SIMULTANEOUS ANAYSIS OF PCBS AND POLYBROMINATED DIPHENYL ETHERS (PBDES) IN HUMAN ADIPOSE USING GC/MS/MS

Brown, F R, Winkler, J[1], Visita, P, Park, J S[1], Petreas, M.
Department of Toxic Substances Control, Environmental Chemistry Laboratory, Berkeley, California, 94710
[1] Public Health Institute, Oakland, California, 94607

Abstract
In a study of possible links between body burdens of halogenated pollutants and breast cancer, our laboratory analyzed samples of breast adipose tissue, collected in the late 1990s from San Francisco Bay Area women, for PCBs and PBDEs. Because of the small amounts of sample, it was necessary to extract the sample into very small final volumes (10 μL) to achieve usable limits of detection. Traditionally, our laboratory analyzes PCBs by high resolution GC/MS, and PBDEs by low resolution GC/MS with electron capture negative chemical ionization (NCI) detection, thus requiring two separate injections for the two classes of analytes. This becomes problematic when the sample volume is limited. This paper describes the simultaneous analysis of these adipose tissue samples for PCBs and PBDEs using GC/MS/MS. The use of GC/MS/MS gives very low limits of detection. GC/MS/MS also provides greater specificity for PBDEs than provided by GC/MS-NCI, as well as high specificity for PCBs, though not as great as with high resolution GC/MS.

Introduction

PCBs and PBDEs are structurally similar bioaccumulative compounds that are found at high concentrations in both humans and wildlife.[1-5] However, when sampling biological specimens, one is frequently restricted to relatively small sample sizes. Thus, despite their increasing concentrations in humans and wildlife, it is generally necessary to concentrate samples to small volumes, typically ~10 μL to achieve adequate limits of detection. This can be problematic when multiple analyses are required on these small samples. The ability to combine analyses would help minimize the number of injections required from these small volumes.

Our laboratory has reported the combined analysis of PCBs and PBDEs by GC/ECNCI[6] and applied that method to the analysis of human breast adipose tissue from Brazilian women.[7] However, ECNCI detection, while very sensitive, is not very selective for PBDEs over other brominated compounds. Thus, we decided to use GC/MS with EI/MS/MS detection to measure both PCBs and PBDEs in human breast adipose samples collected in the late 1990s from San Francisco Bay Area women participating in a study of study of possible links between body burdens of halogenated pollutants and breast cancer.[8,9] The advantages of EI/MS/MS detection over ECNCI include: better specificity for PBDEs; high specificity for PCBs, though not as high as with high resolution MS; EI detection is a cleaner ionization method than NCI; MS/MS gives much improved signal-to-noise ratio over ECNCI; and, MS/MS equipment is less expensive and easier to operate and maintain than high resolution mass spectrometers.

Materials and Methods

Samples collected as a part of this breast cancer study were stored at -20°C until needed. Samples were extracted by homogenizing the samples in 1:1 dichloromethane/hexane, shaking and centrifuging A portion of the extract was taken to determine the lipid content gravimetrically, 90% of the extract was taken for the analysis of dioxins/furans and coplanar PCBs, and the remaining extract was used for the analysis of organochlorine pesticides, PCBs, and PBDEs. This final portion was prepared as follows: extracts were spiked with ^{13}C-labeled pesticide and PCB internal standards. Gel permeation and Florisil column chromatography were used to remove lipids. Recovery standards were then added, and the extract reduced to 10 μL.

PBDEs were not included in the original list of analytes, but were added about half way through the project, so only about half of the samples were spiked with $^{13}C_{12}$-BDE-77 as an internal standard for the PBDE analysis.. However, for the sake on consistency, we decided to use $^{13}C_{12}$ PCB-180 as the BDE internal standard for all samples.

Analysis was carried out on a Varian 3800 GC with electronic flow control and a 1200L mass spectrometer. The column was a Varian VF-5ms 30 m x 0.25 mm id x 0.25 μm film. Helium at 1.0 mL/min was used as the carrier gas. Samples were injected into an 1177 injector at 280°C in splitless mode with no pressure pulse. A deactivated injection port liner was used (Siltek, 4.0 mm id, with glass frit). The GC temperature program was: initial temperature 180°C (2 min hold); 180°C to 300°C at 10°C/min (12 min); final hold at 300°C (4 min hold) for 18 min total run time. The 1200L mass spectrometer was operated in extended dynamic range mode using electron impact ionization and MS/MS detection. Ionization voltage was 70 eV; ionization current was 50 μA; collision cell (Q2) pressure was ~2.3 mTorr of argon. The MS/MS parameters are shown in Table 1.

Table 1. MS/MS Parameters

	Q1 Resolution	Precursor Mass	Collision Energy (V)	Q3 Resolution	Product Mass	LOQ (pg)	Acquisition interval (min)
PCB-118	2	326 (M)	-20	3	255 (M-2Cl)	1	7.5 –11.25
PCB-138	2	360 (M)	-30	3	289 (M-2Cl)	1	7.5 –11.25
PCB-153	2	360 (M)	-30	3	289 (M-2Cl)	1	7.5 –11.25
$^{13}C_{12}$ PCB-153	2	372 (M)	-30	3	301 (M-2Cl)		7.5 –11.25
PCB-180	2	394 (M)	-30	3	324 (M-2Cl)	1	11.25 – 12.75
$^{13}C_{12}$ PCB-180	2	406 (M)	-30	3	336 (M-2Cl)		11.25 – 12.75
PBDE-47	2	486 (M)	-30	3	326 (M-2Br)	2.5	11.25 – 12.75
PBDE-99	2	564 (M)	-30	3	404 (M-2Br)	2.5	12.75 – 14.0
PBDE-100	2	564 (M)	-30	3	404 (M-2Br)	2.5	12.75 – 14.0
PBDE-153	2	643 (M)	-30	3	484 (M-2Br)	2.5	14.0 – 18.0
PBDE-154	2	643 (M)	-30	3	484 (M-2Br)	2.5	14.0 – 18.0

Quality Control

Solvent blank samples were prepared with each batch of six adipose samples. Analytical results were considered valid if the analyte concentration exceeded three times the blank level for that analyte. The accuracy of the new GC/MS/MS method was confirmed by analyzing the National Institute of Science and Technology (NIST) 1588b Cod Liver Oil SRM. To confirm the proper operation of the GC/MS system during this project, a full seven point calibration curve (1.0 pg/μL – 2500 pg/μL for BDEs, 2.5 pg/μL – 5000 pg/μL for PCBs) was created for each batch of samples analyzed. In addition, a check standard was injected each day following injection of the final sample. Finally, the NIST cod liver oil SRM extract was injected every three to four batches.

Results and Discussion

Figure 1 shows the total ion current chromatogram of a mixed PCB/PBDE standard. All congeners are well resolved. Figure 2 shows the total ion current chromatogram (upper trace) and the extracted ion current profiles of PBDEs (lower left trace) and of PCBs (lower right trace) of a human adipose sample. Both the PBDE and PCB traces for their respective individual congeners show very low noise.

The results of the analysis of the NIST SRM 1588b (Cod Liver Oil) are shown in Table 2. The results for the PCBs show good agreement with the NIST certified concentrations. We attribute the higher bias of the PBDE results to the use of $^{13}C_{12}$ PCB-180 as the internal standard, rather than $^{13}C_{12}$ BDE-77. However, as explained above, we opted to use $^{13}C_{12}$ PCB-180 as the internal standard throughout this study, rather than use $^{13}C_{12}$ PCB-180 for part of the samples and $^{13}C_{12}$ BDE-77 for the remaining samples.

Preliminary results of the analysis PCBs and PBDEs in human adipose samples are shown in Table 3. For PCBs, the relative concentration of the four congeners is PCB-153 > PCB-180 ~PCB-138 > PCB-118. For PBDEs, the relative concentration of the five congeners is BDE-47 > BDE-99 > BDE-153 > BDE-100 > BDE-154. This pattern is mostly consistent with previous results of a subset of these samples measured by GC/MS with ECNCI

detection[6], with one exception. BDE-154 was previously reported as being the second most abundant congener, and in this paper, it is the least abundant. We attribute this to potential interference from PBB-153 with ECNI, which is not a factor in this project in which EI/MS/MS detection is used.

Table 2. Results of analysis of NIST 1588b Cod Liver Oil SRM using GC/MS/MS[a]. n = 11

	NIST Value[b]	Mean	Median	Min	Max	Stnd Dev	% RSD	% Bias
PCB-118	172	192	194	176	202	8.83	4.59	11.9
PCB-138	212	218	213	198	242	13.5	6.21	2.73
PCB-153	275	282	281	271	292	7.25	2.57	2.38
PCB-180	98.5	94.4	94.6	87.8	104	4.98	5.28	-4.20
PBDE-47	17.8	23.3	24.1	18.8	25.5	2.15	9.20	31.1
PBDE-99	0.56	1.40	1.43	1.11	1.70	0.171	12.3	149
PBDE-100	1.89	2.42	2.54	1.87	2.78	0.282	11.63	28.3
PBDE-153	NA	1.40	1.43	1.11	1.70	0.171	12.3	
PBDE-154	0.495	0.513	0.507	0.341	0.667	0.097	19.0	3.73

[a] Concentrations in ng/g wet weight
[b] PCB concentrations are certified concentration values. PBDE concentrations are reference concentration values.

Table 3. Preliminary results of analysis of human breast adipose samples[a] n = 155

	Mean	Median	Min	Max	Stnd. Dev.	% > LOD
PCB-118	34.8	23.0	2.69	326	43.1	92.3
PCB-138	112	62.4	22.1	1900	226	87.1
PCB-153	170	106	17.0	3170	364	94.2
PCB-180	119	72.6	22.4	2340	262	85.2
PBDE-47	85.9	21.5	4.27	3140	297	80.0
PBDE-99	35.0	12.1	1.28	470	75.1	54.2
PBDE-100	15.1	4.81	0.78	442	43.0	80.6
PBDE-153	19.9	6.58	0.84	371	49.5	93.5
PBDE-154	3.11	1.01	0.00	82.2	8.98	61.3

[a] concentrations in ng/g lipid weight

Acknowledgements
This work is partially funded by the California Breast Cancer Research Program. The ideas and opinions expressed are those of the authors and do not necessarily reflect the views of the California Department of Toxic Substances Control.

References

1. Robertson L, Hansen, L. in: *PCBs: Recent Advances in Environmental Toxicology and Health Effects*, University Press of Kentucky, 2001.
2. de Witt C. *Chemosphere* 2002, 46:583.
3. Law R, Allchin C, de Boer J, Covaci A, Herzke D, Lepom P, Morris S, de Witt C *Levels and Trends of Brominated Flame Retardants in the European Environment*, Third International Workshop on Brominated Flame Retardants, Toronto, ON, Canada, 2004.
4. She J, Holden A, Tanner M, Sharp M, Adelsbach T, Hooper K *Organohalogen Compounds* 2005, 67: 607.
5. She J, Holden A, Sharp M, Tanner M, Williams-Derry C, and Hooper K *Organohalogen Compounds* 2005 67: 647.
6. Brown FR, Kalantzi O, Winkler J, Dhaliwal J, Petreas M *Combined PBDE/PCB Analysis using GC/MS with ECNCI Detection*, 25th International Symposium on Halogenated Organic Pollutants and POPs, Toronto, ON, Canada, 2005.
7. Kalantzi O, Brown FR, Erdmann C, Caleffi M, Goth-Goldstein R, Petreas M *Polybrominated Diphenyl Ethers (PBDEs) in Human Breast Adipose Tissue Samples From Brazil*, 25th International Symposium on Halogenated Organic Pollutants and POPs, Toronto, ON, Canada, 2005.
8. She J, Petreas M, Winkler J, Visita P, McKinney M, Kopec, D. *Chemosphere* 2002; 46:697.
9. Petreas M, She J, Brown FR, Winkler J, Windham G, Rogers E, Zhao G, Bhatia R, Charles, MJ. *Environ. Health Perspect* 2003; 111:1175.

Figure 1. Total ion current chromatogram of 500/250 pg/μL PCB/PBDE mixed standard.

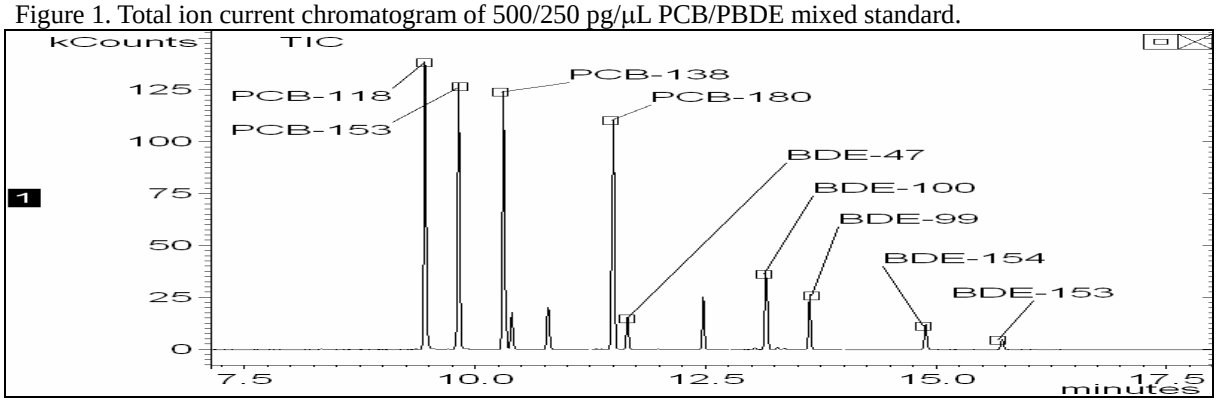

Figure 2. Total ion current and extracted ion current profiles for PBDEs (left) and PCBs (right) for human adipose sample analyzed by GC/EI/MS/MS.