DEVELOPMENT OF ANALYSIS FOR POLYBROMINATED DIPHENYL ETHER IN SEAFOOD AND ACTUAL CONTAMINATION OF SEAFOOD

Shinjiro Hori[1], Kazuhiko Akutsu[1], Mikiya Kitagawa[1], Hajime Oda[1], Hiroyuki Nakazawa[2], Yasuhiko Matsuki[3], Tsunehisa Makino[4],
[1] Osaka Prefectural Institute of Public Health, 1-3-69, Nakamichi, Higashinari-ku, Osaka 537-0025,Japan
[2] Department of Analytical Chemistry, Hoshi University, 2-4-41, Ebara, Shinagawa-ku, Tokyo 142-8501, Japan
[3] Food & Drug Safety Center, ,Hatano Research Institute, 729-5,Ochiai, Hadano, Kanagawa 257-8523, Japan
[4] Department of Obstetries and Gynecology, Tokai University, Bouseidai, Isehara, Kanagawa 259-1193, Japan

Introduction

Polybrominated diphenyl ether (PBDE) has been widely used as a flame retarder of synthetic resins in various products such as containers for electronic apparatuses[1]. However, PBDE has also been considered as a contaminant to the environment[2]. In addition, based on its chemical structure and biological experiments, it is concerned that PBDE may have endocrine disrupting effects[3,4]. In particular, the concentration of PBDE in breast milk has been reported to be on the rise recently in Northern Europe[5], causing a concern for bad effects on infants.

In Japan, tens of thousands of tons of PBDE have been consumed since the 1980s. However, there have been very few reports on concentrations of PBDE in the environment or in biological samples, and contamination by PBDE has not been investigated properly. Thus, prior to the risk assessment of PBDE, contamination by PBDE in Japan needs to be investigated. In this study, we developed a routine analysis for PBDE in fish and investigated concentrations of PBDE in fish.

Material and Methods

Samples: The samples used in this study were horse mackerels, sea eels, sea basses, yellowtails, flounders, gray mullets, and red sea breams caught in the Inland Sea of Japan in October-December in 1998.

Reagents: The reagents used in this study were 3,4,4'-TrBDE (BDE-37), 2,2',4,4'-, 2,3',4,4'-, 2,3',4',6-, 2,4,4',6-, 3,3',4,4'-TeBDE (BDE-47, 66, 71, 75, 77), 2,2',3,4,4'-, 2,2',4,4',5-, 2,3',4,4',6-PeBDE (BDE-85, 99, 119), 2,2',4,4',5,5',- HxBDE (BDE-153) and 2,3,3',4,4',5,6-HpBDE (BDE-190) (products of Cambridge Isotope Laboratories); 2,4,4'-TrBDE (BDE-28), 2,2',4,4',6-PeBDE (BDE-100), and 2,2',3,4,4',5'-, 2,2',4,4',5,6'- HxBDE (BDE-138, 154) (products of Wellington Laboratories); 4,4'-diBDE (BDE-28) (product of Tokyo Chemical Industry Co., Ltd.); and DBDE (BDE-209) (product of Wako Pure Chemical Industries, Ltd.).

GPC (Gel permeation chromatography) conditions: An AS-2000 (product of abc laboratories) was used as GPC equipment. A CLN pak EV-G (100 mm × 20 mmϕ, product of Shodex) was used as a pre-column, and a CLN pak EV-2000 (300 mm × 20 mmϕ, product of Shodex) as a GPC column. Acetone/ cyclohexane (3:7) was used as the GPC mobile phase (flow rate: 5 ml/min). The time program was set to be dump 15 min, collect 13 min, and wash 12 min (total 40 min).

GC/MS with negative chemical ionization: An Auto Mas 120M (JOEL,Tokyo) was connected to a Hewlett-Packard GC 5890. GC condition : column, DB-1 (J&W,California) 0.25mm i.d. x

15m with film thickness of 0.25 μm; column temperature, 140•C (2min), 140-180•C(10•C/min), 180-220•C(3•C/min), 220-325•C(10•C/min), 325•C(5min); carrier gas, He; column pressure, 6 psi; injection temperature, 270•C; injection volume, 2μl; injection mode, splitless. MS conditions; ionization voltage, 70V; filament current, 305μA; detector voltage, -0.8kV; scan time, 100 msec for SIM mode; ion source temperature, 180•C; transfer line temperature, 250•C; chemical ionization gas, isobutane(purity 99.99%); vacuum, 3.5×10^{-1} Torr.

Results and Discussion

In the analyses for PBDE in biological samples that have been reported by now, concentrated sulfuric acid treatments have been employed, or dichloromethane, which is highly toxic, has been used in the extraction and clean-up procedure, making these analyses unsatisfactory as a routine analysis. Accordingly, we aimed to develop a simple and quick pretreatment using gel permeation chromatography (GPC), which is easily automated, and a disposable mini-column. It was found that by employing the extraction and clean-up procedure shown in Figure 1., obstacles to the GC analysis such as fat were efficiently removed.

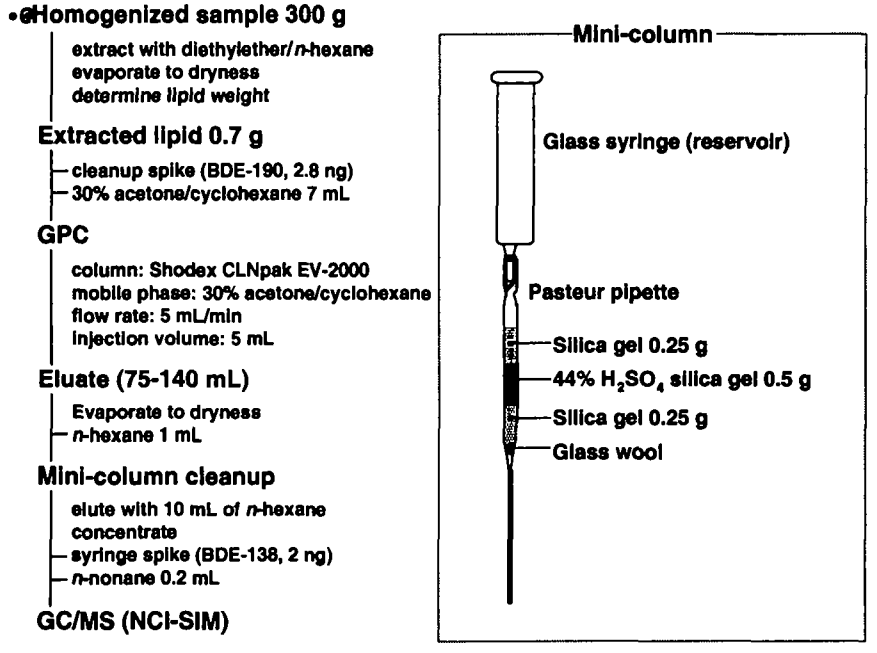

Figure 1. Analytical procedure for PBDEs in Fish

The negative chemical ionization mode GC/MS; NCI-GC/MS, which is considered to be capable of a highly sensitive analysis of organic halogens, was employed as the detection method. It was found that the mode was about ten times as sensitive as EI-GC/MS in analyzing SIM, and that no interference peaks serving as obstacles to quantitative analyses were observed in analyzing actual samples.

In contrast, HRGC/HRMS (E I mode) was found to be capable of a highly sensitive detection, but was considered to be unsuitable for a simple analysis because it is costly and its maintenance is troublesome. Also, in analyzing actual samples having different matrixes, the results by NCI-

GC/MS were almost identical to those by GC/HRMS. Thus, it was concluded that NCI-GC/MS is a simple and useful analysis for PBDE in biological samples.

By using this newly developed analysis, addition and recovery tests and analyses of PBDE in 25 fish samples were performed. Figure 2 shows a typical chromatogram, and Table 1 shows the

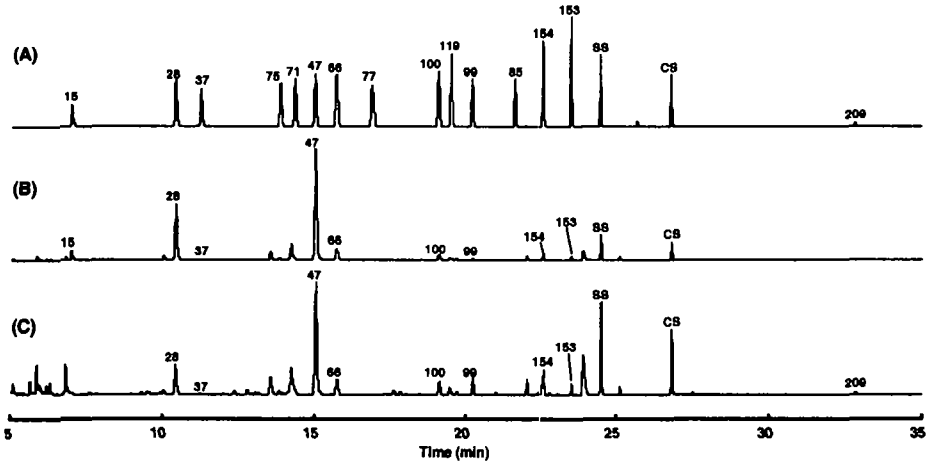

Figure 2. Typical SIM chromatograms of bromide ion isotopes (m/z=79 and 81)
(A) standard solution (each 10ng/ml), (B) grey mullet (sample No. 9),
(C) bastard halibut (sample No. 1)

results of analyses of the 25 samples.

The recovery fell in the range of 70-120% (RSD<10%) when each PBDE was added to fish oil to make the concentration 4 ng/g (n=4).

Among the PBDEs to be measured, BDE-28, 47, 66, 99, 100, 153, 154 were detected from all the analyzed samples. Among these, BDE-47 was detected in large amounts in all the samples. Among the fishes, grey mullets and yellowtails contained PBDEs in high concentration, but there was a clear difference between the PBDE congener compositions of these two types of fish. The PBDE concentrations found in grey mullets were TeBDE (BDE-47 + 66) > Tr BDE (BDE-28) > PeBDE (BDE-99 + 100), HxBDE (BDE-153 + 154), while those found in yellowtails were TeBDE > PeBDE > HxBDE > TrBDE. Also, BDE-15 was detected in grey mullets but not in yellowtails. Based on these results, yellowtails are considered to accumulate relatively highly brominated PBDEs in their body compared with grey mullets.

The concentrations of TeBDE were found to be similar to those reported by Watanabe et al[6]. in the 1980s, but lower than those reported abroad by one digit or two.

Several unknown peaks were detected from the samples, which were supposed to be PBDE. These peaks still remain to be identified and quantified.

In Japan, no TeBDE has been in use since 1991, when the industry banned its use voluntarily. However, fish caught in 1998 were found to contain BDE-47 (2,2',4,4'-TeBDE) in relatively high concentration. It is not known whether the contamination was caused by TeBDE that had been discharged into the environment in the past and that still remained in the environment or it was caused by the ongoing contamination resulting from effluence from resin products buried in landfills or from debromination of highly brominated diphenyl ether. The contamination needs to be continuously monitored.

Tetrabromobisphenol A, which is a reactive bromine flame retarder, was not detected from the fish samples in this study (detection limit: 0.05ng/g).

Table1. Levels (pg/g wet weight) of PBDE in Fish samples

	Concentration (pg/g ww, mean (range))								
Compound	Bastard halibut (N*1, 2*2)	Bastard halibut (C, 3)	Conger eel (N, 3)	Grey mullet (N, 2)	Horse mackerel (N, 3)	Japanese sea bass (N, 3)	Red sea bream (N, 3)	Red sea bream (C, 3)	Yellowtail (C, 3)
BDE-15	NC*3 (<5)	NC (<2)	NC (<3)	180 (74-290)	NC (<5)	NC (<1)	NC (<1)	NC (<6)	NC (<8)
BDE-28	19 (15-23)	48 (36-56)	34 (27-41)	760 (500-1000)	47 (28-65)	57 (38-80)	29 (19-44)	61 (30-87)	95 (66-140)
BDE-37	1.2 (0.69-1.7)	2.0 (1.2-3.4)	NC (<2)	12 (6.7-17)	NC (<3)	NC (<0.5)	NC (<0.6)	NC (<3)	10 (8.0-13)
BDE-47	65 (38-91)	150 (98-200)	88 (67-120)	2100 (1800-2300)	180 (120-240)	140 (91-190)	58 (53-64)	340 (240-470)	1200 (800-1900)
BDE-66	8.5 (4.7-12)	21 (11-36)	6.5 (4.3-10)	150 (110-190)	16 (6.9-25)	16 (12-22)	6.1 (3.0-8.0)	21 (18-27)	100 (81-120)
BDE-71	NC (<0.2)	NC (<1)	NC (<2)	NC (<1)	NC (<3)	NC (<0.5)	NC (<0.6)	NC (<3)	NC (<4)
BDE-75	NC (<0.2)	NC (<1)	NC (<2)	NC (<1)	NC (<3)	NC (<0.5)	NC (<0.6)	NC (<3)	NC (<4)
BDE-77	NC (<0.2)	NC (<1)	NC (<2)	NC (<1)	NC (<3)	NC (<0.5)	NC (<0.6)	NC (<3)	NC (<4)
BDE-85	NC (<0.2)	NC (<1)	NC (<2)	NC (<1)	NC (<3)	NC (<0.5)	NC (<0.6)	NC (<3)	NC (<4)
BDE-99	10 (5.7-14)	23 (12-40)	9.0 (6.3-11)	32 (24-41)	8.1 (1.4-19)	13 (9.5-14)	5.5 (2.5-7.5)	17 (10-23)	290 (120-420)
BDE-100	7.2 (4.4-10)	15 (8.6-21)	8.6 (6.4-13)	110 (87-140)	17 (8.8-29)	16 (11-19)	11 (9.9-12)	50 (42-60)	220 (140-370)
BDE-119	NC (<0.2)	NC (<1)	NC (<2)	NC (<1-1.8)	NC (<3)	NC (<0.5)	NC (<0.6-0.63)	NC (<2-3.3)	NC (<4)
BDE-153	3.3 (2.1-4.5)	4.6 (2.2-8.4)	7.7 (5.4-10)	91 (35-150)	9.1 (3.0-19)	8.3 (5.9-9.7)	1.3 (0.66-2.1)	2.0 (0.74-3.1)	46 (29-56)
BDE-154	6.0 (4.0-8.0)	11 (5.9-16)	15 (12-21)	87 (41-130)	17 (6.5-30)	13 (9.4-16)	12 (8.0-17)	38 (30-48)	120 (85-160)
BDE-209	18 (15-22)	NC (<10)	NC (<20-29)	NC (<10-13)	NC (<30-47)	12 (8.7-17)	NC (<10-20)	NC (<30)	NC (<30-48)

*1 native (N) or cultured (C) *2 number of samples analyzed *3 NC=not calculated

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