DETERMINATION OF PBDEs AND PCBs IN BELGIAN HUMAN ADIPOSE TISSUE BY NARROW BORE (0.1 mm i.d.) CAPILLARY GC-MS

A. Covaci, S. Voorspoels, J. De Boer[1], J.J. Ryan[2], P. Schepens

Toxicological Center, University of Antwerp, 2610 Wilrijk, Belgium
[1]Netherlands Institute for Fisheries Research, P.O. Box 68, 1970 AB IJmuiden, The Netherlands
[2]Health Canada, Bureau of Chemical Safety, Ottawa K1A 0L2, Ontario, Canada

Introduction

Due to their persistence and bioaccumulation potential, organohalogen compounds (such as PCBs and PBDEs) are found in the environment and in humans[1]. While PCBs are routinely measured in human tissues[2,3], PBDEs have been measured only recently in few studies[4-6]. Because concentrations of PBDEs in humans are in the order of ng/g lipid weight (50 to 200 times lower than PCBs), most of the analytical work has been carried out by highly sensitive systems as gas chromatography-high resolution mass spectrometry (GC/HRMS) or gas chromatography-negative chemical ionization low resolution mass spectrometry (GC/NCI-LRMS). Electron impact low resolution mass spectrometry (EI-LRMS) is used for the determination of PCBs in human matrices and of PBDEs in samples with relatively high concentrations[7]. We show here that a combination of narrow-bore (0.1 mm id) capillary column and EI-LRMS with large volume injection for PBDEs (LVI) or cold splitless for PCBs can be used for their determination in human adipose tissue. This is the first report on PBDE levels in Belgian population.

Methods and instrumentation

Human adipose samples (n=20) were obtained by autopsy from the University Hospital of Antwerp, Belgium. One gram of each sample was accurately weighted and mixed with anhydrous Na_2SO_4 till a fine floating powder was obtained. After addition of internal standards (100 µl from a mixture of ^{13}C-BDE 47, 99 and 153, 13.06 pg/µl in iso-octane and 200 µl from a mixture of PCB 46 and 143, 100 pg/µl in iso-octane), the powder was extracted by automated hot Soxhlet for 2 hours with 75 ml of hexane : acetone : dichlormethane = 3:1:1 (v/v). After concentration and determination of lipid content, the extract was subjected to clean-up on 2 successive solid phase cartridges containing acid silica and acid silica : neutral silica : deactivated basic alumina (from top to bottom), respectively. PBDEs and PCBs were eluted with 50 ml hexane. The eluate was concentrated to almost dryness and 100 µl of the recovery standard (bromobiphenyl (PBB) 80 – 18pg/µl in iso-octane) was added, after which it was reconcentrated to approximately 60 µl.

A HP 6890 GC was connected with a HP 5973 mass spectrometer operated at 70 eV in selected ion monitoring (SIM) mode. A 10m x 0.10mm x 0.10μm AT-5 (5% phenyl polydimethyl siloxane) capillary column (Alltech) was used with helium as carrier gas at constant flow of 0.4 ml/min.

For PBDE determination, 20 μl (4x5 μl) were injected in a PTV injector in solvent vent mode (vent flow 100 ml/min for 1 min, injector at 70°C for 1.1 min and then heated with 700°C/min to 270°C) with the split outlet opened after 2.1 min. The run time was 11 min. Dwell times were set at 10 ms. Two most abundant ions were monitored for each level of bromination for native and labeled PBDE. Mean recoveries of internal standards and detection limits are presented in Table 1. The method performance was assessed through analysis of two biota samples (eel and freeze-dried porpoise liver) used for the first interlaboratory test on PBDE[8], which showed a variation of 10-15% from mean values obtained from the participating laboratories.

For PCB determination, two different columns were used. For the 10m x 0.10mm x 0.10μm AT-5, 1 μl was injected in cold splitless. Run time was 8 min and dwell times were set to 10 ms. For the 50m x 0.22mm x 0.25μm HT-8 (SGE), 1 μl was injected in hot splitless. Run time was 50 min and dwell times were set to 50 ms. Two most abundant ions were monitored for each level of chlorination. Mean recoveries of internal standards were 73 and 79 % for PCB 46 and 143, respectively. Detection limits ranged between 0.2 and 0.5 ng/g fat. The method performance was assessed through rigorous internal quality control, which included daily check of calibration curves and regular analysis of procedural blanks and certified material CRM 350 (PCBs in mackerel oil).

Results and discussion

PBDEs

The use of EI-LRMS allows the use of ^{13}C-labeled standards as internal standards (this procedure is not possible when using NCI-LRMS). Moreover, the higher selectivity of EI is important compared to NCI where often only Br ions can be measured. However, the response factors in EI are very different for congeners with different degrees of bromination and detection of highly brominated congeners is problematic in EI-LRMS, due to low concentrations to be measured and poor sensitivity. It is possible to tune manually the MS to obtain increased sensitivity for higher masses. The problem can be overcome by using large volume injection which allows the introduction of a larger amount of extract, thus increased sensitivity. However, the clean-up procedure should be very efficient as interferences may easily disturb the chromatogram. The targeted PBDEs eluted from the GC column between 6.1 and 9.2 min. The short retention times are due to the use of a narrow-bore capillary column (id=0.1mm), which offers the resolution power of conventional column (id=0.25mm), but decreases the analysis time with more than 50% (Figure 1). Furthermore, the smaller id results in a smaller peak width and an increased mass sensitivity (higher S/N ratios for the same amount injected). Moreover, with the introduction of

extract volumes up to 20 µl, low detection limits (DL) can be achieved (Table 1). A good linearity (r^2>0.995) was achieved for each compound between 2 x DL (calculated for a S/N=3) and 10 ng/g fat..

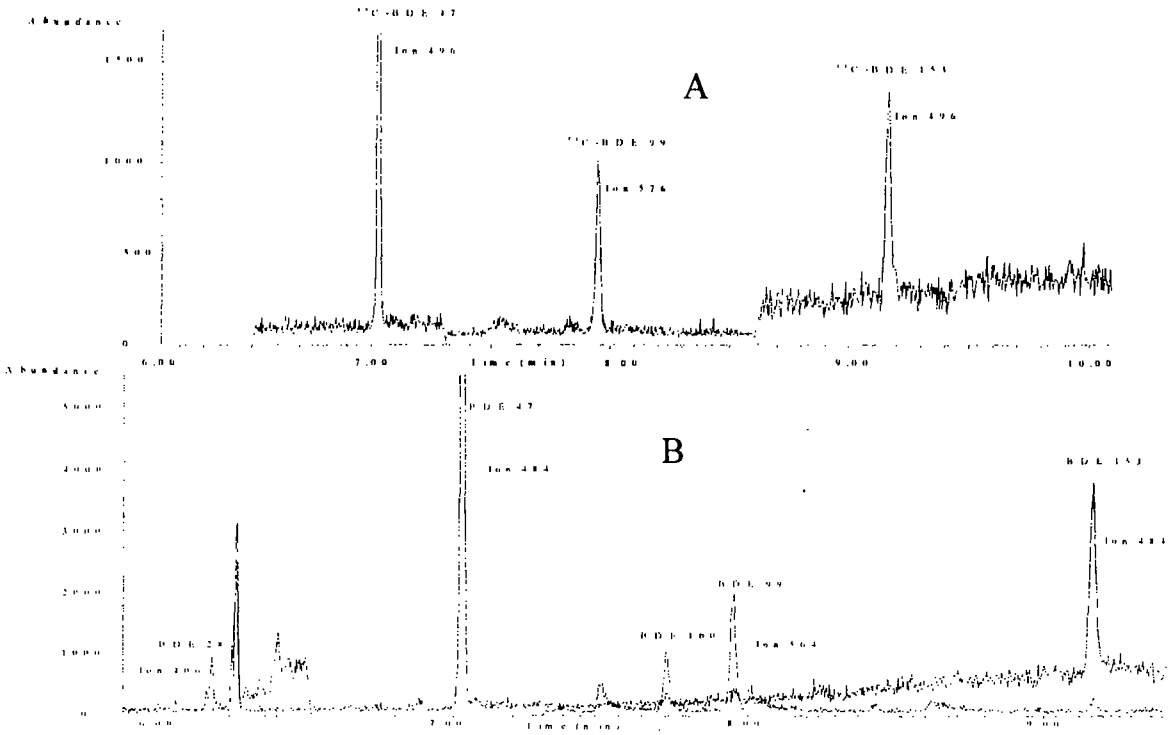
Table 1. Acquisition parameters and concentrations of PBDEs in Belgian human adipose tissue (n=20).

Target compounds	RT (min)	Ions	Recovery (%)	DL (ng/g fat)	Mean ± SD (ng/g fat)	Range (ng/g fat)	Spain[4] (ng/g fat)
BDE 28	6.18	406, 408		0.05	0.11 ± 0.07	nd – 0.26	
^{13}C-BDE 47*	7.03	496, 498	81 ± 12				
BDE 47	7.03	484, 486		0.1	1.45 ± 1.01	0.54 – 4.71	1.36
^{13}C-BDE 99*	7.94	576, 578	84 ± 17				
BDE 99	7.95	564, 566		0.15	0.29 ± 0.38	nd – 1.61	0.42
BDE 100	7.72	564, 566		0.15	0.48 ± 0.34	nd – 1.50	0.51
^{13}C-BDE 153*	9.16	496, 498	103 ± 21				
BDE 154	8.92	484, 486		0.25	nd	nd	nr
BDE 153	9.17	484, 486		0.25	1.47 ± 0.70	nd – 2.79	1.83
Sum BDE					3.73 ± 2.11	1.70 – 10.13	

nd-not detected, nr-not recorded

Concentrations of PBDEs in Belgian adipose tissue were ranging between 1.70 and 10.13 ng/g fat. This is in concordance with concentrations reported in other countries[4-6]. Interestingly, BDE 47 was not always the most abundant congener. Higher values of BDE 153 were obtained in some samples. Blanks were run to check for interference, but no significant contribution to these high values was observed. Higher values for hexa-BDEs were also observed in some samples (n=13) from Spain[4] (Table1).

Figure 1. Selected ion chromatograms of a human adipose tissue extract: ^{13}C-labeled BDEs (chromatogram A) and target PBDEs (chromatogram B).



PCBs

Following the addition of appropriate internal standards, the extraction and clean-up procedure allowed also the determination of PCBs and DDTs on the same sample extract. Concentrations of PCBs measured on 2 different capillary columns are presented in Table 2. Even if several pairs of congeners with the same degree of chlorination were found to coelute on the AT-5 column (namely 28/31, 138/163, 170/190), the AT-5 column was used due to shorter retention times[9]. All these congeners were resolved on the HT-8 column. The 7 marker PCBs constituted approximately 70% from the total PCB burden.

Median concentrations of PCBs in the 20 adipose tissue (mean age of specimens 47.2 years) were higher than the median concentration of 46 samples from young Belgian females[2] (mean age 31.9 years). There was no significant difference between PCB concentrations in men and women.

Low correlation coefficients were obtained between PBDEs and PCBs (r=0.56). Age was not correlated with PBDE levels (r=0.09), but weakly correlated with PCBs and DDTs (Table 3). Meneses[4] has observed in 13 samples that the highest as well as the lowest levels of PBDEs correspond to elder men, while for other persistent organohalogenated contaminants (PCBs, DDTs) older persons were found to have higher levels due to bioaccumulation and long-half lives of the compounds.

Table 2. Concentrations of POPs (ng/g fat) in Belgian human adipose tissue (n=20) measured by 2 capillary columns.

	Mean ± SD	Median	Range
Sum PCBs (AT-5) (20 congeners)	830.8 ± 428.5	802.4	263.4 - 1756.4
Sum PCBs (HT-8) (35 congeners)	879.7 ± 444.8	840.5	285.8 - 1801.5
Sum 7 markers* (AT-5)	567.5 ± 300.2	567.3	174.2 – 1197.1
Sum 7 markers (HT-8)	510.5 ± 262.6	504.4	163.9 - 1027.8
Sum DDTs (pp-DDE + pp-DDT)	437.5 ± 598.8	290.1	47.0 - 2802.2
HCB	51.1 ± 30.4	51.0	13.9 - 100.1

*-IUPAC n° 28, 52, 101, 118, 138, 153, 180

Table 3. Pearson correlation coefficients between different groups of POPs.

	BDE 47	Sum BDE	PCB 153	Sum PCB	DDTs	age
BDE 47	-	0.95 *	0.44*	0.42*	0.28	0.07
Sum BDE		-	0.57*	0.56*	0.31	0.09
PCB 153			-	0.98*	0.81*	0.56*
Sum PCBs				-	0.77*	0.59*
DDTs					-	0.40*
age						-

*- $p<0.05$

Conclusions

GC/EI-LRMS in combination with LVI and narrow bore capillary column was found suitable for the determination of PCB and major PBDE congeners in 20 human adipose tissue samples from Belgium.

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