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 pharmacokinetics or exposure routes.

Keywords: Dietary exposure, breast milk, serum, 6-hydroxy-BDE47, 6-methoxy-BDE47

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, bioaccumulate, 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 (Nomiya 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 et al., 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). 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 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 [$^{13}\text{C}_6$], 4-hydroxy-2,2',3,4',5,5',6-heptachlorobiphenyl (4-OH-CB187 [$^{13}\text{C}_{12}$]), 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153 [$^{13}\text{C}_{12}$]) and α -endosulfan [$^{13}\text{C}_9$] 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 hours prior to use. All solvents used were of pesticide-grade quality.

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 [$^{13}\text{C}_6$] and 4-OH-CB187 [$^{13}\text{C}_{12}$] for phenolic analytes (0.2 ng of each), and α -endosulfan [$^{13}\text{C}_9$] (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 on comparison with CB-153 [¹³C₁₂], 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 (¹³C-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 2 to 200 pg g⁻¹ lipid (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 were higher in the order of TriBP > TBBPA > PenCP > 6-OH-BDE47 > 4-OH-CB187 in the 2004 diet study, whereas in the order of TriBP > 6-OH-BDE47 > TBBPA > PenCP > 4-OH-CB187 in the 2009 study (Table 2). For the methoxylated analogs, the dietary intake was higher in the order of TriBA > 6-MeO-BDE47 > PenCA in the 2004 study, whereas in the order of TriBA > PenCA > 6-MeO-BDE47 in the 2009 study. 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 organohalogenes 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 ($r=0.655$, $p<0.01$) but not with the other contaminants. TBBPA was associated with PenCA ($r=0.531$, $p<0.01$), but not PenCP ($p>0.05$). 6-OH-BDE47 was not associated with 6-MeO-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 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).

4. Discussion

4.1. PenCP, PenCA, and OH-PCB

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 wet weight (mean 216 pg/g 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 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 (Nomiya et al., 2010; Kawashiro et al., 2008). 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., 2011), 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 bw 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 et al., 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-PBDEs 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 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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493

Figure and Table Captions

Fig. 1 Daily intake of phenolic and methoxylated organohalogenes 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 organohalogenes 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.

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

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

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

Fig. 1

Fig. 2

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	Okinawa	Male	4	2004	32.2 (20-50)
(duplicate diet study)		Female	6		
diet 2009	Okinawa	Male	10	2009	36
(duplicate diet study)					
breast milk	Okinawa	Female	9	2005-2006	31.4 (26-39)
serum	Okinawa	Female	10	2006	43.7 (40-47)

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
<i>trans</i> -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	1.03	<LOQ	7.08	9.48	<LOQ
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-hour 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
<i>Halogenated compounds</i>								
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
<i>trans</i> -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	
<i>Phenolic OHCs</i>								
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
<i>Methoxylated OHCs</i>								
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	
<i>Ratios</i>								
PenCA/PenCP		0.05	0.06			0.02	0.02	
TriBA/TriBP		0.37	0.38			0.40	0.55	

^a number detected

Concentrations below the LOQ were treated as 1/2 LOQ for the mean and median values.