ANALYSIS OF PBDEs IN SEAL BLUBBER AND HUMAN BREAST ADIPOSE TISSUE SAMPLES

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Introduction

Brominated flame retardants are used extensively in modern life. The most frequently used are the polybrominated biphenyls, tetrabromobisphenol-A, and the polybrominated diphenyl ethers (PBDEs). Due to their high lipophilicity and persistence, PBDEs are expected to bioaccumulate. A substantial part of these compounds will eventually reach the marine environment and ultimately the human body. Residual levels of PBDEs have been found in sewage sludge[1,2], sediment[3], biota[4] and human tissues[5,6]. A continuous increase in the levels of PBDEs was shown in human milk samples[7]. Most of the studies on levels of PBDEs have been focusing on Europe, especially northern Europe. Very few data are available about levels of PBDEs in the USA. To explore the levels of PBDEs in biota and humans in California, harbor seal (*phoca vitulina*) blubber and human adipose tissue samples were analyzed. An electron capture, negative chemical ionization GC/MS method was developed for the determination of the PBDEs.

Materials and Methods

A random subset of breast adipose tissue samples was selected for PBDE analysis. The breast adipose samples were collected in the late 1990s from Northern California women as part of a case-control study on organochlorines and breast cancer. Harbor seal blubber samples were collected between 1989–1998, from beach-cast harbor seals found along the San Francisco Bay shoreline. The seals are being studied for their organochlorine body burdens as indicators of pollution in the Bay.

Harbor seal blubber and human adipose tissue samples were processed in a similar way, with minor exceptions. Harbor seal blubber samples (1-3 g) were homogenized and extracted in 1:1 hexane : methylene chloride, shaken and centrifuged to separate phases. The extract was spiked with all 17 $^{13}C_{12}$-labelled 2,3,7,8-substituted PCDD/PCDFs, PCBs 77, 126, 169, 28, 52, 47, 101, 105, 118, 153, 180, 194, and 209; $^{13}C_6$ β-HCH, $^{13}C_4$ dieldrin, $^{13}C_8$ mirex, $^{13}C_6$ HCB, $^{13}C_{12}$ p, p'-DDT, $^{13}C_{12}$ p, p'-DDE and $^{13}C_{12}$ -PBDE 77. Nine tenths of the extract was analyzed for polychlorinated dibenzodioxins and furans, and one tenth of the extract was cleaned up for PBDE, pesticides and PCBs. The human adipose tissue samples originally were not spiked with $^{13}C_{12}$-PBDE 77 and PBDE quantitations were based on $^{13}C_{12}$ PCB 180. After obtaining the $^{13}C_{12}$ -PBDE 77 standard, all subsequent samples were spiked with it. In this abstract we concentrate on those early samples of human tissues. The fat in the sample was removed with gel permeation and Florisil chromatography in a single automated system (Fluid Management Systems). Theanalytes were concentrated in the presence of 4 ng of $^{13}C_{12}$ PCB 128 and 178 and $^{13}C_6$ -α-HCH (recovery standards) to 10 µl for GC/ECNI analysis. The analysis was carried out using a Finnigan - 4510

GC/MS system. Samples were introduced through a splitless injector connected to a 60 m x 0.25 mm, 0.25 μm film thickness, DB - 5ms column with helium as the carrier gas. Research grade methane was used as the reagent gas. The MS was operated in the electron capture, negative chemical ionization, multiple ion monitoring mode. The ion source pressure was held at 0.6 Torr and ion source temperature was 150°C. The electron energy was typically 70 eV and the electron emission current was kept at 0.3 mA. The bromide ion (m/z 79) of PBDEs was chosen as the quantitation ion.

Results and Discussion

1) PBDE in human breast adipose tissue: Table 1 lists the lipid concentration of PBDEs in some of the human tissue samples. At the beginning of the study, for the determination of PBDE in the human tissue samples, ^{13}C-labeled PCB 180 was used as an internal standard, due to its structural similarity and availability at the time when samples were processed. Tetra- to hexa- PBDEs were measured in all breast adipose tissue samples. Among them, PBDE 47 (tetraPBDE) was the highest. The levels of PBDEs in human adipose tissue examined were in the same order of magnitude as reported for Swedish and Spanish human tissue samples[8, 9]. The levels of hexa-PBDE in the human adipose tissue samples examined were comparable with previously estimated levels in the USA[6]. While these are very limited and preliminary data, it appears reasonable to say that the background level of PBDEs in the general population is in the low ppb range (less than 100 ppb).

Table 1. Concentration of PBDEs in human adipose tissue samples (ng/g lipid)

Sample No.	% Lipid	PBDE 47	PBDE 99	PBDE 153
1	44.6	23	7.3	2.3
2	50.8	11	3.6	1.6
3	85.6	7.0	3.1	1.5
4	89	28	6.6	2.4
5	84.5	20	4.1	3.2
Mean	70.9	18	4.9	2.2
SD	21.3	8.6	1.9	0.69

2) PBDEs in harbor seal blubber: Due to their position at the top of the aquatic food chain and their rather long life-spans, marine mammals are an important tool to monitor long-term effects of pollution on the marine environment worldwide. They can also be used as global pollution indicators and can be considered as model systems for low-dose, long-term effects of environmental pollution.

Table 2 shows the lipid content and lipid concentration of PBDEs in 11 harbor seal samples. The concentration of PBDE 47 in harbor seals ranged from 50 ppb to 7 ppm. PBDE 99 and 153 were found in seal blubber at a lower concentration than that of PBDE 47. For the determination of PBDE in seal blubber, 13C-labeled PBDE 77 was used as an internal standard.

Contrary to the data on human adipose tissue, there are more reports about the level of PBDEs in seals. It should be noted that the published reports refer to different subspecies from different

geographic locations, and therefore, care should be taken when data are compared and interpreted. Female ringed seals collected in 1981 from Svalbard contained ΣPBDE levels of 40-51 ng/g[10]. Baltic Sea harbor seals contained 90 ng/g and harbor seals from the North Sea contained 10 ng/g PBDEs. Female gray seals from the Baltic Sea collected in 1979 - 1985 contained 730 ng/g PBDEs. Blubber from Baltic gray and ringed seals collected between 1981 and 1988 contained 419 and 350 ng/g, respectively. Harbor seals from the Dutch coast contained 605 - 6010 ng/g PBDEs[11].

The concentration of PBDEs in seal blubber in this study provides the first information on PBDEs in the San Francisco Bay. The level of PBDEs in the harbor seals can be seen as an indicator of environment pollution by flame retardants in fish eating marine mammals in the San Francisco Bay Area[12].

Table 2. Lipid content and concentration of PBDEs in harbor seal samples (ng/g, lipid)

Sample No.	% Lipid	PBDE 47	PBDE 99	PBDE 153
1	66.9	277	86	11
2	73.5	349	151	35
3	96.9	331	51	16
4	95.8	308	149	42
5	47.2	2343	172	160
6	98.6	46	17	4
7	72.4	87	24	7
8	74.4	299	96	26
9	94.3	956	102	60
10	39.6	692	26	31
11	27.9	6682	303	155
Mean	71.6	1124	107	49.7
SD	24	1952	85	55

The major congeners found in human adipose tissue and harbor seal blubber are the same: PBDE 47, 99, and 153. This may suggest that these three congeners are more easily bio-accumulated than the others. Examination of their molecular structure reveals the absence of meta- or para-vicinal hydrogen, believed to facilitate PCB metabolism.

Conclusions:

GC/MS in ECNI mode provided a very sensitive tool for monitoring trace levels of PBDEs in biological matrices. PBDEs were found both in human adipose tissue and seal blubber samples. The levels of PBDEs in the samples were in the same order of magnitude as those reported in other studies. The dominance of PBDE 47 (tetra-) over PBDEs 99 (penta-) and 153 (hexa-) may indicate that tetrabrominated biphenyl ethers bioaccumulate more than the higher brominated congeners.

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