REAL SITUATION OF CONTAMINATION BY POLYBROMINATED DIPHENYL ETHERS AS FLAME RETARDANT IN MARKET FISH AND MOTHER MILK OF JAPAN

Souichi Ohta, Daisuke Ishizuka, Hajime Nishimura, Teruyuki Nakao, Osamu Aozasa, Yoshiko Shimidzu[1], Fumie Ochiai[1], Takafumi Kida[2] and Hideaki Miyata

Faculty of Pharmaceutical Sciences, Setsunan University, 45-1, Nagaotoge-cho, Hirakata, Osaka, 573-0101, Japan
[1]School of Nursing University of Shizuoka, 52-1 Yada, Shizuoka 422-8526, Japan
[2]Kanto Chemical Co., Inc., Marusan BLDG. 11-5, Nihonbashi Honcho 3-chome, Chuo-ku, Tokyo, 103-0023, Japan

Introduction

Many goods in modern life are equipped with flame-retardants for purpose of fire protection. Especially, polybrominated diphenyl ethers (PBDEs) are used in large quantities as flame-retardant additives for many applications such as television sets, computers, radios, textiles, new synthetic building materials and car etc. Therefore, there are large amount of PBDEs in environment, which was released from their manufacturing or operating process[1-3]. Further, when the products containing such flame-retardant were incinerated, it has estimated to be able to act as precursors for polybrominated dibenzo-p-dioxins and -furans. Consequently, we have usually intake these contaminants from food, air and water etc, and accumulated them in our body.

The PBDEs were first discovered in pike, eel and sea trout samples of Sweden in 1981[4]. Since then, many researchers have confirmed the PBDEs in the various samples of environment in Canada and European countries, detecting main isomers as tetra- and penta- BDEs[5-7]. In humans, it was first reported that there are high concentration of PBDEs in adipose tissue in 1990[8]. Similarly, many studies with human contamination by PBDEs have been also reported[9-10]. Therefore, it has been much concern about the adverse effect on the future health in the above countries, because they are extremely liphophilic and stable substances in vivo. However, little is known about the pollution of brominated flame-retardants in Japan[11].

In this paper, we have first established the new analytical methods for PBDEs by using the isotope dilution method, as same as the analytical method for dioxin analogues, and then investigated the levels of PBDEs contamination in fishes and mother milk of Japan. Finally, it has been considered the relationship between the intake of fishes and their concentrations in mother milk.

Materials and Methods

1) Samples

The fish samples were purchased from two markets in Hirakata city of Osaka prefecture. Five kinds of marine fishes (natural yellowtail, cultured young yellowtail, natural salmon, natural yellow-fin tuna and natural mackerel; 3 samples for each kind) and one kind of shellfish (natural

short-necked clam) were selected as marine foods, which Japanese usually preferred to eat. After cutting the muscle as edible part and liver from the above fishes, both samples were fully homogenized. Then, the samples of mother milk were collected from six primiparae at one month after delivery. The samples of fish homogenates and mother milk were used in this study. Further, after they were classified the samples of mother milk by the intake frequency of fishes as large amount of fish-consumer and small amount of fish-consumer, and we tried to compare the relationship.

2) Analytical method

In this study, in order to increase the accuracy of measurement, the isotope dilution methods for the analysis of PBDEs were firstly adopted using the standards of $^{13}C_{12}$-labelled BDE isomers (2,2',4,4'-TeBDE, 2,2', 4,4'5-PeBDE, 2,2',4,4'6-PeBDE, 2,2',4,4'5, 5'-HxBDE, 2,2',4,4'5,6'-HxBDE; Wellington laboratories). Similarly, unlabelled native standards (2,4,4'-TriBDE (#28), 2,2',4,4'-TeBDE (#47), 2,2',4,4'5-PeBDE (#99), 2,2',4,4'6-PeBDE (#100), 2,2',4,4'5,5'-HxBDE (#153), 2,2',4,4'5, 6'-HxBDE (#154); Wellington laboratories) were also used. Determination was performed by the method of relative calibration curves using the above both standards. Next, 20 g of this fish homogenates and 50ml of mother milk were used for the analysis of PBDEs; after addition of internal $^{13}C_{12}$-labelled BDE standards (each 2 ng) to the samples, each sample was saponified by 150ml of 1N KOH/EtOH containing 10% H_2O for 2hr with shaking. The PBDEs were partitioned and extracted with 120ml of n-hexane. After first elution of n-hexane (180 ml), the PBDEs in the extracts were cleaned up on a multi-layer column containing Na_2SO_4 (2.0 g), 10%(w/w) $AgNO_3$-silica (6.0 g), silica (0.9 g), 22%(w/w) H_2SO_4-silica (6.0 g), 44%(w/w) H_2SO_4-silica (4.5 g), silica (0.9 g), and 2%(w/w) KOH-silica (3.0 g), silica (0.9 g) with second eluent of n-hexane (200 ml). The elute was concentrated and purified by active carbon column with first eluent of 25% methylene chloride/n-hexane (200 ml). The purified extract was dissolved in 20 µl of n-nonane and analyzed for PBDEs in EI-SIM mode using a HP 5873 mass spectrometer connected to HP 6890 GC (HRGC-LRMS) equipped with an autosampler. It was also determined by use of HP6890 GC-JEOL JMS700 MS (HRGC-HRMS) at high-resolution condition over 5000, and the analytical data were compared. The extract was analyzed on a Supelco SPB-5 (30 m x 0.32 mm, 0.25 µm film thickness) with the rate of heating condition as follows; held for 2min at 120°C programmed to 215°C at 10°C /min, to 270°C at 3°C /min, and to 310°C at 10°C /min, finally held for 10min at 310°C. The concentrations of PBDE isomers were corrected with the recoveries of their respective internal standards. The lipid content of each sample was determined by the method of Haglund et al.[2] The above experiments were performed twice for each sample.

Results and discussion

As shown in Table 1, the accumulation levels of PBDEs in muscle and liver of five kinds of fishes and one kind of shellfish on the market of Japan were compared. With respect to the PBDE concentration in muscle, it was observed that total PBDE concentration was in a wide range of 21.5 to 1554 pg/g fresh weight, and the concentration becomes higher in the order, natural yellow-fin tuna (1.1% as lipid content in the muscle), natural short-necked clam (1.8%), natural salmon (1.6%), natural yellowtail (3.9%), natural mackerel (8.2 %) and cultured young yellowtail (3.5%) and. Young yellowtail or mackerel as higher concentration were captured by the cultured fishery or the coastal fishery, respectively, whereas yellow-fin tuna (1.1 %) as the lowest concentration was captured by pelagic fishery. Therefore, it was suggested that PBDE

concentrations observed in the fishes and shellfish were strongly influenced by their living marine environmental factors such as the PBDE level in the seawater and their intake amount of

Table 1. Comparison of the levels of PBDE concentration in muscle or liver of five kinds of fish and one kind of shellfish on the market of Japan

	yellowtail	young yellowtail		salmon	mackerel		yellowfin tuna	short-necked clam
	muscle	muscle	liver *	muscle	muscle	liver *	muscle	muscle
Compound	PBDEs concentration (pg/g fresh wt.)							
2,4,4'-TriBDE	53.3	79.6	132	39.5	46.1	62.9	3.29	10.8
2,2',4,4'-TeBDE	650	1000	1545	359	695	833	5.07	22.7
2,2',4,4',5-PeBDE	174	116	227	130	282	390	4.37	12.1
2,2',4,4',6-PeBDE	157	187	368	84.2	136	184	2.74	3.29
2,2',4,4',5,5'-HxBDE	42.9	35.5	145	32.5	37.4	70.2	2.01	3.35
2,2',4,4',5,6'-HxBDE	110	136	590	88.4	39.8	75.7	4.07	3.35
Total	1190	1554	3008 *	734	1236	1616 *	21.5	55.6

*The PBDEs analysis in livers were done for young yellowtail and mackerel

Table2. Comparison of PBDEs concentration (pg/g lipid base) in the mother milk collected from six primiparae at one month after delivery by the difference of intake frequency of fish and shellfish

	Large amount of fish-consumer				Small amount of fish-consumer	
	A	B	C	D	E	F
Compound	PBDEs concentration (pg/g lipid base)					
2,4,4'-TriBDE	185	157	105	265	125	83.6
2,2',4,4'-TeBDE	540	565	375	349	216	182
2,2',4,4',5-PeBDE	131	122	104	87.0	89.2	92.8
2,2',4,4',6-PeBDE	181	167	157	119	79.3	72.4
2,2',4,4',5,5'-HxBDE	399	386	494	315	238	194
2,2',4,4',5,6'-HxBDE	46.8	26.3	43.1	22.7	19.5	30.2
Total	**1480**	**1420**	**1280**	**1160**	**766**	**655**

A: (27 years old, The frequency of intake (F.I.); 3-4 day/week), B: (29 years old, F.I.; everyday), C: (33 years old, F.I.; everyday), D: (24 years old, F.I.; 5-6 day/week), E: (30 years old, F.I.; 1-2 day/week), F: (25 years old, F.I.; 1-2 day/week)

artificial feed, small fish or plankton etc., rather than by their respective lipid content. Then, when it was compared the accumulation level of PBDEs in muscle or liver of mackerel and young yellowtail, the PBDE concentrations in their livers were higher than that in their muscles. Irrespective of the similar level of contamination in muscle of both samples, on the other hand, the contamination in the liver of young yellowtail exhibited about two times for that of mackerel. It was estimated that the difference of accumulation level in both livers might be depend on the activities of the cytochrome P450 enzymes, which each kind of fish induce against various PBDE congeners[12]. Then, when the contribution of each congener for total PBDE concentration was compared, the 2,2',4,4'-TeBDE as main isomer was observed in all samples analyzed (Table1 & Fig.1). Further, except the above six isomers, 11 kinds of BDE congeners (from tri- to hexa-brominated congener) which was estimated from the pattern of their respective mass spectra, could be detected on the chromatogram of fish samples, showing over 700 pg/g fresh weight as total concentration (data not shown). However, in this study, we determined only six isomers that can completely identify based on the isotope dilution method, because one of our purposes was performed to determine each PBDE isomer under reliable analytical conditions.

Table 2 shows the comparison of PBDE concentration in the mother milk collected from six primiparae at one month after delivery by the difference of intake frequency of fish and shellfish as marine foods. Certainly, it was recognized that the average concentration (1335 pg/g lipid base) in the mother milk from large amount of fish-consumers was higher than that (711 pg/g lipid base) from small amount of fish-consumer. For all samples of mother milk, the abundant congeners were the 2,2',4,4'-TeBDE and 2,2',4,4'5,5'-HxBDE. Therefore, when it was compared to the composition ratio of PBDE congener in fish and mother milk, we considered that the 2,2',4,4'5,5'-HxBDE exhibits high accumulation potency in human body. From the results, it has much concerned about their adverse effect on health, because many Japanese usually preferred to eat the large amount of fish and selfish in their food life.

Hereafter, we plan to clarify the main sources and pathways for the PBDE contamination of human and environment in Japan.

References

1. Boer J., Wester P.G., H.J.C. Klamer, Lewis W.E. and Boon J.P. (1998) Nature, 394, 28.
2. Haglund P.S., ZooK D.S., Buser H.-R. and Hu J. (1997) Environ Sci Technol, 31, 3287.
3. Lindstrom G., Wingfors H., Dam M. and Bavel B. (1999) Arch Environ Contam Toxicol, 36, 355.
4. Andersson O. and Wartanien A. (1981) Chemosphere, 10, 775.5. Lindstrom G., Wingfors H., Dam M. and Bavel B. (1999) Arch Environ Contam Toxicol, 36, 355.
5. Luross J. Sergeant P.B. and Alaee M. (1999) Organohal Comp, 40, 9.
6. Sellstrom U., Jansson B., Kierkegoard A and Wit C. (1993) Chemosphere,
7. Asplund L., Athanasiadou M., Eriksson U., Sjodin A., Borjeson, Bergman A. (1997) Organohal Comp, 33, 355.
8. Remmers J.C., Breen J.J., Schwemberger J., Stanly J.S., Cramer P.H and Thornburg K.R. (1980) Orhanohal Comp, 14, 211.
9. Lindstrom G., Bavel B., Hardell L. and Liljegren G. (1997) Onc Rep, 4, 999.
10. Bergman A., Ostman C., Nybom R., Sjodin A., Carlsson H., Nilsson U. and Wachtmeister C.A. (1997) Organohal Comp, 33, 414.
11. Watanabe I., Kashimoto T., and Tatsukawa R. (1987) Chemosphere, 50, 2389.
12. Boon J.P., Meer J., Allchin C.R., Law R.J., Klungsoyr J., Leonords P.E.G., Spliid H. and Storr-Hansen E. (1998) Arch Environ Contam Toxicol, 33, 298.