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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.