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Determination and occurrence of polybrominated diphenyl ethers in maternal adipose tissue from inhabitants of Singapore

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Abstract

Polybrominated diphenyl ethers (PBDEs), as a specific group of brominated flame retardants (BFR), are used in a variety of consumer products including electronics and household furnishings. In recent years, a marked increase in the levels of PBDEs in human biological tissues and fluids, especially breast milk, has been reported in several countries. However, few data are available from countries in the Asia-pacific region, including Singapore. This study presents a validated method procedure and the first available data of the concentrations of PBDE congeners: PBDE-47 (2,2,4,4-Tetrabromodiphenyl ether), PBDE-99 (2,2′,4,4′,5-Pentabromodiphenyl ether), PBDE-100 (2,2′,4,4′,6-Pentabromodiphenyl ether), PBDE-153 (2,2′,4,4′,5,5′-Hexabromodiphenyl ether), PBDE-154 (2,2′,4,4′,5,6′-Hexabromodiphenyl ether) in maternal adipose tissue collected from inhabitants of Singapore. Microwave-assisted extraction (MAE) of PBDEs spiked adipose tissues coupled with GC–MS analysis achieved comparable recoveries to a conventional Soxhlet Extraction (SE) procedure of between 70 and 130%. MAE also yielded comparable precision data (variance less than 13%) relative to the SE procedure. Spiked Carbon-13 PBDE congeners were also used as surrogates for MAE quality assurance and confirmed the efficiency of the procedure. PBDE congeners were detected in all of 16 maternal adipose tissues collected in Singapore, where levels were comparable to available data from Belgium.

Keywords: Flame retardants; Polybrominated diphenyl ethers; Soxhlet extraction; Microwave-assisted extraction; Adipose tissue; Women; Singapore

1. Introduction

Polybrominated diphenyl ethers (PBDEs) have been extensively used as flame retardants in the last few decades [1]. PBDEs are blended with polymers used in electrical and electronic equipment, and in other plastic goods, coatings, cables, construction materials, and textiles. PBDEs are persistent, toxic, and bioaccumulative chemicals of anthropogenic origin, thus presenting a potential threat to wildlife and human health [2]. High PBDE levels have also been reported in a range of environmental media and biota including fish, treated sewage sludge and even household dust [3]. Long-term risks for human health exist because the chemicals are highly lipophilic and can persist in the human body for up to 30 years [3].

Asia accounted for 50, 40, and 3% of the estimated world market demand [4] for Deca, Octa, and Penta-BDEs in 2001, respectively. The data in Table 1 indicate that the population in Asia was an important consumer of PBDEs.

To date, PBDEs data for human tissue are mainly derived from Europe, North America, and the Arctic. For Asia, data from Japan has been reported to evaluate human exposure to the PBDEs [5]. Median and range of concentrations of seven PBDE congeners -28, -47, -100, -99, -154, -153, -183 from Japanese human adipose tissue was 1.288 and 0.466–2.753 ng/g [6]. Singapore is an industrially developed and highly urbanized country in Southeast Asia, with a population of four million people within a confined land area of approximately 700 km^2. The country is home to the world's third largest petroleum refinery and has extensive pharma-

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Table 1
Estimated world market demand for PBDEs in 2001 given in metric tons [4]

	Asia	Europe	Americas	Rest of the world	Total
Penta-BDE	150	150	7100	100	7500
Octa-BDE	1500	610	1500	180	3790
Deca-BDE	23,000	7600	24,500	1050	56,100

ceutical and electronics manufacturing industries. Data on the extent of PBDE contamination are limited, although recent studies have reported the presence of flame retardants in green mussels in the coastal marine environment [7].

In this study, the concentration level of five PBDE congeners were determined in maternal adipose tissue collected from pregnant women undergoing caesarian section at the National University Hospital in Singapore from August 2003 to August 2004. Here, we report on the development of a quality assured method for the accurate quantification of PBDEs in human adipose tissue using microwave-assisted extraction (MAE) in conjunction with GC–MS analysis. Analytical data on the concentrations of PBDE congeners in human adipose tissue are also presented and compared to available data for other countries.

2. Materials and methods

2.1. Chemicals

A PBDE standard containing PBDE congeners -47, -99, -100, -153 and -154 was obtained from Accustandard (New Haven, CT, USA). Five ^{13}C PBDE congeners -47, -99, -100, -118 and -153 for use as surrogate standards, as well as an internal standard of ^{13}C PCB 208 were obtained from Cambridge Isotope Laboratories (Andover, USA). Ultra pure silica gel (60 Å) was purchased from Silicycle Inc. (Canada), and sulfuric acid (95–97%) for impregnating silica gel was from Fluka (Switzerland). The silica gel and anhydrous sodium sulfate (Granulated, ACS, 99+%, Switzerland) were dried at 450 °C prior to use. All organic solvents used were of pesticide residue analytical grade (Fisher Scientific, Singapore). Water was deionized and purified using a Milli-Q cartridge system (Millipore, MA, USA).

2.2. Sample collection and preparation

Approximately 10 g of abdomen adipose tissues were procured via biopsy from 16 volunteer expectant mothers admitted to the National University Hospital, Singapore for caesarian section delivery. All adipose samples were placed in glass bottles and stored at minus 20 °C prior to analysis. The volunteers ranged in age from 22 to 41 years. All women were in a healthy condition without any complications pertaining to their pregnancy. The study was approved by the Institutional Ethics Review Board, Singapore and informed written consents were obtained from the women antenatally.

2.3. Soxhlet extraction (SE) procedure

An empty cellulose Soxhlet thimble (Winzer, Germany) was refluxed with 150 mL of *n*-hexane–dichloromethane (DCM) (1:1, v:v) for 6 h in the Soxhlet extraction equipment to ensure decontamination prior to sample extraction. Approximately 0.5 g adipose tissue sample and 3 g sodium sulfate were placed into this cellulose Soxhlet thimble and spiked with the surrogate standard. The sample was then extracted with 150 mL of *n*-hexane–DCM (1:1, v:v) for 6 h. The extracts were concentrated to 5 mL using a rotary evaporator (Heidoph, Germany) and then purified by the clean-up procedure described below (Section 2.5). An aliquot of extract was taken for lipid determination after SE (Section 2.7).

2.4. Microwave-assisted extraction procedure

Approximately 0.5 g of adipose tissue was homogenized at 6000 rpm in an Ultra-turrax homogenizer (Ika, Janke and Kunkel, Germany). The sample was spiked with the surrogate standard, and then extracted with 25 ml of *n*-hexane–DCM (1:1, v/v) in the presence of 3 g sodium sulfate using a Mars X (CEM, Matthews, NC, USA) microwave oven. The oven was programmed for a temperature increase to 115 °C over a 10 min period, which was maintained for 15 min [8]. The subsequent sample clean-up procedure is described in Section 2.5. An aliquot (2 mL) of extract was taken for lipid determination after MAE (Section 2.7).

2.5. Clean-up procedure

Lipids in the extracts derived from both the SE and MAE procedures were removed using a 20 g acidified silica gel (silica gel/H_2SO_4; w/w, 2:1) glass column (25 cm length, 15 mm i.d., Fischer & Porter Co., USA); and the constituents of interest were then eluted with 100 ml of *n*-hexane and subsequently with 50 mL of *n*-hexane–DCM (2:3, v/v). The extracts were then concentrated to around 500 μL using a rotary evaporator. The final volume of the extracts was adjusted to 25 μL in dodecane (99+%, Aldrich, Germany) after addition of the internal standard substance (Carbon-13 unlabeled PCB 208) using a Reacti-Vap Evaporator (Pierce, Rockford, IL, USA) under a gentle flow of nitrogen.

2.6. GC–MS conditions

The identification and quantification of all analytes was performed using a Shimadzu QP-2010 (Shimadzu Asia-Pacific, Singapore) gas chromatograph coupled with a quadrupole mass spectrometer. Analytes were separated on a DB-5ms (J&W Scientific, USA) capillary column (30 m length, 0.25 mm i.d., 0.25 μm film thickness) with a helium flow of 20 cm/min. The spectrometer was operated in electron impact ionization (EI) mode with selected ion monitoring (SIM). Ion masses 485.7, 483.7 were selected for PBDE-47, and masses 563.6, 565.6 for PBDE-99 and -100, and

masses 641.5, 643.5 for PBDE-153 and -154 [9]. The GC oven program was as follows: 50 °C (1 min), 20 °C/min up to 150 °C (5 min), 3 °C/min up to 250 °C, and 10 °C/min up to 300 °C (10 min). A 2 μL extract was injected to GC–MS for each sample using a splitless injection. The injection port, ion source and interface temperature were set as 250, 200 and 300 °C, respectively. Within each batch of eight samples, one blank sample, the absence of a tissue sample in the extraction vessel was then subjected to the whole experimental procedure to determine any analytical contamination.

2.7. Lipid determination

An aliquot (2 mL) of the extracts from SE and MAE vessels were transferred to 10 mL test tubes which were then placed in an aluminum test-tube block holder on a hotplate (Chiltern Scientific, New Zealand) and maintained at 40 °C overnight to dryness. The dry residue was weighed and used to calculate the percentage of lipid in the samples. The method used for gravimetric lipid weight determination had been described in detail [10]. The lipid content determination gravimetrically was determined by the ratio: lipid content% = dry lipid residue weight (g)/original tissue weight (g) × 100%.

3. Results and discussions

3.1. Quality control

3.1.1. Comparison of lipid extraction between SE and MAE

The lipid content was determined in adipose tissue for SE and MAE procedures, based on triplicate analysis of a single adipose tissue, were 80.73% ± 0.62 and 81.26% ± 0.43, respectively. One-way ANOVA (Minitab Version 14) used with a 95% confident level, indicates that there are no significant differences ($p = 0.319 > 0.05$) in lipid values between the two extraction methods. MAE therefore represents an effective and efficient method for determining lipid content in human adipose tissues compared to conventional SE procedures.

3.1.2. PBDEs' recoveries in self-spiked tissue using SE and MAE

The percentage recoveries of PBDE congeners -47, -99, -100, -153 and -154 spiked at concentrations of 5 and 10 ng/g in tissue, based on triplicate analysis of the same adipose tissue sample, for MAE and SE procedures are shown in Table 2. Recoveries were in the range of 70–130% for MAE and SE. The recovery intervals expressed by average percent recovery (R) and the standard deviation of the percent recovery (S_R) were from $R - 2S_R$ to $R + 2S_R$, which is acceptable according to EPA method 1668a for PCB congeners in tissues [11]. MAE efficiency is therefore comparable to analyte recoveries using a conventional SE procedure and provides a similar high level of quality assurance.

Table 3
Recoveries of mean and S.D. of spiked-surrogates in sixteen adipose tissue samples using MAE

Congeners	Mean ± S.D. (%)
13CPBDE47	72 ± 9
13CPBDE99	80 ± 10
13CPBDE100	82 ± 10
13CPBDE118	84 ± 7
13CPBDE153	90 ± 5

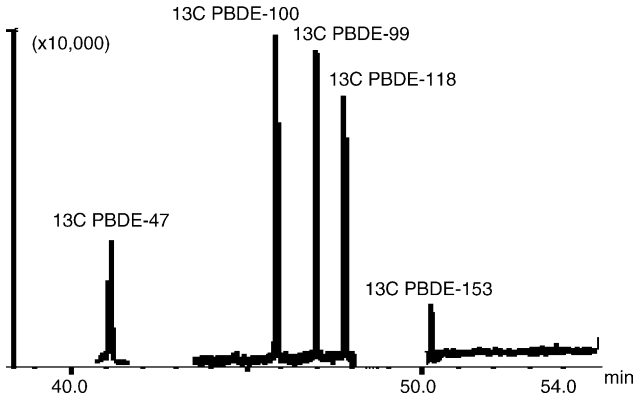

Fig. 1. GC–MS EI chromatogram of five ^{13}CPBDEs surrogates of 200 ng/g solution in dodecane.

3.1.3. Recoveries of surrogate substances using MAE

Surrogate substances recoveries, shown in Table 3, confirm that there was no unacceptable loss of analytes during the entire analytical procedure for MAE, where the mean of surrogate recovery ranged between 72 and 90%. The chromatogram of five surrogate standards subjected to GC–MS EI analysis is shown in Fig. 1, where good separation and peak identification was readily achieved. Selected ion mass (SIM mode) was: 338.0, 340.0 for ^{13}C PBDE-47; 576.0, 416.0 for

Table 2
Recoveries' intervals of PBDE congeners obtained in spiked samples using MAE and SE coupled with GC–EI–MS

	MAE		SE	
PBDE congeners in samples	Recovery (5 ng/g in tissue) mean ± S.D. (%)	Recovery (10 ng/g in tissue) mean ± S.D. (%)	Recovery (5 ng/g in tissue) mean ± S.D. (%)	Recovery (10 ng/g in tissue) mean ± S.D. (%)
PBDE-47	113 ± 5	112 ± 3	115 ± 4	119 ± 4
PBDE-99	114 ± 3	104 ± 2	117 ± 4	121 ± 2
PBDE-100	111 ± 5	111 ± 4	109 ± 5	109 ± 3
PBDE-153	82 ± 4	84 ± 3	82 ± 4	82 ± 3
PBDE-154	80 ± 5	82 ± 3	81 ± 5	86 ± 4

Table 4
Lipid content data from MAE and SE

Sample ID	Age	Fat (%) MAE	Fat (%) SE	PBDE-47	PBDE-99	PBDE-100	PBDE-153	PBDE-154	ΣPBDE
030810	32	65	67	5.07	ND	2.12	ND	ND	7.19
030920	33	94	92	4.62	ND	2.79	ND	ND	7.41
031016	34	90	89	2.11	ND	1.20	ND	ND	3.31
031017	30	83	84	1.22	ND	<DL	ND	ND	1.22
030310	36	85	85	6.56	ND	ND	ND	ND	6.56
030911	41	84	85	9.01	ND	3.29	ND	ND	12.30
031010	29	82	83	2.02	ND	ND	ND	ND	2.02
031205	26	79	80	1.65	<DL	ND	ND	ND	1.65
040813	28	76	77	1.50	ND	ND	ND	ND	1.50
040526	22	76	77	2.44	ND	1.01	ND	ND	3.45
040622	34	84	85	2.03	<DL	ND	ND	ND	2.03
040804	23	82	84	1.70	ND	<DL	ND	ND	1.70
040714	37	76	77	1.69	ND	1.69	ND	ND	3.38
040607	38	74	76	1.05	ND	ND	ND	ND	1.05
040601	24	75	77	2.12	ND	<DL	ND	ND	2.12
040708	31	68	72	1.86	ND	<DL	<DL	ND	1.86
Minimum	22	65	67	0.50	ND	ND	ND	ND	0.50
Maximum	41	94	92	9.01	<DL	3.29	<DL	ND	12.32
Mean	31	80	81	2.89	NA	NA	NA	NA	3.63
S.D.	5.6	7.5	6.4	2.34	NA	NA	NA	NA	3.17

Concentrations of PBDE congeners in 16 maternal adipose tissue samples (ng/g lipid basis). The lipid content of blank sample was undetectable. The concentration data reported have been corrected for blank interferences. ND, not detected; DL, detection limit; NA, not available.

^{13}C PBDE-100, -99, -118; and 496.0, 655.8 for ^{13}C PBDE-153.

3.2. GC–MS calibration and limits of detection

Internal linear calibration curves were obtained for PBDE congeners -47, -99, -100, -153, -154 over a 0–100 ng/g range with an r^2 coefficient of greater than 0.99. The MDL on a lipid weight basis was defined as the procedural blank peak area plus three times the standard deviation. The MDL was 0.5 ng/g for PBDE-47, 0.8 ng/g for PBDE-99, 0.7 ng/g for PBDE-100, 1.2 ng/g for PBDE-153, 1.0 ng/g for PBDE-154.

3.3. Sample analysis

3.3.1. Comparison of tissue lipid contents for SE and MAE

The lipid content of 16 adipose tissue samples collected from Singaporean women undergoing caesarian section are given in Table 4. Lipid contents for MAE ranged between 65 and 94% and for SE between 67 and 92% with similar levels achieved for both methods for the same sample.

As seen in Fig. 2, the correlation of lipid content for SE and MAE is highly significant ($r^2 = 0.9871$ and $p = 0.670 > 0.05$), confirming the extraction efficiency of MAE, where MAE lipid extraction is achieved within 30 min compared to 6 h for SE, and solvent extraction volume is only 25 mL compared to 150 mL for SE. The lipid data from MAE for 16 maternal adipose tissue samples were analyzed using the statistical Shapiro–Wilk test, which supports the hypothesis that the data fit a log normal distribution ($W = 0.967 > W_{(0.05,16)} = 0.887$) with a geometric mean of 79% lipid content.

3.3.2. PBDEs content of maternal adipose tissue samples using MAE

The concentrations of PBDE congeners -47, -99, -100, -153 and -154 in maternal adipose tissues are shown in Table 4.

The dominance of PBDE-47 over the other congeners with respect to total PBDEs tissue burdens indicates that this congener is readily bioaccumulated in human adipose tissues. The study conducted in the USA reported that this congener was dominant in human adipose tissues, and may indicate that PBDE-47 bioaccumulates more readily than the higher brominated congeners, although the exact mechanism is still

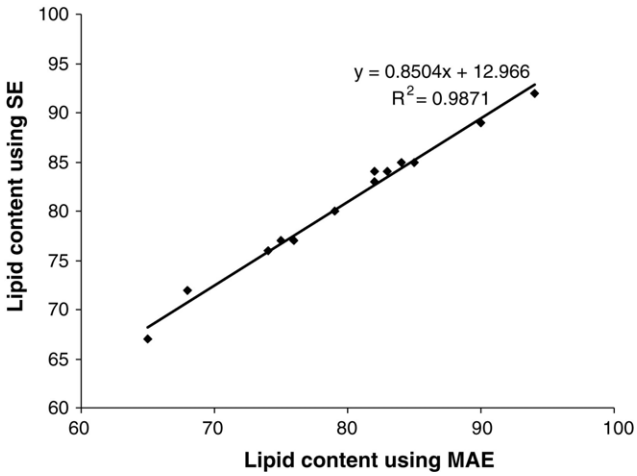

Fig. 2. The correlation of lipid content between MAE and SE.

under investigation [12]. PBDE-47 concentrations in adipose tissue reported for the Sweden population, for a total of 77 individuals, in the period of 1995–1997, were in the range of 3.8–16 ng/g, with a mean concentration of 4.5 ng/g [13,14]. This compares to PBDE-47 concentrations range of 0.50–9.01 ng/g and a mean of 2.89 ng/g in adipose tissue samples from Singapore. In contrast, the range of 0.7–4.7 ng/g in the adipose tissues of nine females from Belgium with a mean of 1.8 ng/g is lower than for Singapore [15]. Therefore, based on our analysis, the level of PDBE-47 in individuals in Singapore is in the mid-range of values reported from Sweden and Belgium.

The study in Belgium reported a total of PBDE congeners (PBDE-28, -47, -99, -100, -153) for adipose tissue of nine females from 2.4 to 11.7 ng/g with a mean of 5.3 ng/g in 2000 [15]. The available data of the sum of PBDE congeners in this study i.e. 0.50–12.32 ng/g, with a mean of 3.63 ng/g, are comparable to the data from Belgium. It is not possible to state with absolute certainty differences exist between countries, but contrasts in food culture, safety standards and the commercial use of the chemicals are likely reasons.

4. Conclusion

MAE coupled with GC–EI–MS has proven to be an efficient and quantitative method to detect and analyze trace PBDE congeners level in human adipose tissues relative to conventional SE procedures. A similar level of quality assurance can be achieved with MAE relative to SE with respect to the recoveries of self-spiked PBDE congeners, surrogates and the accuracy of sample analysis. However, a low solvent extraction volume of only 25 mL for MAE, compared to 150 mL for SE, and a sample extraction time of 30 min compared to 6 h for SE, makes MAE the favored method for the rapid quantification of flame retardants in human adipose tissues. Using the quality assured MAE procedure on a 16 maternal adipose tissues collected in Singapore indicates that prevailing levels of PBDE in females are slightly lower than available data from Sweden, and comparable to the data from Belgium.

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