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Hexabromocyclododecane Levels in Foodstuff and Human Breast Milk in Osaka, Japan

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Abstract—Hexabromocyclododecane (HBCD) is a type of brominated flame retardant. As with other persistent organic contaminants, exposure to HBCD is a cause for concern during breast-feeding. The consumption of animal products is assumed to be one of the main routes for exposure to HBCD. The purpose of this study was to determine the level of dietary exposure to HBCD in Japanese women, and to investigate the HBCD levels in their breast milk.

We used liquid chromatography-tandem mass spectrometry (LC/MS/MS) to investigate the stereoisomer (α, β, and γ)-specific concentrations of HBCD in pooled milk lipid samples collected in 2007 and 2008. α-HBCD was dominant and was detected in all the samples, while γ-HBCD and β-HBCD were found in none of them. The α-HBCD concentrations ranged from 1.4 to 1.9 ng·g^{-1} lipid weight.

Next, we determined the dietary HBCD exposure levels on the basis of market basket study samples obtained in 2007. From among 14 food groups, HBCD was detected only in the fish group; the HBCD level was 6 ng·kg^{-1} body weight·day^{-1} (α, 42%; β, 1%; and γ, 57%).

Keywords: hexabromocyclododecane, flame retardant, human breast milk, food

INTRODUCTION

HBCD is primarily used as a protective additive in the polystyrene foam used in upholstery and building materials (de Wit, 2002) and the flameproofing of textiles. It has been used since the early 1980s and is now used extensively throughout the world. In Japan, more than 2000 metric tons of HBCD has been used in industry each year since 2000, and this figure increased to 2400 tons in 2004, an amount that is nearly 4 times higher than it was in 1986 (The Chemical Daily Co., 1987–2006). HBCD is lipophilic and has the potential for bioaccumulation (de Wit, 2002), and has been detected worldwide in various

Table 1. Milk sample information.

Sample ID	Year	*N**	Age range (avg.)	% Lipid range (avg.)
2007	2007	16	25–29 (27.4)	2.6–6.5 (4.2)
2008	2008	23	25–29 (27.2)	2.4–5.6 (3.7)
2007b	2007	33	30–40 (33.2)	1.1–7.2 (3.9)
2008b	2008	24	30–41 (33.8)	2.2–6.1 (3.7)

*Number of breast milk donors in pooled sample.

Table 2. Concentrations of HBCD isomers in human breast milk samples ($ng \cdot g^{-1}$ lipid weight).

Sample ID	α	β	γ	$\sum$HBCD
2007	1.4	<0.1	<0.2	1.4
2008	1.7	<0.1	<0.2	1.7
2007b	1.6	<0.1	<0.2	1.6
2008b	1.9	<0.1	<0.2	1.9

2007b–2008b: samples from women ≥30 years.

environmental matrices and aquatic/terrestrial animals. In human samples, HBCD was found in the breast milk of Norwegian women (Thomsen *et al.*, 2003, 2005), and stereoisomer-specific concentrations of HBCD have been reported in the breast milk of women in North America (Ryan *et al.*, 2006). HBCD was also detected in serum samples of workers whose occupations exposed them to HBCD at an industrial plant (Thomsen *et al.*, 2007), and in the wives of Swedish fishermen (Weiss *et al.*, 2006). Human exposure to HBCD takes place through multiple routes. For individuals who are not exposed to the compound in their occupations, the major intake route was considered to be from food and indoor air or dust (Remberger *et al.*, 2004; Covaci *et al.*, 2006). Remberger *et al.* (2004) studied HBCD concentrations in foodstuffs and reported that the concentration was highest in fish (48 $ng \cdot g^{-1}$ lipid weight), followed by egg yolks (9.4 $ng \cdot g^{-1}$ lipid weight), and chicken (6.5 $ng \cdot g^{-1}$ lipid weight). HBCD was also detected in fish oil supplements, with a maximum concentration of 67 $ng \cdot g^{-1}$ whole weight (the sum of α, β, and γ) found in sardine oil (Kakimoto *et al.*, 2008a). There have been several reports on the metabolic pathway, toxicology, and bioavailability of this compound. It is suspected that HBCD affects the thyroid hormone receptor-mediated gene expression and is a potential disruptor of endocrine functions (Yamada-Okabe *et al.*, 2005). Another study reported that HBCD induces cytochrome P450 activity in rats (Germer *et al.*, 2006). Monohydroxy metabolites were detected as initial reaction products of HBCD biotransformation by the cytochrome P450 system in an *in vitro* study of the hepatic microsomes of rats,

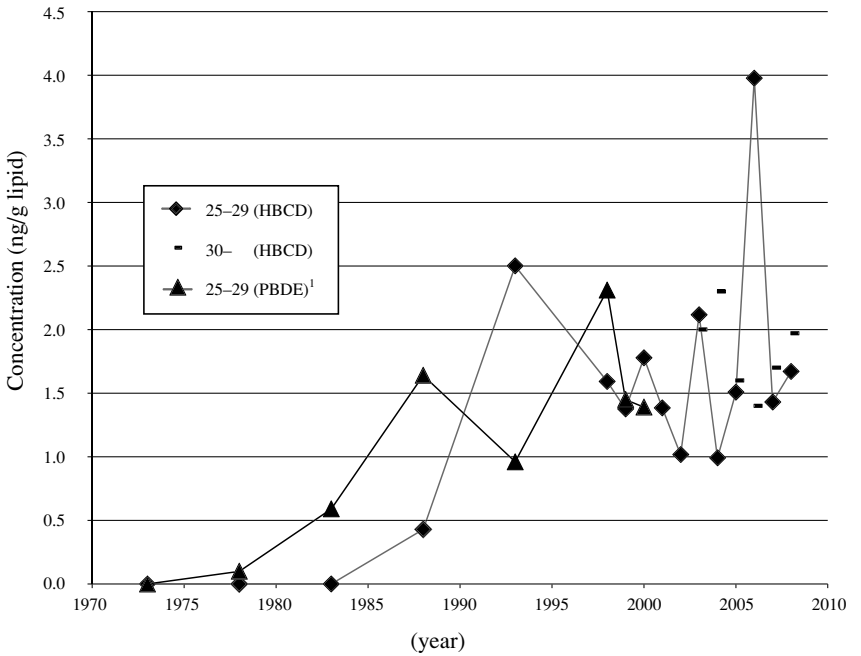

Fig. 1. Time trend in the concentrations of ∑HBCD (α, β, and γ stereoisomers) and ∑PBDEs in the breast milk of Japanese women. [1]∑13PBDEs: #28, #37, #75, #47, #66, #77, #100, #99, #85, #154, #153, #138, and #183 (Akutsu *et al.*, 2003).

and a significant decrease in β-HBCD and γ-HBCD was observed after incubation (Zegers *et al.*, 2005).

To our knowledge, no studies have been conducted on the level of HBCD in human samples from the Japanese population or in food products marketed in Japan. Furthermore, no time-trend data are available.

As is the case for other persistent organic contaminants, exposure to HBCD during breast-feeding is a cause for concern. The consumption of animal products is assumed to be one of the main routes of human exposure to HBCD. The purpose of this study was to determine the levels of HBCD exposure in human food products, and to investigate the HBCD levels in the breast milk of Japanese women.

We have already reported the time-trend data for HBCD in human breast milk samples from 1978 to 2006 (Kakimoto *et al.*, 2008b). In this article, we have added the data for 2007 and 2008. We have also determined dietary HBCD exposure levels on the basis of market basket study samples obtained in Osaka, Japan in 2007.

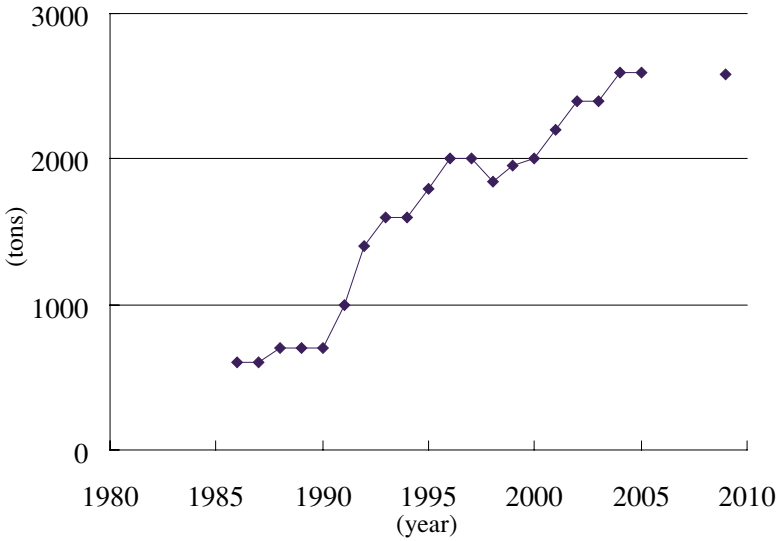

Fig. 2. Trend in the annual consumption of HBCD in Japan.

MATERIALS AND METHODS

Human milk samples

Human milk samples were collected in Osaka in 2007 and 2008. The human milk sample information is shown in Table 1.

Food samples

A total of 126 food samples were purchased from two supermarkets in Osaka in 2007. The market basket study samples were prepared on the basis of official food classification and consumption data obtained from the National Nutrition Survey. These food samples were cooked or prepared for consumption in a typical manner and were then blended to form 14 food group composites. These food groups were designated as groups I–XIV, as shown in Table 3.

Chemical analysis

The details of the analytical methods used for the human milk samples are described in reference (Kakimoto *et al*., 2008b). For the extraction of HBCD from the food products, we modified the guideline method that was formulated for the analysis of dioxin in food products by Japan's Ministry of Health, Labor and Welfare. For groups I, II, V, VI, VII, VIII and IX, acetone : *n*-hexane (1:1) 20 mL was used for extraction. For groups III, IV, X, XI and XIII, diethyl ether : *n*-hexane (1:2) 20 mL was used. For group XII, Lipid was extracted from samples using a mixture of potassium oxalate, ethanol, diethyl ether, and *n*-hexane. For

Table 3. Information of 14 food groups of the market basket study in Osaka, 2007.

Food group	Composition	No. of food variety	Lipid content (%)	Daily intake per capita (g/day)	HBCD concentration (ng/g wet weight)
I	Rice and rice products	2	0.2	341	N.D.
II	Grains, seeds, and tubers	15	1.5	174	N.D.
III	Sugar and confectioneries	6	9.4	35	N.D.
IV	Oils and fats	4	93	11	N.D.
V	Legumes and their products	6	6	58	N.D.
VI	Fruits	12	0.2	121	N.D.
VII	Brightly colored vegetables	13	0.3	93	N.D.
VIII	Other vegetables, mushrooms and seaweeds	13	0.3	184	N.D.
IX	Beverages	7	0	616	N.D.
X	Fish, shellfish, and their products	23	7.7	82	3.6 (α1.5, β0.03, γ2.1)
XI	Meat and eggs	8	18	121	N.D.
XII	Milk and dairy products	5	6.7	143	N.D.
XIII	Seasonings and other processed foods	11	8.8	93	N.D.
XIV	Dinking water	1	0	250	N.D.

Detection limit 0.007 ng/g.
Abbreviations: N.D., not detected.

group XIV, dichloromethane was used for extraction. Following preparation processes after lipid extraction were the same as the human breast milk samples. We used 10 g of each food group for the measurement of HBCD.

RESULTS AND DISCUSSION

Human milk

The levels of HBCD concentration in human breast milk are shown in Table 2. α-HBCD was dominant and was detected in all the samples (2007, 2008, 2007b, 2008b), while γ-HBCD and β-HBCD were found in none of them. The α-HBCD concentrations ranged from 1.4 to 1.9 ng·g^{-1} lipid weight.

As shown in Fig. 1, the time trend of HBCD concentrations in the breast milk samples appeared to be related to the time trend of the technical HBCD consumption levels in Japan (Fig. 2). In recent years, the level of HBCD has remained roughly constant. This result is assumed to be due to the recent flat trend of consumption of HBCD in Japan (2,577 tons in 2009; 84% for polystyrene foam, 15% for textiles, and 1% for other uses, Ministry of Economy, Trade and Industry of Japan). The time point of elevation for the total concentration of HBCDs appeared to be later than that of the elevation for the total concentration of polybrominated diphenyl ethers (Akutsu *et al.*, 2003) in breast milk samples collected in Osaka, Japan.

The estimated average level of HBCD intake by breast-feeding infants (aged 0 days to 3 months) was 7.5 ng·kg^{-1} body weight·day^{-1}.These values were derived using a mathematical formula (EU risk assessment report, 2007) used for breast milk samples collected from women aged 25–29 in 2007.

Market basket study

Among 14 food groups, HBCD was detected only in the fish group (Table 3), in which the HBCD level was 3.6 ng·g^{-1} wet weight (6 ng·kg^{-1} body weight·day^{-1}) and γ-HBCD was dominant (α, 42%; β, 1%; and γ, 57%). The elevated level of γ-HBCD in the fish food group indicates that the fish was recently exposed to the technical mixture. In the technical mixture, γ-HBCD was the most dominant isomer, followed by α-HBCD and β-HBCD, and γ-HBCD appeared to have a relatively shorter environmental and biological half-life than α-HBCD.

The HBCD levels in both breast milk and food products were much lower than the no-observed-adverse-effect-level (NOAEL) of 10.2 mg·kg^{-1} body weight·day^{-1} for HBCD (Ema *et al.*, 2008).

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