DEVELOPMENT OF AN ANALYSIS METHOD FOR POLYBROMINATED DIPHENYL ETHERS AND THEIR LEVELS IN JAPANESE HUMAN MOTHER'S MILK

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Introduction

Polybrominated diphenyl ethers (PBDEs) have been widely used as excellent noncombustible materials in synthetic resins. Recently however, these materials have been identified as residual environmental pollutants which may possibly disturb the endocrine system and concern is growing that they may have a serious influence on the health of the fetus and of infants.

In Japan, in total, about 100,000 tons of PBDEs have been used from the 1980s. In spite of this, there has been almost no research on PBDEs as pollutants and the situation regarding possible health effects remains unclear. In the previous year, in a pioneering analysis of human somatic materials, we have developed a simple analysis method for PBDEs in fishery products and have analyzed the level of PBDEs in edible fish from the Seto Inland Sea[1]. This year, to elucidate the exposure situation of infants to PBDEs, we have carried out a highly-sensitive analysis on PBDEs isomers in mother's milk.

Materials and Methods

Samples

Ten samples of mother's milk (No. 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10) and one sample of serum (No. B-10) were collected in the nearby hospital of the Medical Department, Tokai University (named Tokai university Hospital in the following) in Kanagawa Prefecture, and three samples of mother's milk(No.11,12,13) collected in Okayama Prefecture in 1999 were used as the materials. The total fat in the materials was extracted and weighed in advance and then a portion of about 0.5g was used for the analysis.

As materials for the addition and recovery experiment, the fat extracted from the mother's milk collected in Osaka Prefecture and the plasma for fractionation, a product of the Red Cross Society of Japan have been used.

In addition, eight fat samples from mother's milk collected and frozen in Osaka Prefecture from 1973 to 2000 (from a stock of 19~35 samples taken per year) have also been analyzed.

Analytical method for PBDEs in human mother's milk

Analysis of PBDEs in human mother's milk was performed according to the procedure shown in Figure 1.

Results

We have investigated a high-sensitive analysis method using isotope dilution and gas chromatography (GC)/HRMS. To the fat extracted from mother's milk, or the plasma for fractionation, PBDEs in the form of 16 kinds of the native 3~7 brominated type and 5 kinds of the ^{13}C-labeled 3~6 brominated type were added and pretreated by alkaline decomposition at room temperature and by using a multiple layer silica gel column. The recovery of PBDEs (a revised value) after these pretreatments was about 80~110 % and the RSD value was below 10 %, showing a satisfactory result..The detection limitation for each isomer was set to be 0.1ng/ml in the detection solution (equivalent to 0.01 ppb in mother's milk) according to the results of control experiments.

Using this method, we have analyzed the ten samples collected in Tokai University Hospital and the three ones collected in Okayama Prefecture. We have found that 7 kinds of PBDEs (of the 3~7 brominated form) are detected in all the samples. In sample (No. 10) from Tokai University Hospital, a level of PBDEs two decades higher than in other samples was detected (Table 1). For further investigation, the serum (8.36 g) of this sample was also analyzed. Similarly, a high level of PBDEs was detected in the serum, indicating a high level of PBDEs pollution in the donor of this sample (135 ng/g fat). Since this was an especially high value, even compared to those reported abroad, we tried to specify the origin. We have inquired the professional career, diet, medicine in use and inhabitation environment of this donor with the cooperation of the doctor in charge, but have not found any specific cause for the high exposure to PBDEs. The health conditions of both the mother and the child were good.

In addition, we have analyzed the eight fat samples from the stock of Osaka Prefecture and investigated the change as a function of the year in the PBDEs concentration. The results showed that the level of PBDEs had increased significantly from 1973 to 1988 (ND~1.6 ppb) and afterwards changed in the range of about 1~2 ppb (Table 1).

BDE-209 is the main component of deca-BDE (the 10 brominated form, an original industrial material) which is widely used throughout the world in large quantities. The sensitivity and accuracy of electron ionization (EI)/HRMS was not high enough for its analysis and its accumulation was so low that so far there have been no reports on its detection at high level from somatic materials. Therefore this was excluded from the detection subjects of high-sensitive isomer analysis in this study. Nevertheless, in a preliminary analysis using negative chemical ionization (NCI)/quadrupole-based mass spectrometry, both BDE-209 and BDE-15 (4, 4'-DiBDE) added to the materials were recovered similarly to other PBDEs in the pretreatment process shown in Figure 1. Therefore we have analyzed the remaining samples and have found that peaks corresponding to BDE-209, BDE-15 and an unidentified 9, 8 brominated form are detected in several samples. However, all of these were at an amount from trace to several ppb/g fat and the accumulation of these PBDEs in the living body is supposed to be lower than those of 3~6 brominated ones.

Guosheng Chen et al.[2] have reported the relative EROD-inducing potency (REP) and the relative binding affinity (RBA) to AhR with those of tetrachlorodibenzo-p-dioxin (TCDD) in various cells. Using the highest RBA, we have evaluated the PBDE concentration in mother's milk. The results show that except for only one sample, the PBDE level in recent mother's milk was 0.2~2.7 pg-TEQ/g fat and less than 1/10 of the dioxin concentration.

Discussions

In Japan, the use of the low brominated form of PBDEs has been discontinued from the beginning of 1990s due to self-regulation by the companies involved. Recently, the use of low accumulative octa/deca-BDE noncombustible materials has also been on a decreasing trend. Ohta et al. at Setsunan

University have reported in a recent academic meeting a decreasing tendency of PBDEs pollution in the fish of Osaka Bay from the latter half of 1980s. Based on these findings, it is considered that the background concentration of PBDEs in mother's milk hereafter is not likely to increase significantly from the present level of several ppb in Japan. On the other hand, there are many points which are unclear regarding the outflow of PBDEs from reclaimed resin products and the behavior of PBDEs in environment, such as their movement, half-life and decomposition pathway etc. It is difficult to estimate at present how long the pollution will last. Therefore, it is desirable to continue investigating the pollution situation and to chase the transition of pollution in mother's milk in our country.

Acknowledgments

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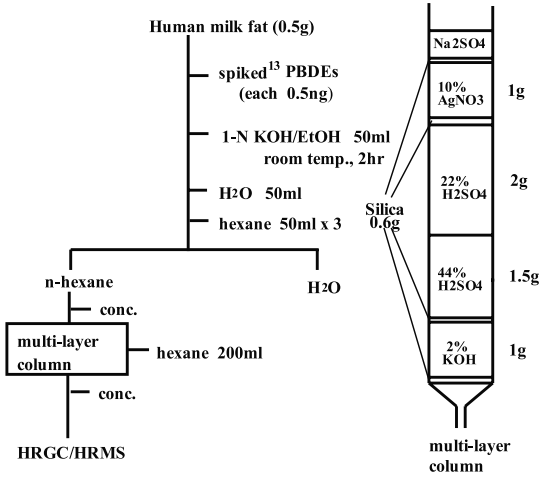
References

1. Kazuhiko Akutsu, Hirotaka Obana, Masahiro Okihashi, Mikiya Kitagawa, Hiroyuki Nakazawa, Yasuhiko Matsuki, Tsunehisa Makino, Hajime Oda, Shinjiro Hori, GC/MS analysis of polybrominated diphenyl ethers in fish collected from the Inland Sea of Seto, Japan, Chemosphere, 44, 1325-1333, 2001
2. Guosheng Chen, Alexandre D.Konstantinov, Brock G. Chittim, Elizabeth M. Joyce, Niels C. Bols, and Nigel J. Bunce, Synthesis of polybrominated ethers and Their capacity to induce CYP1A by the Ah receptoe mediated pathway, Environ. Sci. Technol. 35, 3749-3756, 2001



GC/MS Analysis

HRGC/HRMS: JEOL JMS-700 (M-station; R=10000)

The HRGC conditions were as follows; Column: J&W DB-1 15 m x 0.25 mm i.d., 0.25μm thickness, column temperature program: 140? (1 min), 140-180? at 10?/min, 180- 220? at 3?/min, 220-325? at 10?/min, 325? (5 min),, carrier gas: He, injection temperature: 275?; injection volume: 2μl (splitless). The MS conditions were as follows; detection mode: EI, ion source temperature: 260?, ionization current: 600μA, ionizing energy: 38eV, accelerating voltage: 8kV. The results were corrected for the recovery of $^{13}C_{12}$-labeled internal

Figure 1 analysis method for polybrominated diphenyl ethers

Table 1 Concentrations of PBDEs in human milk samples

Sample ID	Congener(ng/g fat)																	TEQ(pg/g fat)	
	#28	#37	#75	#71	#47	#66	#77	#100	#119	#99	#85	#154	#153	#138	#183	#190	ƒPBDEs	AhR	Hep G2
1	0.09	0	0	0	0.57	0.02	0	0.17	0	0.16	0.01	0.03	0.43	0	0.09	0	1.57	0.73	0.04
2	0.3	0.04	0	0	0.37	0	0	0.11	0	0.12	0	0.02	0.31	0	0.09	0	1.36	0.62	0.03
3	0.28	0.02	0	0	2.15	0.02	0	0.28	0	0.39	0.03	0.03	0.27	0	0.04	0	3.51	2.24	0.03
4	0.03	0	0	0	0.3	0	0	0.08	0	0.14	0	0.02	0.17	0	0.04	0	0.78	0.24	0.02
5	0.14	0.02	0	0	0.52	0.01	0	0.1	0	0.11	0	0.02	0.35	0	0.06	0	1.33	0.53	0.03
6	0.18	0.02	0	0	0.82	0.06	0	0.21	0	0.23	0	0.06	0.26	0	0.08	0	1.92	0.87	0.03
7	0.05	0	0	0	0.2	0	0	0.05	0	0.06	0	0.01	0.16	0	0.03	0	0.56	0.20	0.02
8	0.23	0.03	0	0	2.25	0.04	0	0.38	0	0.45	0.05	0.06	0.4	0	0.08	0	3.97	2.69	0.04
9	0.12	0	0	0	0.58	0	0	0.1	0	0.11	0	0.02	0.14	0	0.03	0	1.10	0.50	0.01
10	37.7	1.1	0.49	0	186	3.24	0.11	27.2	0	21.1	2.05	1.33	10.2	0.22	0.08	0	291.40	200.70	1.26
11	0.22	0	0	0	1	0.02	0	0.12	0	0.14	0	0.03	0.28	0	0.03	0	1.84	0.97	0.03
12	0.14	0.01	0	0	1.06	0.05	0	0.21	0	0.55	0.04	0.07	0.33	0.02	0.05	0	2.53	1.74	0.03
13	0.03	0	0	0	0.27	0	0	0.07	0	0.08	0	0.02	0.24	0	0.02	0	0.73	0.22	0.02
Year																			
1973	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0.00	0.00
1978	0.07	0	0	0	0.03	0	0	0	0	0	0	0	0	0	0	0	0.10	0.10	0.00
1983	0.15	0.01	0	0	0.26	0.02	0	0.02	0	0.04	0	0.01	0.07	0	0.01	0	0.59	0.38	0.01
1988	0.47	0.09	0	0	0.67	0.06	0	0.05	0	0.08	0	0.02	0.18	0	0.02	0	1.64	1.08	0.02
1993	0.17	0.02	0	0	0.32	0.02	0	0.07	0	0.06	0	0.03	0.21	0	0.06	0	0.96	0.46	0.02
1998	0.1	0.01	0	0	1.03	0.02	0	0.22	0	0.53	0.04	0.05	0.29	0	0.02	0	2.31	1.60	0.03
1999	0.11	0.01	0	0	0.62	0.02	0	0.18	0	0.16	0.01	0.03	0.29	0	0.02	0	1.45	0.76	0.03
2000	0.09	0.01	0	0	0.53	0.02	0	0.17	0	0.15	0.01	0.03	0.34	0	0.04	0	1.39	0.69	0.03

TEQ(AhR;rat) were calculated on RBA(EC50 of TCDD/EC50 of PBDE for Ah receptor binding)

TEQ(Hep G2;human hepatoma cell line) were calculated on RBA(EC50 of TCDD/EC50 of PBDE for EROD induction)