NATURAL PERSISTENT ORGANOHALOGENS IN BREAST MILK FROM JAPAN

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Abstract
Japanese breast milk from four regions (Miyagi, Wakayama, Kochi and Okinawa) in 2004 was investigated for natural persistent organohalogens. Lipophilic natural components were dominated by heptachlorinated methyl bipyrrole (Cl_7-MBP, 0.08-1.68 ng/g lipid), mixed halogenated dimethyl bipyrroles (Br_4Cl_2-DBP, 0.25-4.98 ng/g lipid), methoxylated tetrabromodiphenyl ethers (2'-MeO-BDE68, 0.01-2.77 ng/g lipid) and dimethoxylated tetrabromobiphenyl (2,2'-diMeO-BB80, 0.01-0.69 ng/g lipid). The concentrations of Br_4Cl_2-DBP were higher in mothers from the northern Japan (Miyagi), whereas those of 2'-MeO-BDE68 were higher in mothers from the southern Japan (Okinawa). These natural organohalogens were present at one order of magnitude lower levels than PCB153, but at comparable levels to a major component of PBDEs, BDE47 (0.13-3.10 ng/g lipid), in all cases. The dietary source of these components is most probably marine fish and seafood commercially available in Japan.

Introduction
Synthetic halogenated compounds, including persistent organic pollutants (POPs), have been identified as global environmental and human contaminants over the past 30 years. Polybrominated diphenyl ethers (PBDEs) have also been detected in various biological samples such as freshwater or marine fishes[1], from aquatic or terrestrial animals[2], and even from humans.[3,4] Recent work has described the environmental existence of persistent bioaccumulative organohalogens that have a natural origin[5]. Heptachlorinated methyl bipyrrole (Cl_7-MBP, referred as Q1), mixed halogenated dimethyl bipyrroles (HDBPs) and methoxylated tetrabromodiphenyl ethers (MeO-BDEs) have been reviewed recently.[6] Cl_7-MBP has been identified at the ppm range in marine mammals and at the ppb range in fish and human milk.[7] HDBPs have been detected in biota sampled from locations around the world and are thought to undergo long-range transport.[8] MeO-BDEs have been found in mammals all over the world.[2,9]

In Japan, odontocetes (beaked whales, dolphins, and porpoises), and mystecetes (mainly minke whales) have been hunted and sold for human consumption. In a recent survey, highly contaminated levels of natural persistent products, e.g. HDBPs, which have similar lipophilicity to PCBs, have been found in whale products.[10] Fish samples available in Japan are also contaminated with natural halogenated compounds (data not shown), although the source is still unknown. The purpose of this study was to determine whether or not Japanese women have higher body burden of natural persistent organohalogens, as compared to anthropogenic POPs. For this purpose, we used human milk samples collected throughout Japan. In the present study, a total of 65 individual samples from four sampling sites areas (Miyagi, Wakayama, Kochi and Okinawa) were analyzed for natural products (MBP, HDBPs, MeO-BDEs etc) as well as PCBs and PBDEs. The quantitative results were compared between natural and anthropogenic substances and between four regions. This is the first survey on the natural persistent organohalogens in Japanese breast milk.

Materials and Methods
Sample collection: We used breast milk stored in The Kyoto University Human Specimen Bank, where a total of 1000 individual breast milk samples had been collected from 13 regions throughout Japan.[3] A total of 65 milk samples collected in 2004 was selected from four regions (Miyagi, Wakayama, Kochi and Okinawa) in this study. Information on age and occupation was obtained from participants. Samples were stored at -20ºC prior to usage.

Chemicals: Three internal standards, 2,3,4,5,6,3',4',5'-octachlorobiphenyl (CB205), 2,3,4,2',4',5'-hexabromodiphenyl ether (BDE138, IS-2) and 4'-MeO-2,4,6,3',5'-pentabromodiphenyl ether (4'-MeO-BDE121, IS-1) were used for the determination of PCB, PBDE and natural organohalogens, respectively. Three HDBP congeners (Br_4Cl_2-, Br_3Cl_3- and Br_3Cl_2-DBPs) were prepared according to the method of Gribble et al., and Cl_7-MBP was by the method of Wu et al. Four MeO-PBDEs (2'-MeO-BDE68, 6-MeO-BDE47, 4'-MeO-BDE121 and 2',6-diMeO-BDE68) and 2,2'-diMeO-BB80 were donated by Dr G. Marsh (Stockholm University).
Chemical analysis: Before extraction, each internal standard was added to milk samples (2 ng of CB205, 0.2 ng of BDE138 and 4'-MeO-BDE121). Human milk (5 mL) was three times extracted with *n*-hexane, after adding 5 mL water, 1 mL potassium oxalate solution, 10 mL ethanol and 5 mL diethyl ether. After solvent evaporation, gravimetric lipid determination was performed. The lipid was subjected to gel permeation column (Bio Beads S-X3) and the eluate was purified by silica gel column (Wako gel S-1). The final extract was reduced in a volume of 50 μL for GC/MS analysis.
Instruments and Quantificaiton: GC-MS analysis of the samples and reference standards were performed on an Agilent GC/MSD 9573i (Agilent technologies) provided with a 6980N gas chromatograph. A capillary column used was HP-5MS (30 m × 0.25 mm i.d. and 0.25 μm film thickness, J&W Scientific). Splitless injections were performed. The GC temperature programs were: 70ºC (1.5 min) - 20ºC/min - 230ºC (0.5 min) - 4ºC /min –280ºC (5 min) with helium as carrier gas (head pressure of 3 psi). The injector and transfer line temperatures were 250ºC and 280ºC, respectively. The electron energy was 70 eV and the temperature of the ion source was 230ºC for all MS techniques. Masses between 60-680 *m/z* were scanned on ECNI full scan runs for PCBs. The identification was based on GC retention time and correct isotope ratio of reference standards. The quantification was performed in SIM mode using *m/z* 79, 81 and 161 for PBDE, MeO-BDEs and HDBPs, and *m/z* 386 for Cl_7-MBP by using internal standards (IS-1 and IS-2).

Results

A mass chromatogram (ECNI) in the SIM mode using *m/z* 79 for brominated compounds in the neutral fraction of a milk sample is shown in Fig. 1. Each congener was identified in the retention order, as Cl_7-MBP (12.30 min), 2'-MeO-BDE68 (17.54 min), 2,2'-diMeO-BB80 (17.73 min), Br_4Cl_2-DBP (17.81 min), 6-MeO-BDE47 (18.09 min), and 2',6-diMeO-BDE68 (19.27 min). Table 1 shows the concentrations (means and ranges) of these organohalogens in breast milk from four regions.

Cl_7-MBP levels in milk samples ranged from 0.08 to 1.68 ng/g lipid, with a mean of 0.46 ng/g lipid. There were no geographical differences in the levels. HDBPs were dominated by Br_4Cl_2-DBP as observed for the surveys of mammals.[10] In 8 of 65 samples, Br_3Cl_2-DBP and Br_3Cl_3-DBP were detected. The mean concentration of Br_4Cl_2-DBP was 1.36 ng/g lipid (range; 0.25-4.91 ng/g lipid). The levels were the highest in women from northern Japan (Miyagi, 1.79 ng/g lipid) and the lowest in women from southern Japan (Okinawa, 1.04 ng/g lipid). Methoxylated PBDEs were dominated by 2'-MeO-BDE68, followed by 6-MeO-BDE47, both of which have been found frequently in fish and mammals, were present at mean concentrations of 0.42 and 0.10 ng/g lipid, respectively. The concentrations of 2'-MeO-BDE68 were 3-fold higher in mothers from Okinawa (0.77 ng/g lipid) than in those from Wakayama (0.26 ng/g lipid). 2,2'-diMeO-BB80 and 2',6-diMeO-BDE68 were present at the range of 0.39 and 0.10 ng/g, respectively. The former was 3-fold higher in mothers from Kochi (0.64 ng/g) than in those from Miyagi (0.19 ng/g). The level of each natural compound was comaparable to those of authentic BDE47 (range, 0.13-3.10 ng/g lipid), one of the major PBDE pollutants, although one order of magnitude lower than PCB153 residue (4.7-61 ng/g lipid).

Discussion

In the present study, six organohalogen compounds, supposed to be of natural origin, were identified in breast milk samples from 65 individual women in the general population from four regions in Japan 2004. The result shows that the natural products are present at the comparable levels to PBDEs and the other organohalogens in fish from Japanese environment. Two recent studies[3,4] have described the current PBDE levels in human milk in Japan. PBDE levels in mother's milk used in this study were identical (major congener, BDE47: 0.13-3.10 ng/g

lipid, n=65) to the previous studies, supporting that the concentration range in Japanese breast milk are similar to those from mothers in Europe.

Halogenated bipyrroles have been reported earlier in the environment as natural organohalogen compounds[7]. This study shows that MBP levels are not as abundant as in human milk from the Faroe Islands (southeast of Iceland)[6], where the levels were estimated to be 12-230 ng/g lipid. High Cl_7-MBP levels in mother's milk from the Faroe Islands were thought to be due to the consumption of whale meat and blubber, which contained high levels of MBP. In our recent survey on fish and marine products commercially available in Japan, Cl_7-MBP was highly distributed in imported fish (e.g. bluefin tuna) or seafood from Australia. In this study, the highest levels of natural products were Br_4Cl_2-HDBPs, which have been found in wildlife from different regions as well as fish and seafood from Canada.[11] The present study indicates that HDBP residues may be more abundant in mothers in northern Japan, although the geographical and biological sources of HDBPs are still unclear. It should be noted that *in vitro* assay, HDBPs bind to the aryl hydrocarbon receptor (AhR) and induce CYP1A in chick embryo hepatocytes in the same manner as mono-*ortho* PCBs.[12] On the other hand, methoxylated PBDEs have been detected in algae, marine sponges and marine mammals all over the world.[6] In this study, the ratio of 2'-MeO-BDE68/6-MeO-BDE47 was about 4 (Table 1). The high ratio of 2'-MeO-BDE68 indicates the environmental distribution in the Pacific Ocean. 2'-MeO-BDE68 and 2',6-diMeO- BDE68 have been isolated in green alga *Cladophora fascicularis*[13], and marine sponge *Phyllospongia dendyi*[14], respectively. A major source of 6-MeO-PBD47 may also be marine products, although it is not excluded that 6-MeO-BDE47 is derived from metabolites of BDE47. 2,2'-diMeO-BB80 has been quantified first in whale products sold in Japan.[9] This component may be formed by bacterial O-methylation[15] of dihydroxy-BB80, which produced by a novel marine bacterium, *Pseudoalteromonas phenolica*.[16]

In conclusion, the present survey indicates that both halogenated bipyrrole derivatives and methoxylated organobromines are present at the sub-ppb levels in breast milk of Japan. The dietary source of these compounds is most probably marine fish or seafood in Japanese market. Although toxicological implications of these components is still unclear, it is essential that marine organisms including mammals and humans should be monitored for understanding of the geographical sources, biotransformation process and toxic impact of these natural products. Our survey of the concentrations of natural POPs in fish from the Pacific is in progress.

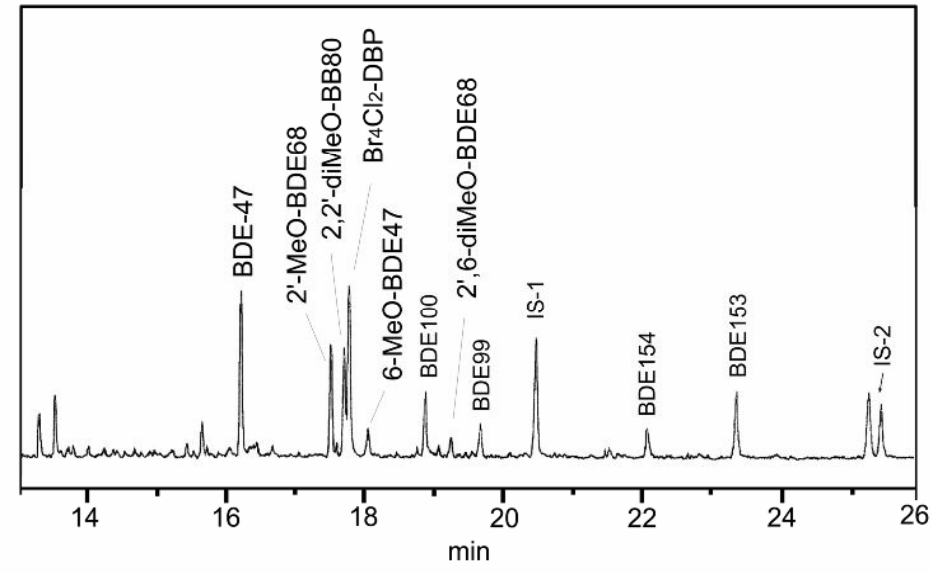

Fig 1. SIM profiles (*m/z* 79, ECNI) of brominated organohalogens in human milk from Japan

Table 1. Determination of brominated organohalogens in human milk from Japan

Lipophilic compounds	Mean concentration (ng/g lipid)					
	Total		Miyagi (n=15)	Wakayama (n=20)	Kochi (n=15)	Okinawa (n=15)
Cl_7-MBP (Q1)	0.46	(0.08-1.68)	0.38	0.50	0.66	0.36
Br_4Cl_2-DBP	1.36	(0.25-4.91)	1.79	1.55	1.09	1.04
2'-MeO-BDE68	0.42	(0.01-2.77)	0.29	0.26	0.48	0.77
6-MeO-BDE47	0.10	(0.01-0.65)	0.08	0.07	0.12	0.14
2',6-diMeO-BDE68	0.10	(0.01-0.69)	0.10	0.11	0.13	0.04
2,2'-diMeO-BB80	0.39	(0.11-1.45)	0.19	0.29	0.64	0.53
Total	2.81	(0.62-5.82)	2.48	2.37	2.97	2.78
BDE47	1.11	(0.13-3.10)	1.11	1.22	1.06	1.16
PCB153	16.9	(4.71-60.9)	16.7	19.6	19.4	11.9
ratio						
2'-MeO-BDE68 / 6-MeO-BDE47	4.2		3.6	3.7	4.0	5.5
6-MeO-BDE47 / BDE47	0.09		0.09	0.06	0.11	0.12
Br_4Cl_2-DBP / PCB153	0.08		0.11	0.08	0.06	0.09

Acknowledements
Human milk samples were provided by The Kyoto University Human Specimen Bank (Prof. Akio Koizumi), and MeO-PBDE standards were by Stockholm University (Dr. Göran Marsh), who are acknowledged. This work was mainly supported by a Grant-in-Aid for Scientific Research (17404006 K.H., 18602002 T.E.) from Japan Society for the Promotion of Science.

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