Wako gel S-1) by elution with 15 mL of DCM:*n*-hexane (12:88, v/v). Each fraction was concentrated to 200 μL prior to GC/MS analysis.

2.4. Instruments and quantification

Twelve analytes were measured by GC/MS/ECNI using an Agilent GC/MSD 5973i (Agilent Technologies, Santa Clara, CA, USA) coupled to a 6890N gas chromatograph. The GC/MS conditions and target ions for determination of analytes are summarized in Supplementary Table S1. Quantification of the compounds was based on signals in the mass chromatograms and in comparison with CB-153[$^{13}C_{12}$], which was used as a syringe spike. The concentrations of chemicals are reported as picogram per gram (pg g^{-1} wet weight for serum, pg g^{-1} lipid weight for breast milk).

2.5. Quality control and quality assurance

The extraction, cleanup, and fractionation steps were evaluated by the measurement of the absolute recoveries of the compounds (^{13}C-labeled internal and native surrogate standards) that were spiked and passed through the entire analytical procedure. Procedural blanks were analyzed simultaneously with every batch of 10 samples to evaluate for interference or contamination from solvents and glassware. For recovery tests, two levels (2.0 and 10.0 ng g^{-1}) of the 11 analytes were spiked into cow milk and determined based on GC/MS-selected ion monitoring (GC/MS-SIM). The recoveries were between 87 and 99% with a relative standard deviation of <10% (n = 5). The limits of quantification (LOQ), defined as 10-fold that of the noise, ranged from 1 to 200 pg g^{-1} (Supplementary Table S1). When the levels of the target chemicals were less than their LOQs, we allocated half of the LOQ as the value for analysis. The calibration (0.1 to 5 ng mL^{-1} of each analyte) was linear and characterized by good correlation coefficients (>0.99) for all of the studied compounds. The quality of the method under validation was verified by Standard Reference Materials (non-fortified human serum, SRM1974, NIST) for PCBs and selected pesticides. Data from the current study were within 12% of the certified values of SRM1974.

2.6. Statistical analysis

The data were analyzed using SPSS version 18.0 for Windows 2007 (SPSS Inc., Chicago, IL, USA). Spearman's rank correlation coefficients were used to test the relationship among concentrations of analytes. Probability values of less than 0.05 were considered to indicate statistical significance.

3. Results

3.1. Duplicate diet study

Daily intakes (ng day^{-1}) of neutral, phenolic and methoxylated OHCs in the duplicate diet studies from Okinawa in 2004 and 2009 are shown in Table 2. For the analyzed neutral OHCs, 4,4′-DDE was the predominant contaminant in both sampling years, followed by α-endosulfan, and then hexachlorobenzene (HCB). Some of the dietary homogenates were contaminated with higher levels of BDE-99 than BDE-47 in 30% of samples. The maximum intake of BDE-99 was estimated to be 914 ng day^{-1} in the 2009 study, although the median intake of BDE-47 exceeded that of BDE-99 in both sampling years. The dietary exposure to BDE-99 in most cases could originate from commercial penta-BDE products that have been previously used as a BFR in Japan until 1999, whereas the unusual high levels of BDE-99 may be from another source, i.e. indoor environment.

For the phenolic OHCs, we detected PenCP and TriBP in all analyzed samples, whereas TBBPA, 4-OH-CB187 and 6-OH-BDE47 were detected at frequencies of 80, 20 and 35%, respectively, from the dietary samples. Of the methoxylated OHCs, PenCA and TriBA were found in all samples, whereas 6-MeO-BDE47 was present at a 50% frequency. Methoxy-CBs and dimethoxylated TBBPA were not detected in any of the dietary homogenates.

The mean dietary intake of phenolic OHCs in the 2004 diet study was higher in the order of TriBP > TBBPA > PenCP > 6-OH-BDE47 > 4-OH-CB187, whereas those in the 2009 was higher in the order of TriBP > 6-OH-BDE47 > TBBPA > PenCP >4-OH-CB187 (Table 2). For the methoxylated analogs, the dietary intake in the 2004 study was higher in the order of TriBA > 6-MeO-BDE47 >PenCA, whereas those in the 2009 was higher in the order of TriBA > PenCA > 6-MeO-BDE47. The ratios of methoxylated and phenolic OHCs were estimated to be 0.15 for TriBA/TriBP, 0.33 for PenCA/PenCP, and 0.69 for 6-MeO-BDE47/6-OH-BDE47 in the 2004 diet study (Table 2).

The estimated daily intakes (EDIs; ng kg body $weight^{-1}$ day^{-1}) of neutral and phenolic OHCs via diet for adults are shown in Supplementary Table S2. The average EDIs of organohalogens for adults were lower by at least a factor of 1000 than the intake guidelines established by World Health Organization (van Oostdam et al., 1999).

The correlations in concentrations among selected contaminants are shown in Supplementary Tables S3 and S4. Lipids (%) in the diet were independent of the contaminant levels. For phenolic OHCs, TriBP was positively associated with TBBPA ($r = 0.809$, $p < 0.01$) and α-endosulfan ($r = 0.619$, $p < 0.01$) but not with the other contaminants. TBBPA was associated with PenCA ($r = 0.531$, $p < 0.01$), but not PenCP ($p > 0.05$). 6-Meo-BDE47 was not associated with 6-OH-BDE47 and the other contaminants. For neutral OHCs, BDE-47 were significantly associated with BDE-99 ($r = 0.988$, $p < 0.01$) but not with the other neutral OHCs.

3.2. Breast milk

The concentrations of neutral, phenolic and methoxylated OHCs in breast milk from Okinawa in 2005 are shown in Table 3. The concentrations of neutral contaminants were in the order of 4,4′-DDE > HCB > α-endosulfan. The concentrations of phenolic OHCs were present in the order of TriBP (mean, 1167 pg g^{-1} lipid) > TBBPA (1035 pg g^{-1} lipid) > PenCP (577 pg g^{-1} lipid) > 4-OH-CB187 (29 pg g^{-1} lipid), whereas 6-OH-BDE47 was below the LOQ in all samples. The methoxylated analogs were present in the order of TriBA (445 pg g^{-1} lipid) > 6-MeO-BDE47 (249 pg g^{-1} lipid) > PenCA (37 pg g^{-1} lipid). The ratios of TriBA/TriBP and PenCA/PenCP in breast milk were 0.38, and 0.06, respectively (Table 3). The EDIs of the analytes via human milk for nursing infants

Table 2
Daily intake of contaminants (ng day^{-1}) in the Okinawa area of Japan.

	Freq	2004 (N = 10)			2009 (N = 10)		
	(%)	Median	Mean ± SD	Range	Median	Mean ± SD	Range
Age		32.0	32.2 + 8.7	20–50	36.0	36.0 ± 0.0	36.0–36.0
Total homogenate (g)		2340	2360 ± 230	2060–2850	1690	1700 ± 403	1075–2452
Fat (%)		2.6	3.0 ± 1.5	1.2–5.6	3.7	4.0 ± 2.4	0.7–9.3
Halogenated compounds							
4,4′-DDE	100	165	168 ± 69	48–303	82	132 ± 134	30–457
HCB	100	22.1	26.2 ± 10.1	18.0–44.7	19.4	27.9 ± 23.3	9.2–86.6
trans-Nonachlor	100	1.2	1.2 ± 0.6	0.3–2.4	1.6	2.7 ± 2.8	0.6–9.4
α-Endosulfan	100	36.3	45.0 ± 26.1	28.8–118	28.2	30.3 ± 12.5	14.6–53.5
CB-153	100	19.3	20.0 ± 9.6	7.1–32.9	12.8	27.4 ± 30.5	5.0–91.6
CB-187	100	2.0	2.6 ± 1.5	1.2–4.7	1.9	5.2 ± 7.1	0.2–21.1
BDE-47	85	7.47	7.51 ± 3.25	1.04–12.1	2.47	16.8 ± 40.3	<LOQ–131
BDE-99	30	0.14	5.9 ± 10.6	<LOQ–30.4	0.09	93.2 ± 288	<LOQ–914
BDE-153	10	0.12	0.71 ± 1.88	<LOQ–6.07	0.09	3.95 ± 12.2	<LOQ–38.7
Phenolic OHCs							
PenCP	100	5.2	7.0 ± 5.0	2.3–12.4	1.8	2.2 ± 1.8	0.2–5.6
4-OH-CB187	20	0.06	0.07 ± 0.03	<LOQ–0.14	0.05	0.40 ± 0.72	<LOQ–2.26
TriBP	100	165	181 ± 83	60–373	48	52 ± 26	13–110
TBBPA	80	10.6	15.6 ± 12.3	3.9–40.2	2.7	3.0 ± 2.8	<LOQ–7.5
6-OH-BDE47	35	0.12	0.12 ± 0.01	<LOQ–0.14	1.83	11.1 ± 22.5	<LOQ–72.6
Methoxylated OHCs							
PenCA	100	1.4	1.5 ± 0.88	0.45–3.7	0.77	1.1 ± 0.71	0.43–2.4
4-MeO-CB187	0	<LOQ	<LOQ		<LOQ	<LOQ	
TriBA	100	5.1	7.3 ± 6.8	1.8–22.8	20.3	21.5 ± 14.7	3.6–44.9
diMeO-TBBPA	0	<LOQ	<LOQ		<LOQ	<LOQ	
6-MeO-BDE47	50	0.12	1.0 ± 2.4	<LOQ–7.6	7.08	9.48 ± 11.1	<LOQ–35.1
Ratio							
PenCA/PenCP		0.27	0.27		0.43	0.50	
TriBA/TriBP		0.03	0.04		0.42	0.41	

Daily intake was calculated as total content (ng) in 24-h diet concentrations of analytes (ng g^{-1} wet) × total content of homogenate (g). Concentrations below the LOQ were treated as 1/2 LOQ for the mean and median values.

Table 3
Concentrations of organohalogen contaminants in human breast milk and serum in Okinawa.

Analytes	Breast milk, ng g^{-1} lipid (n = 9)				Serum, pg g^{-1} wet weight (n = 10)			
	n[a]	Median	Mean ± SD	Range	n[a]	Median	Mean ± SD	Range
Halogenated compounds								
4,4′-DDE	9	76.1	91.1 ± 51.1	30.9–177	10	1850	1950 ± 1490	250–5250
HCB	9	7.15	7.0 ± 3.2	2.77–12.6	10	134	136 ± 47	59–214
trans-Nonachlor	9	52.4	66.9 ± 35.9	29.3–144	10	83	110 ± 71	27–254
α-Endosulfan	9	1.03	1.11 ± 0.56	0.35–1.95	10	133	157 ± 83	96–373
CB-153	9	16.0	13.9 ± 6.35	4.19–20.8	10	235	215 ± 66	64–286
CB-180	9	9.53	7.78 ± 4.20	2.30–13.9	10	133	123 ± 45	23–180
CB-187	9	4.44	3.88 ± 1.96	1.02–6.29	10	38	35 ± 14	9–56
CB-183	9	1.00	0.83 ± 0.35	0.23–1.20	10	9	10 ± 6.9	4–27
BDE-47	9	0.57	0.87 ± 0.74	0.093–1.99	5	1	1.5 ± 0.4	<LOQ–3
BDE-99	8	0.33	0.54 ± 0.51	<LOQ–1.13	0	<LOQ	<LOQ	
BDE-153	9	0.40	0.38 ± 0.18	0.13–0.65	0	<LOQ	<LOQ	
Phenolic OHCs								
PenCP	9	0.63	0.58 ± 0.37	0.087–1.09	10	143	216 ± 166	87–633
4-OH-CB187	2	<LOQ	0.029 ± 0.59	<LOQ–0.16	10	42	45.3 ± 21.3	21–79
TriBP	9	1.06	1.17 ± 0.75	0.13–2.73	10	26	40.2 ± 30.1	18–100
TBBPA	9	0.72	1.04 ± 0.65	0.39–2.22	3	1.0	40.5 ± 78.0	<LOQ–238
6-OH-BDE47	0	<LOQ	<LOQ		9	90	172 ± 184	<LOQ–542
Methoxylated OHCs								
PenCA	9	0.033	0.037 ± 0.018	0.007–0.068	10	2.5	5.4 ± 8.6	1–29
4-MeO-CB187	0	<LOQ	<LOQ		0	<LOQ	<LOQ	
TriBA	9	0.39	0.45 ± 0.25	0.028–0.85	10	10.5	22.1 ± 28.1	4–87
diMeO-TBBPA	0	<LOQ	<LOQ		0	<LOQ	<LOQ	
6-MeO-BDE47	8	0.19	0.25 ± 0.19	<LOQ–0.53	0	<LOQ	<LOQ	
Ratios								
PenCA/PenCP		0.05	0.06			0.02	0.02	
TriBA/TriBP		0.37	0.38			0.40	0.55	

Concentrations below the LOQ were treated as 1/2 LOQ for the mean and median values.
[a] Number detected.

were calculated, based on 5-kg infants and 750 mL of daily dietary milk (Supplementary Table S2). The average EDIs via milk of all analytes were less than one-tenth of the acceptable daily intake (ADI) and provisional tolerable daily intake (PTDI) established by WHO (van Oostdam et al., 1999), except for *trans*-nonachlor, which was a relatively high concentration (mean 52% of ADI).

3.3. Serum

Serum concentrations of neutral, phenolic and methoxylated OHCs in Okinawan females are shown in Table 3. The neutral contaminants were present in the order of 4,4′-DDE > CB-153 > HCB > α-endosulfan. The phenolic OHCs in serum were quantified in the order of PenCP (216 pg g^{-1} wet weight) > 6-OH-BDE47 (152 pg g^{-1} wet weight) > 4-OH-CB187 (45 pg g^{-1} wet weight) > TriBP (41 pg g^{-1} wet weight) > TBBPA (40 pg g^{-1} wet weight). The methoxylated analogs were detected at the levels of 22 pg g^{-1} wet weight for TriBA and of 5.4 pg g^{-1} wet weight for PenCA, whereas the levels of 6-MeO-BDE47 were below the LOQ in all samples. 4-MeO-CB187 and TBBPA dimethyl ether were not detected in any of the samples. The ratios of TriBA/TriBP and PenCA/PenCP in serum were 0.55 and 0.02, respectively (Table 3).

3.4. Association between diet and serum/milk

Dietary intake of phenolic and methoxylated OHCs are illustrated in Fig. 1. Their profiles and concentrations in serum and breast milk are collectively illustrated in Fig. 2. Dietary PenCP and PenCA were present at a ratio of 0.03 and 0.27, respectively. The concentration ratios of TriBP and TBBPA between breast milk and serum were higher than those of the other phenolic OHCs. 6-OH- and 6-MeO-BDE47 were at similar levels (1:1 and 1:4 ratios in the dietary homogenates), respectively, whereas 6-MeO- and 6-OH-BDE47 were selectively partitioned between breast milk and serum, respectively (Table 2, Fig. 2).

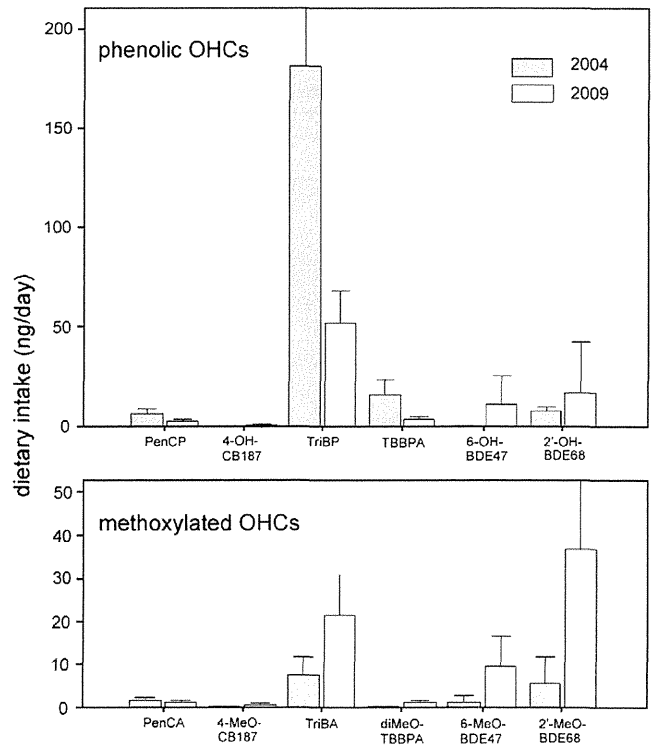

Fig. 1. Daily intake of phenolic and methoxylated organohalogens investigated in a duplicate diet study. PenCP = pentachlorophenol; PenCA = pentachloroanisole; TriBP = 2,4,6-tribromophenol; TriBA = 2,4,6-tribromoanisole; TBBPA = tetrabromobisphenol A; diMeO-TBBPA = TBBPA-dimethylether.

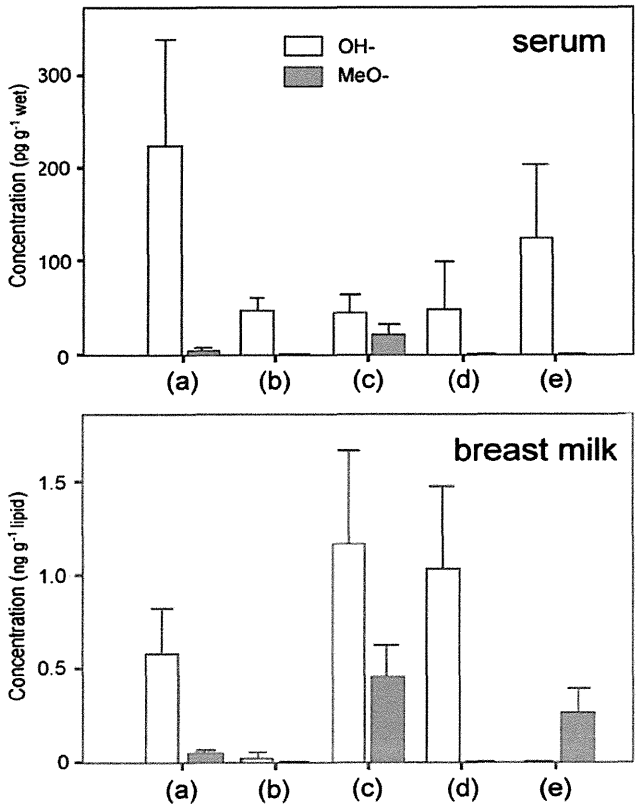

Fig. 2. Comparison of concentrations of phenolic and methoxylated organohalogens between human breast milk and serum in Okinawa. (a) = PenCP and PenCA; (b) = 4-OH-CB187 and 4-MeO-CB187; (c) = TriBP and TriBA; (d) = TBBPA and diMeO-TBBPA; (e) = 6-OH-BDE47 and 6-MeO-BDE47.

4. Discussion

4.1. PenCP, PenCA, and OH-CB

PenCP has been used as an herbicide in agricultural chemicals and preservatives in Japan until 1990 (Suzuki et al., 2008). The present data demonstrate that the dietary intake of PenCP was low compared with other OHCs. The reduced dietary exposure to PenCP may result in the lower levels in breast milk. In fact, the current levels (577 pg g^{-1} lipid) are much lower than those in a Chinese study (Hong et al., 2005). In contrast, the serum concentrations of PenCP ranged from 87 to 633 pg g^{-1} wet weight (mean 216 pg g^{-1} wet weight), as high as CB-153. Selective retention of PenCP in serum is most likely because of the specific binding potency to human TTR (Suzuki et al., 2008). The current values are still lower than those in European countries (Glynn et al., 2011; Guvenius et al., 2003; Meijer et al., 2008; Rylander et al., 2012). The primary source is considered to be dietary because PenCP has been reported in fishery products from China (Ge et al., 2007). Indoor air has been also reported as a possible source of PenCP in Japan (Suzuki et al., 2008), but the intake rates from both exposure routes are unknown.

PenCA, in contrast, has been reported as a microbiological transformation product from PenCP in marine fish, shellfish, and sediments in Japan (Watanabe et al., 1983a). This compound is expected to be more persistent than PenCP and has been found in rainbow trout (Glickman et al., 1977). However, the PenCA/PenCP ratios (0.27 and 0.50) in the dietary homogenate resulted in lower ratios in serum (0.02) and breast milk (0.06). This may be explained by the exposure to PenCP from other sources e.g., indoor environment (Harrad et al., 2010), which may have led to the increased ratios in the body tissues.

Hydroxylated PCBs (OH-CBs) are formed by cytochrome P450-mediated oxidation in hepatic microsomes after dietary ingestion of PCBs (Rylander et al., 2012). In this study, we selected 4-OH-CB187 to monitor the contamination status of phenolic PCBs in diet, serum and milk because this metabolite has been commonly observed at a high level in humans (Sandau et al., 2002). 4-OH-CB187 was detected in 20% of samples, at a 0.03:1 ratio of 4-OH-CB187:CB-187 in the diet, which was not negligible. 4-OH-CB187 was retained in blood at the second highest level. This is because of the binding affinity of phenolic OHCs to human TTR (Lans et al., 1993), a property which is in common with PenCP. The current serum concentrations of 4-OH-CB187 (45 pg g^{-1} wet weight) may reflect the contamination status of phenolic PCBs in humans because these levels are comparable to those of Japanese women (Kawashiro et al., 2008; Nomiyama et al., 2010). The serum levels of 4-OH-CB187 were well correlated with those of CB-187 ($r = 0.774$, $p < 0.01$, Supplementary Table S3), indicating that the variation of 4-OH-CB187 in serum depends on dietary exposure to parent PCBs and the individual metabolic capacity for PCB.

4.2. TriBP, TriBA, and TBBPA

Dietary intake of TriBP varied between 2004 and 2009. The large variation suggests diverse exposure routes of TriBP and food preference (i.e. deviated habitual consumption of organic foods or seafood). Dietary TriBP would be most likely derived from marine biota, i.e., ocean fish (Whitfield et al., 1999) and marine algae via the food chain (Haraguchi et al., 2010). The release of TriBP from BFR may be another source (Watanabe and Sakai, 2003). In the body, TriBP was more abundant in breast milk compared with serum. This is in contrast to PenCP and 4-OH-CB187, which demonstrated selective retention in blood. The serum levels of TriBP in Okinawa were similar to those from Norway (Thomsen et al., 2002) and India (Eguchi et al., 2012), although these are much higher than those detected in pregnant women in Japan (Kawashiro et al., 2008). Therefore, the thyroid-disrupting effects for populations exposed to TriBP should be addressed in future studies.

TriBA is a known endproduct of microbial O-methylation of TriBP (Allard et al., 1987; Whitfield et al., 1997) and is a major cause of mustiness in food (Whitfield et al., 1997). It has been detected in wine (Giannikopoulos and Whitfield, 2009) and in seafood from Japan at similar levels to PenCP (Watanabe et al., 1983a). In the present study, although the ratio of TriBA/TriBP in the diet was largely different between the 2004 and 2009 diet studies, the ratios (0.42) in 2009 were comparable to those in seafood in 1983 (Watanabe et al., 1983a). Because of the higher lipophilicity of TriBA, higher ratios were expected in the body but were not found to be elevated in either breast milk or serum (0.37–0.40). This may support the hypothesis that TriBA is more lipophilic than TriBP and can transfer to breast milk and that dietary intake is a major contributor of human exposure to TriBA.

The production of TBBPA has been reported to be 31,000 tons in 1999 in Japan, and it has been widely distributed in the environment via many flame retarded consumer products (Watanabe and Sakai, 2003). The present survey demonstrated that the possible source for human exposure to TBBPA is diet. The EDI of TBBPA via diet for adults (50 kg body weight) was calculated as a maximum of 804 pg kg^{-1} body weight day^{-1}, which was 4 orders of magnitude lower than the PTDI values (1 mg kg^{-1} body weight day^{-1}) established by The UK Committee on Toxicity (COT, 2004). The present EDIs of TBBPA (mean, 185 pg kg^{-1} body weight day^{-1}) were lower than those in the total diet study in China in 2009, ranging from 232 to 280 pg kg^{-1} body weight day^{-1} (Shi et al., 2009). However, they are consistent with the EDIs of people from Belgium (Dirtu et al., 2008), France (Cariou et al., 2008) and Norway (Thomsen et al., 2002). The levels of TBBPA (390–2220 pg g^{-1} lipid) in human breast milk were lower compared with those in China (5100 pg g^{-1} lipid) (Shi et al., 2009). The EDIs of TBBPA may predict its concentration in breast milk and serum. In contrast, the milk/serum partition ratio was

relatively higher in TriBP and TBBPA compared with other phenolic OHCs (Table 3), indicating these phenolic BFRs are preferentially transferred from blood to breast milk.

The Asian population is exposed to several BFRs, most likely with food as a major source (Shi et al., 2009). However, higher plasma concentrations of TBBPA have been reported in workers at a dismantling plant (Thomsen et al., 2001), indicating an occupational exposure to TBBPA for these individuals via ingestion of indoor dust. The dietary intakes of TBBPA were significantly associated with those of TriBP but not to the other phenolic OHCs, implying a similar exposure source between TBBPA and TriBP. Because TBBPA has a short half-life (two days) in human plasma (Hagmar et al., 2000), the occurrence of TBBPA in human serum more likely reflects recent exposure rather than past exposure (Covaci et al., 2009; Sjödin et al., 2003).

TBBPA can also be methylated by bacteria in the environment to form mono- or dimethylether derivatives (George and Häggblom, 2008). Because of its lipophilicity, we expected the occurrence of TBBPA dimethylether, but failed to detect it in food, breast milk, and serum. Further research using human serum samples from a larger number of individuals is necessary for a more complete assessment of the exposure kinetics of TBBPA.

4.3. 6-OH-BDE and 6-MeO-BDE

The present study revealed that dietary homogenates were contaminated with both OH- and MeO-BDEs at similar ratios (1:1 to 1:3). The mean serum concentration of 6-OH-BDE47 (172 pg g^{-1} wet weight) was higher than that of 4-OH-CB187, whereas 6-MeO-BDE47 was below the LOQ. In contrast, 6-MeO-BDE47 was more abundant in breast milk, but the hydroxylated analogs were not detected in the serum samples. Therefore, OH-BDEs and MeO-BDEs seem to be differentially partitioned between serum and breast milk, respectively. A possible explanation is that after dietary exposure, OH-BDEs are retained in blood because of an ability to bind to human TTR, whereas MeO-BDEs were preferentially transferred to breast milk because of their lipophilicity. The ortho-substituted OH-BDEs, such as 6-OH-BDE47, are considered to be of natural origin rather than metabolites of PBDEs (Haraguchi et al., 2010). The major source of 6-OH-BDE47 in human exposure is likely seafood from the Japanese coastal water or east Asian islands (Fujii et al., 2012; Zhang et al., 2012) because it has been distributed in marine sponges or algae in the Asia-Pacific region (Haraguchi et al., 2011), and in blood of fish (Marsh et al., 2004). The present serum levels are high compared with those from Chinese and Indian studies (Eguchi et al., 2012; Wang et al., 2012). OH-BDEs may be formed in part by demethylation in hepatic microsomes after exposure of MeO-BDEs (Wan et al., 2009). The toxicological implication of human exposure to OH-BDEs remains unknown, but one study observed a reduction of thyroid hormone levels in vivo (Malmberg et al., 2005).

5. Conclusions

The present study revealed that the Okinawan diet is contaminated with toxic persistent phenolic OHCs and suggests a positive relationship between dietary exposure to both phenolic and methoxylated OHCs via diet, which are positively related to the corresponding methoxylated OHCs making up the body burden of OHCs. The available data indicate that dietary exposure to phenolic OHCs leads to the selective retention of PenCP, 4-OH-CB187 and 6-OH-BDE47 in serum, whereas methylated derivatives transfer to breast milk. In particular, 6-OH-BDE47 and 6-MeO-BDE47 were differentially partitioned between serum and breast milk, respectively. These patterns may be due to the different kinetics between hydroxylated and methoxylated PBDEs. To evaluate the potential for thyroid-disrupting effects caused by phenolic OHCs, continuous monitoring of human exposure to phenolic OHCs should be performed.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.envint.2013.10.016.

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Methylmercury Monitoring Study in Karakuwacho Peninsula Area in Japan

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Abstract Methylmercury (MeHg) is a worldwide concern owing to its adverse health effects. To explore MeHg exposure burdens and the potential contributing factors in different subpopulations in a peninsula area (Karakuwacho) in Japan, a cross-sectional survey was performed. This study included 189 individuals from 102 families. The geometric means of total hair mercury (THg) were 5.74, 3.78 and 2.37 μg/g for adult males, females and children, respectively, of which 56.5 %, 30.9 % and 12.9 % had hair THg exceeding 5 μg/g, respectively. Tuna and mackerel were the common fish species that were positively correlated with hair THg levels in different subpopulations (standardized coefficient ranged from 0.20 to 0.58, $p < 0.05$). Frequent consumption of these fish species and a large amount of fish intake are likely major contributors of MeHg exposure in this area. Local-scale risk evaluation and risk communication should be highlighted in future studies.

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Keywords Hair mercury · Methylmercury · Fish species · Japanese population

Mercury is a recognized toxic pollutant of public health concern. Its global distribution is caused by natural processes and anthropogenic activities. Inorganic mercury can be transformed into organic forms (mainly MeHg), which can be bioaccumulated and biomagnified in aquatic food chains (Matthews 1983; Mergler et al. 2007). Various studies have determined that fish consumption is the main source of human exposure to MeHg (Hightower and Moore 2003; Knobeloch et al. 2005). MeHg exposure can lead to various adverse health effects, especially neurological symptoms (Guallar et al. 2002; Harada 1995; Mahaffey 1998; Mozaffarian and Rimm 2006). These adverse health effects may occur at low levels that were previously thought to be safe (Karagas et al. 2012; Maruyama et al. 2012).

In Japan, people habitually consume more fish products than other countries. Historically, several catastrophes (i.e., Minamata disease and Niigata Minamata disease) have been caused by MeHg-contaminated fish consumption (Harada 1995). Several monitoring studies have indicated higher exposure levels in Japan compared with reference values or other populations (Yasuda et al. 2005; Yasutake et al. 2004). Therefore, identifying the high-risk areas and the major contributors is essential for developing effective reduction strategies. In a previous monitoring study (unpublished data), we identified that peninsula or island areas had higher mercury exposure levels. To further investigate the exposure pattern and the potential contributing factors in different subpopulations in such areas, we performed a family-based study of MeHg exposure in Karakuwacho, a peninsula area in the northeast of Miyagi prefecture in Japan.

Materials and Methods

This study was performed in Karakuwacho and included 189 individuals from 102 families. There were ten families composed of father, mother and children; 28 families composed of mother and children; and the other families we collected one individual or two non-parent–child paired individuals. Generally, there were 104 adults (81 females and 23 males) and 85 children (Table 1). For each individual, hair samples (0.1–1.0 g) were cut from the base of the scalp, behind the ear, and the samples were washed with neutral detergents, rinsed twice with acetone, and then dried at room temperature and stored in a desiccator in Kyoto University Human Specimen Bank (Koizumi et al. 2009) until they were analyzed for total mercury (THg). At the time of hair sampling, body weight was measured by a investigator using a standard scale. A questionnaire was administered to the participants to collect information regarding their age, gender, total fish consumption frequency, and commonly consumed fish species. All participants were fully informed about the purposes of the study and provided written consent. The study was approved by the Institutional Review Board and Ethics Committee of the Kyoto University School of Medicine, Japan.

For hair THg analysis, the preconditioned dry hair sample was cut into small pieces (<2 mm) with scissors. Aliquots of samples (15–20 mg) were dissolved in 0.5 mL 2 N NaOH while being heated at 60°C for 1 h. Ten or twenty microliter of the solution was used to analyze the THg levels by the oxygen combustion–gold amalgamation method and an MD-1 atomic absorption detector (Nippon Instruments, Co., Ltd., Osaka, Japan) (Yasutake et al. 2003). This technique of quantification is based on a pyrolysis process of the sample using a combustion tube heated at 700°C under an oxygen atmosphere. Vaporized mercury was transferred to gold-absorber at a carrier gas flow rate of 0.5 L/min. Gaseous compounds other than mercury was eliminated from the system. Gold-absorber was then heated at 700°C for 2 min to vaporize and transfer concentrated mercury to detector. The concentration of mercury was determined by measuring the absorbance of gas at 253.7 nm emitted from mercury-vapor lamp. The limit of detection was 0.1 μg/g-hair (signal to noise ratio: 3) and the limit of quantitation was 0.3 μg/g-hair. All the individuals had hair THg levels above the limit of quantitation. The external standard was 2.5 nM mercuric chloride (0.5 μgHg/mL) in 0.5 M L-cysteine/2 % bovine serum albumin solution. To ensure precision of instrument, standard solution was analyzed in every ten analyses. The analysis was qualitatively confirmed by analyzing a certified reference material of human hair, NIES CRM No. 13 (hair reference material for MeHg, THg and other trace elements, National Institute for Environmental Studies, Japan) with a certified THg value of 4.42 ± 0.2 μg/g (http://www.nies.go.jp/labo/crm/hair.html). The THg level from our method above was 4.55 ± 0.05 μg/g. No detectable contamination was observed from procedural blank samples in every 20 samples. The hair THg levels were compared with various limit levels (1.0, 2.2, 2.7 and 5.0 μg/g) proposed by Japan or other international authorities (US EPA.2000; WHO/JEFCA 2004; The Food Safety Commission, Japan, 2005).

Statistical analysis was performed using STATiSTiCA64 (Supplied by Statsoft, OK, USA). Normally distributed

Table 1 Hair THg levels (μg/g) in 189 individuals in Karakuwacho

Variables	Ten father–mother–children paired families			28 mother–children paired families		Total		
	Fathers	Mothers	Children	Mothers	Children	Adult female	Adult male	Children
No	10	10	15	28	38	81	23	85
Age (years)								
Mean ± SD	40.7 ± 7.2	39.7 ± 5.9	7.8 ± 4.0	35.0 ± 4.8	5.0 ± 3.2	47.2 ± 14.9	43.3 ± 9.8	5.76 ± 3.3
Range	27–50	31–48	2–14	27–47	1–14	21–82	23–63	1–15
Male/female	–	–	–	–	–	–	–	42/43
Hair THg (μg/g)*								
Min	2.21	1.93	0.64	1.12	0.6	1.08	2.21	0.6
Max	12.24	6.79	10.76	13.78	8.38	15.95	14.98	10.76
GM	6.71	3.75	1.92	3.45	2.57	3.78	5.74	2.37
Correlation analysis	F versus M	M versus C	F versus C	M versus C				
Spearman coefficient	0.079	−0.442	0.267	0.290				
p value	0.828	0.200	0.455	0.134				

F versus M, father versus mother; M versus C, mother versus children; F versus C, father versus children

* Kruskal–Wallis test, $p < 0.001$

variants were described by their means and standard deviations. The minimum, maximum, and geometric mean (GM) were used to describe log-normally distributed variants. Non-parametric Kruskal–Wallis tests were conducted to compare THg differences between different populations. Spearman correlation analysis was conducted to explore the relationship between hair THg and fish consumption frequency. Stepwise multiple linear regression analysis was employed to assess the relationship between log THg levels in hair and covariates, such as age, sex, body weight and commonly consumed fish species. The significance level was set at less than 0.05.

Results and Discussion

Mercury, especially MeHg, is a worldwide concern owing to its adverse health effects. In order to ensure the population health, effective reduction strategies should be developed in the high exposure areas. With the aim to identify the detail exposure pattern and the major contributing factors in such a high exposure area, a cross sectional survey was performed in Karakuwacho, a peninsula area in Japan.

Generally, wide variation in hair THg levels was observed for different members of the same family. Fathers had the highest hair mercury level, follow by mothers, and then children (Kruskal–Wallis test, $p = 0.001$). In the same household, there were no correlations in hair mercury levels between family members (correlation analysis, all $p > 0.05$) (Table 1). Considering the potentially different fish consumption patterns, we divided the participants into three subgroups (adult males, adult females and children) for further analysis. Adult males had higher hair THg levels than adult females and children (GMs of THg were 5.74, 3.78 and 2.37 μg/g, respectively, Kruskal–Wallis test, $p < 0.001$) (Table 1). The hair THg levels in this area were significantly higher than the national average levels in Japan (GM values of 2.42 and 1.37 μg/g for males and females, respectively) (Yasutake et al. 2005) and the United States (GM values of 0.12 and 0.20 μg/g for children and women, respectively) (McDowell et al. 2004). There were 30.9 %, 56.5 % and 12.9 % of adult females, adult males and children with hair THg levels that exceeded the least strict limit (5.0 μg/g), respectively (Fig. 1). When considering the at-risk population (women of childbearing age, n = 50), 76 % of them had hair THg exceeding the corresponding limit in Japan (2.7 μg/g), and 26.0 % had hair THg levels exceeding 5.0 μg/g. These levels are significantly higher than levels in other countries (Mahaffey et al. 2009; Kim and Lee 2010). Because MeHg accounts for more than 80 % of THg in hair, THg in hair is thought to be a reliable indicator of MeHg exposure (Cernichiari et al. 1995). The general population in this area commonly faces high risk of MeHg exposure.

To elucidate the potential contributing risk factors, fish consumption frequency and commonly consumed fish species were investigated. We found that hair THg levels significantly increased with the frequency of fish consumption (Fig. 2). This may explain the hair THg distribution difference between different subpopulations. Adults tended to consume fish more frequently than children; thus, higher hair THg levels were observed (Fig. 2). In present study, we found 25 species of fish were commonly consumed in the survey area (Fig. 3). According to monitoring data released by the government (Online document. MHLW.2010), a majority of these fish species had THg concentrations with less concern (<0.1 ppm), but increasing concern was raised about commonly consumed high-end predatory fish including marlin, tuna, and alfonsino in which the THg concentration was significantly higher than the permitted limit in Japan (>0.4 ppm) (Nakagawa et al. 1997) (Fig. 3).

Multivariate analyses revealed that tuna or canned tuna were the common fish species positively correlated with hair THg levels in different subpopulations (Table 2). Tuna, which is a carnivorous fish with high mercury accumulation, is often consumed in Japan. Yasutake et al. (2004) determined that in the Miyagi area, the average fish consumption was 96 g/person/day, and 69 % of participants in their survey frequently consumed tuna. In the present study, we similarly found that 52.9 % of individuals commonly consumed tuna, and 20.3 % of individuals consumed tuna more than once per week. High tuna consumption tended to be the major contributor to high MeHg exposure in this area.

It is important to note that even fish species contaminated with low levels of mercury, if consumed frequently and eaten in large amounts, may increase the cumulative risk. In the present study, we observed a positive correlation between mackerel consumption and hair THg levels among adult population, even though mackerel has relatively low mercury levels (mean THg level of 0.11 μg/g) (Table 2; Fig. 3). In Japan, the government released a fish advisory in 2003 and further revised it in 2005 (Ser and Watanabe 2012). The advisory mainly focused on ocean fish species. There was no restriction for species with low mercury content. However, in developing a risk reduction strategy, we should consider the contamination level as well as fish consumption amount at the same time.

Although consumption of fish represents a major source of dietary MeHg exposure for the inhabitants of the Karakuwacho peninsula region, this food source also provides high-quality protein and is also rich in unsaturated fatty acids. Consequently, reducing fish consumption as a mean of reducing exposure to MeHg must weigh both the risks and benefits associated with consuming fish as a

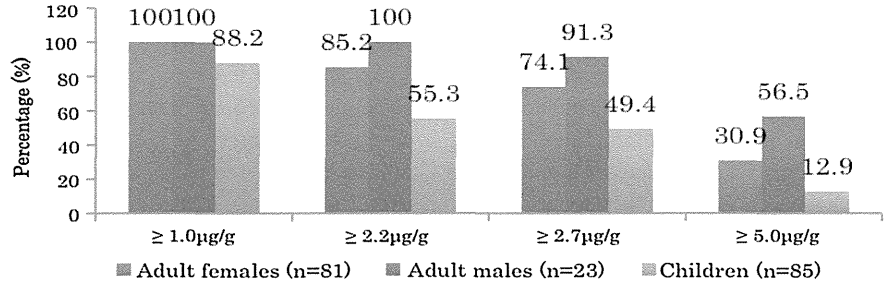

Fig. 1 The percentage of hair THg levels above the limits in different subpopulations of Karakuwacho. Limits: 1.0, 2.2, 2.7 and 5.0 μg/g for hair THg levels correspond to the US EPA, JECFA, and Japanese Food Safety Commission proposed intake limits for pregnant women and the general population (0.7, 1.6, 2.0 and 3.4 μg/kg bw/week), respectively

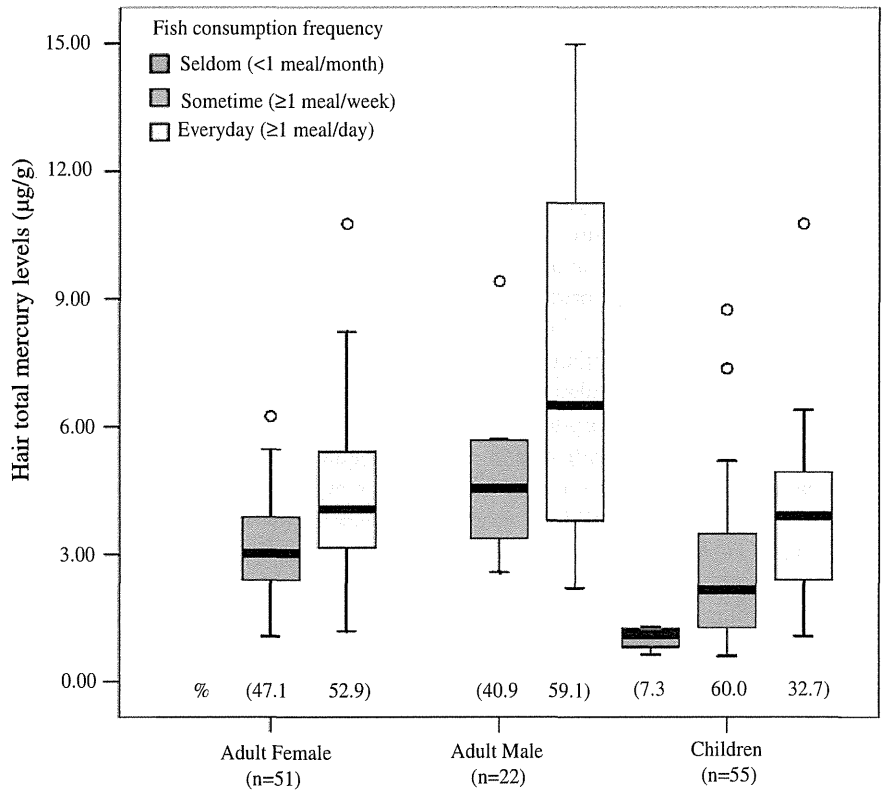

Fig. 2 Correlation between hair THg levels and fish consumption frequency. Hair THg levels significantly increased with fish consumption frequency. *Boxes* depict 25th, 50th and 75th percentiles, and whiskers depict minimum and maximum values, excluding outliers. *Circles* depict the outliers. The *number* below the *boxes* indicates the proportion of each fish consumption frequency in the corresponding subpopulation

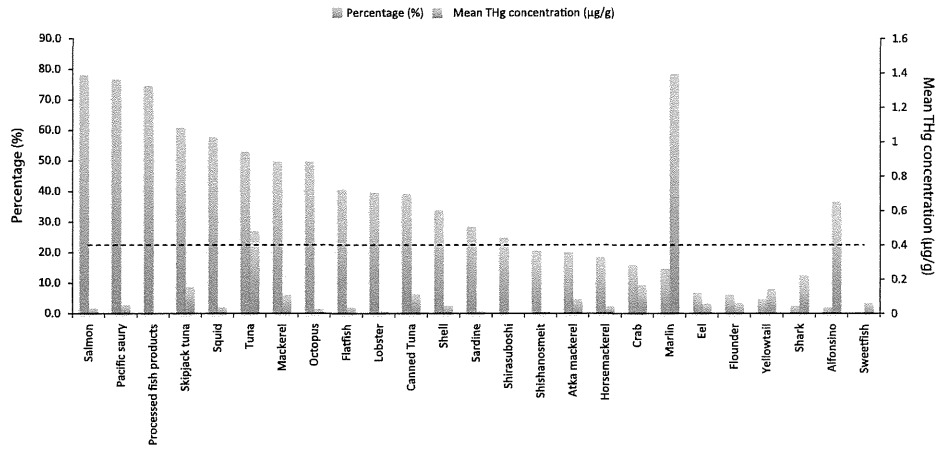

Fig. 3 Fish species commonly consumed by the survey population in Karakuwacho and the means THg concentrations of these fish species, which are cited from the newly released monitoring summary data in Japan (MHLW.2010)

Table 2 Stepwise multiple linear regression analysis between log hair mercury levels and other covariants

Model	Unstandardized coefficients		Standardized coefficients	*t*	*p*	Model summary	
	B	*SE*	*B*			Adjusted R^2	*p*
Adult female (n = 81)							
Constant	0.60	0.06	–	10.16	<0.001	0.254	<0.001
Atka mackerel	−0.13	0.06	−0.21	−2.09	0.041		
Tuna	0.15	0.05	0.31	2.94	0.004		
Octopus	0.17	0.05	0.35	3.18	0.002		
Pacific saury	−0.19	0.07	−0.31	−2.66	0.010		
Mackerel	0.12	0.05	0.24	2.24	0.028		
Squid	−0.12	0.06	−0.23	−2.02	0.047		
Children (n = 85)*							
Constant	0.26	0.05	–	5.70	<0.001	0.101	0.002
Tuna	0.21	0.07	0.34	3.16	0.002		
Adult male (n = 23)							
Constant	0.50	0.09	–	5.28	<0.001	0.337	0.008
Canned tuna	0.30	0.09	0.58	3.16	0.005		
Mackerel	0.23	0.10	0.43	2.35	0.030		

Dependent variable: log hair mercury levels. Independent variables: age, body weight, various commonly consumed fish species

SE standard error

* In this regression model, age, sex, body weight and various commonly consumed fish species were included as independent variables

dietary staple. In this study, we determined that pacific saury was negatively correlated with hair THg levels in the female adults (Table 2). According to mercury exposure monitoring data, Pacific saury had a low THg level of 0.052 ppm. Therefore, according to fish consumption patterns and mercury monitoring levels, populations can be guided to choose fish species having low levels of contamination (i.e., salmon and Pacific saury) and avoid species that may contain high mercury levels (i.e., marlin, alfonsino and tuna). Meanwhile, the total amount of fish consumption should always be considered.

In conclusion, the level of MeHg exposure is high in Karakuwacho peninsula region. Consumption of fish with high mercury contamination (e.g., tuna or canned tuna) and high consumption of fish with low mercury contamination (e.g., mackerel) are likely the major contributors. Therefore, both qualitative and quantitative aspects of fish consumption should be addressed to achieve effective reduction in MeHg exposure.

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Toxicokinetics of perfluoroalkyl carboxylic acids with different carbon chain lengths in mice and humans

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Abstract: Toxicokinetics of perfluoroalkyl carboxylic acids with different carbon chain lengths in mice and humans: Yukiko FUJII, *et al.* Department of Health and Environmental Sciences, Kyoto University Graduate School of Medicine—Objectives: Perfluoroalkyl carboxylic acids (PFCAs) consist of analogs with various carbon chain lengths. Their toxicokinetics have remained unexplored except in the case of perfluorooctanoic acid (8 carbon chemicals). This study aimed to investigate the toxicokinetics of PFCAs with six to fourteen carbon atoms (C6 to C14) in mice and humans. **Methods:** We applied a two-compartment model to mice administered PFCAs intravenously or by gavage. The time courses of the serum concentration and tissue distribution and elimination were evaluated for 24 hours after treatment. For human samples, urine from healthy volunteers, bile from patients who underwent biliary drainage, and cerebral spinal fluid (CSF) from brain drainage were collected. **Results:** The mouse experiment showed that short-chained PFCAs (C6 and C7) were rapidly eliminated in the urine, whereas long-chain PFCAs (C8 to C14) accumulated in the liver and were excreted slowly in feces. Urinary clearance of PFCAs in humans also decreased with increasing alkyl chain lengths, while biliary clearances increased. C9 to C10 had the smallest total clearance for both mice and humans. However, disparities existed in the magnitude of the total clearance between mice and humans. A slightly higher partition ratio (brain/serum) was observed for long-chained PFCAs in mice, but this was not observed in the corresponding partition ratio in humans (CSF/serum). **Conclusions:** The large sequestration volumes of PFCAs in the liver seem to be attributable to the liver's large binding capacity in both species. This will be useful in evaluating PFCA bioaccumulation in other species.
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Key words: Human, Mice, Perfluoroalkyl carboxylates, Perfluorooctanoic acid, Toxicokinetics

Perfluorochemicals, such as perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid [PFOA, chemicals with eight carbon atoms (C8)], have been detected in the environment, and their toxicokinetics have been examined extensively. Their biological half-lives are significantly longer in humans than in other laboratory animal models[1, 2]. The reason for the longer biological half-lives in humans remains unknown.

C8 PFOA has been found to cause hepatotoxicity, developmental toxicity, immunotoxicity and endocrine disruption[3]. Consequently, perfluoroalkyl carboxylic acids (PFCAs) other than C8 PFOA with shorter chain lengths, such as perfluorobutanoic acid and perfluorohexanoic acid (C4 to C6), have been used for commercial applications[4]. These short-chained PFCAs seemed to be less toxic than C8 PFOA[5, 6], possibly stemming from their relatively short half-lives compared to the C8 PFOA[7, 8]. In contrast, long-chained PFCAs, such as perfluorononanoic acid (PFNA, C9) and perfluorodecanoic acid (PFDA, C10), showed relatively longer half-lives than PFOA in rodents[1, 9, 10]. It is well known that straight-chain PFCAs are not metabolized biologically[11]. Furthermore, several *in vitro* studies have found that biological activities are dependent on the alkyl chain length of the parent

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Supplementary tables and figures: refer to J-STAGE: https://www.jstage.jst.go.jp/browse/joh

compounds[12–14]. Nevertheless, increasing levels of long-chained PFCAs have been found in the human serum[15, 16] and daily diet[17] in recent decades.

The present study aimed to investigate the toxicokinetic differences of C6 to C14 PFCAs in mice and humans. Serum concentration and tissue distribution and elimination were evaluated for 24 hours after intravenous (IV) and gavage PFCA administration in mice. Urinary clearance, biliary clearance and cerebral spinal fluid (CSF) partitions of PFCAs in humans were examined for comparison. No such comparison has ever been reported, despite its toxicological importance.

Material and Methods

Animal experiments

1) Animals. All experiments were performed with mice aged 8–10 weeks (body weight 20–30 g). FVB/NJcl mice were purchased from CLEA Japan, Inc. (Tokyo, Japan), and housed in the Kyoto University Institute of Laboratory Animals. A standard commercial lab chow diet (F-2, 3.73 kcal/g, Funahashi Farm Corp., Chiba, Japan) was used. All animals were maintained at an ambient temperature of 24 ± 2°C and 50 ± 10% humidity with a 12-h light/dark cycle (lights on at 7:00 a.m.). Mice were individually placed in metabolic cages and were provided with free access tap water and food.

2) Sample collection. Each PFCA was administered by IV or gavage. PFCAs were dissolved in ethanol/water/dimethyl sulfoxide (5:4:1) and prepared with Milli-Q water. In this study, both IV and gavage administration were applied to evaluate the absorbed ratios of PFCAs. Single doses of PFCAs were administered through the tail vein (IV dose 0.31 μmol/kg, injection volume 10 m*l*/kg) or orally (gavage dose 3.13 μmol/kg, injection volume 10 m*l*/kg). Each group contained 18 mice: 9 males and 9 females.

To observe the time course of the serum PFCA concentrations, whole blood samples (10 μl) were collected from the tail veins at 0, 1, 3, 6, 12 and 24 hours after IV or gavage administration. An additional collection was made at 0.5 hours for IV administration. The study protocol is summarized in Table S1.

After 24 hours, urine and feces were collected in metabolic cages. Mice were then placed under sevoflurane anesthesia and euthanized by cervical dislocation. A portion of the whole blood was collected and centrifuged (370 g) to isolate the serum. Liver, kidney and brain tissues were collected and weighed. Adipose tissue was collected from the abdominal mesenteric fat. The total serum in the mice was estimated to be 56 m*l*/kg mouse body weight for male mice and 65 m*l*/kg mouse body weight for female mice[18]. The total adipose tissue was assumed to be 2.3% of the total body weight of mice[18]. All experimental procedures were approved by the Kyoto University Animal Research Committee (MedKyo11067).

Paired human samples: urine, bile and CSF serum pairs

All paired human samples (bile-serum, CSF-serum and urine-serum) were obtained from the archived samples in the Kyoto University Human Specimen Bank[19, 20]. The characteristics of the participants are summarized in Table S2. Bile samples were taken by nasobiliary drainage, percutaneous transhepatic biliary drainage or percutaneous transhepatic gallbladder drainage for 24 hours. Paired 5-m*l* blood samples were collected from the cubital vein into polypropylene tubes on the same day. CSF samples were taken by cerebral drainage, spinal drainage, ventriculoperitoneal shunt or duraplasty. Ten milliliters of blood was also donated from the donor on the same day. Healthy volunteers were requested to collect 24-h pooled urine samples and to donate 10 m*l* of blood at the end of urine collection. The research protocol was reviewed and approved by the ethics committee of Kyoto University (E25). Written informed consent was obtained from all participants before sample collection.

Determination of PFCA concentration in biological samples

1) Sample homogenization and preparation. Mouse tissue and feces were weighed and diluted with Milli-Q water/methanol (1:1) at a ratio of 15 m*l* water/methanol per gram of mouse tissue. The sample was homogenized using a homogenizer. Part of the homogenate (0.1–1 m*l*, depending on the concentration) was transferred into a 15-m*l* polypropylene tube. For whole blood, serum and urine samples, approximately 10–100 μl of each sample and 1 m*l* of methanol were placed in a 1.5 m*l* microcentrifuge tube and mixed for 3 hours. Part of the resulting solution (0.1–1 m*l*, depending on the concentration) was then transferred into a 15 m*l* polypropylene tube. For the human samples, approximately 0.5–30 m*l* of each sample was directly transferred into 15 or 50 m*l* polypropylene tubes.

2) Determination of PFCAs. Determination of PFCA concentrations in all samples was performed using a method previously reported[17]. Target chemicals included perfluorohexanoic acid (PFHxA, C6), perfluoroheptanoic acid (PFHpA, C7), PFOA (C8), PFNA (C9), PFDA (C10), perfluoroundecanoic acid (PFUnDA, C11), perfluorododecanoic acid (PFDoDA, C12), perfluorotridecanoic acid (PFTrDA, C13) and perfluo-

rotetradecanoic acid (PFTeDA, C14). Procedural blank controls were analyzed after every 10 samples. The method detection limit (MDL) was defined as the concentration that produced a signal three times that of the blank (Table S3). Total recoveries are shown in Table S4.

Toxicokinetic analysis of PFCAs

The ratio of PFCAs between whole blood and serum at 24 hours was used to convert PFCA concentrations in whole blood samples into serum PFCA concentrations. Serum concentration data were analyzed using a two-compartmental model described by the following equation:

$$C(t)=C_1 exp(-\lambda_1 * t)+C_2 exp(-\lambda_2 * t). \quad Eq\ (1)$$

To obtain C_1, C_2, λ_1 and λ_2, PFCA levels in the serum were fitted into a two-compartment toxicokinetic model by nonlinear optimization with a least-square approach[21]. The volume distribution in the IV injection study was defined as follows:

$$Volume\ distribution = Dose / C(0). \quad Eq\ (2)$$

PFCA clearance in mouse and human samples

Mouse urinary clearance ($CL_{u\text{-}mice}$) was determined by dividing the total amount excreted in the urine during a 24-h period with the area under the curve (AUC) of the serum concentration of each PFCA between 0 to 24 hours. Mouse fecal clearance ($CL_{f\text{-}mice}$) was determined by dividing the total amount excreted in the feces during a 24-h period with the AUC of the serum concentration of each PFCA between 0 to 24 hours.

Human urinary ($CL_{u\text{-}humans}$) and biliary clearance ($CL_{b\text{-}humans}$) of each PFCA was determined by dividing the cumulative urine or bile excretion in a 24-h period with the serum concentration of each PFCA.

Statistical analysis

Concentrations lower than the detection limits were given a value half that of the detection limit for statistical analyses. Differences between mean values of each PFCA in human CSF were tested using the Student's *t*-test. Values of $p<0.05$ were considered statistically significant.

Results and Discussion

Toxicokinetic analyses in mice after IV administration

The ratios of whole blood to the serum concentrations of each PFCA (mean ± SD) were 0.60 ± 0.1 for C8, 0.43 ± 0.1 for C9, 0.50 ± 0.1 for C10, 0.53 ± 0.1 for C11, 0.70 ± 0.2 for C12, 0.88 ± 0.2 for C13, and 1.05 ± 0.2 for C14. The mean ratio of each chemical was multiplied by the whole blood concentrations to calculate the corresponding serum concentration.

The time course and fitted curves for PFCAs in logarithmic scale are shown in Fig. 1. As C6 was not detected in the serum at even 1 hour after administration, its serum kinetics was not analyzed. For the other PFCAs (C7 to C14), the serum levels were above the MDLs. As shown in Fig. 1, C7 disappeared from the serum in a time-dependent manner. The other compounds (C8 to C14) demonstrated very unique kinetic profiles characterized by slow elimination from the serum (Table 1). The two-compartment model successfully described the kinetics of PFCAs in mice. The parameters obtained from the serum PFCA concentrations are depicted in Table 1.

The volume distributions of the PFCAs (C7 to C14) exhibited no differences between sexes, with the volume increasing as a function of length in both males and females (Fig. S1). The distributions corresponded roughly to the total volume of blood with C7, extracellular water with C8 and C9 and body water with C11 and C12. Tissue binding was suggested for C13 and C14. These results indicated that chain length was a determining factor for volume distribution (Table 1). The AUCs reached their maximums at C8 and decreased with increasing chain length (Table 1).

Table S5 shows the tissue distribution of PFCAs 24 hours after administration. Total recoveries for all C6 to C14 were greater than 76% in males and somewhat lower in females (greater than 58%). For C6 and C7 PFCAs, almost all of the administered doses were recovered in the urine after 24 hours (101 and 99% for males, 66 and 79% for females), with only a small portion excreted in the feces (5 and 3% for males, 16 and 13% for females). In contrast, only a small portion of C8 was excreted in the urine (6% for males, 7% for females), and even less was excreted in the feces (<1% for both sexes); the majority was retained in the serum and liver (80% for males, 62% for females), with a discernible amount retained in the kidney (1% for both sexes). For C9 to C14, the distribution pattern was similar to that of C8. However, C9 to C14 excretion in the urine and feces for both males and females was much lower than that of C8; most were retained in liver (64–80% for males, 46–55% for females).

Toxicokinetics of PFCAs in mice after gavage administration

After gavage administration, C6 was not detected in the serum at any sampling points. Thus, a two compartment analysis was not conducted for C6. As shown in Fig. 2, the time courses for C7 to C14 were well simulated by the two-compartment toxicokinetic models with no differences in sex (Table 1). The