COMPARISON OF SOIL PBDE LEVELS USING HRGC-HRMS AND MAGNETIC PARTICLE ENZYME IMMUNOASSAY

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Introduction

Brominated flame retardants (BFR) are ubiquitous environmental contaminants that can accumulate through the food-chain. Polybrominated diphenyl ethers are the major class of compounds in the BFR. There are three major formulations of PBDEs, namely pentaBDEs, octaBDEs, and decaBDEs used as flame retardants in different materials.

Because of the low levels and complex mixtures of the PDBEs found in the environment analysis, these have been done utilizing GC coupled with mass spectrometry to achieve the specificity and sensitivity required. Unfortunately, although these methods allow multicongener quantitation, they require sophisticated instrumentation with the associated complexities in their operation, as well as extensive sample cleanup prior to analysis. These factors render the instrumentation incapable of the high throughput analysis required for environmental and food monitoring.

Enzyme immunoassays (EIAs) have provided analytical techniques with properties matching the requirements of high throughput environmental samples with sufficient sensitivity to analyze the low concentrations typically observed in most of the analytes of interest. Magnetic particle-based EIAs have been applied for the analysis of pesticides and other environmental contaminants [1-3] in many different sample matrices. Many aspects of the magnetic particle analysis lead to the ease of use and superior analytical sensitivity of this format. This paper describes the application of a magnetic particle EIA to detect PBDEs in soil samples.

Materials and Methods

Materials and Instrument. A rabbit was immunized with 4-(2, 4-dibromo-5-(2, 4-dibromophenoxy)phenoxy)butyrate-KLH [4] to produce an anti-PDBE serum. Superparamagnetic particles of approximately 1 μm diameter were obtained from Seradyn (Indianapolis, IN). N-hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide (EDAC) were purchased from Sigma-Aldrich (St. Louis, MO). The PBDE ligand and horse radish peroxidase (HRP) were conjugated via a NHS/EDAC activation to yield PBDE-HRP conjugates (Abraxis, Warminster, PA). TMB peroxidase was obtained from BioFx (Randallstown, MD). The BDE-47 for ELISA was obtained from Chem Service (West Chester, PA). Magnetic separation was performed using a magnetic separation rack (Abraxis, Warminster, PA). The absorbance was read by a Photometric Analyzer at 450 nm (Abraxis, Warminster, PA).

Soil collections. A facility where furniture and other combustibles were burned for training firefighters was chosen as the soil sampling site. The top soil samples (0-15 cm) from different directions with different distance (ie 0, 3, 6, 9, or 12 ft) from the burning site were collected in July 2005. In addition, 3 different sites along old rail road tracks where pentachlorophenol treated wood was used and dioxins levels were elevated were used as control sites. The soil samples were dried, ground, and sieved through a 2 mm screen and stored in the dark at room temperature until analyzed.

PBDE soil extraction and cleanup procedures for ELISA. A mixture of one gram of soil, 1.2 g of anhydrous sodium sulfate and 2 mL of 20% acetone in hexane were shaken for 10 minutes. After removing the organic extract, 0.8 mL of sulfuric acid was added, the mixture vortexed, and the organic layer separated. The process was repeated until the acid phase was colorless. The organic solvent was evaporated and the residue dissolved in 1 mL of DMSO. The sample was diluted at least 1:50 in 50% MeOH (for higher concentrations greater dilution was required) and the ELISA performed. The ELISA procedure was described previously. [5]

Soil sample cleanup and GC-MS analysis. Into a sample bottle, 1g of soil was weighed and the 13-C labeled standards (13C-BDE-28, 13C-BDE-47, 13C-BDE-99, 13C-BDE-100, 13C-BDE-153, 13C-BDE-154, 13C-BDE-183, 13C-BDE-197, 13C-BDE-207, and 13C-BDE-209) were added. To each bottle 10 mL of tolulene:acetone (70:30) was added and the slurry was sonicated for 1 hr. The solvent was decanted through funnels containing 20g of pre-wetted sodium sulfate/glass fiber filters. The soil samples were extracted 2 more times using 5 mL fresh toluene: acetone (70:30) and sonicated for 30 minutes each time. The filtrates were combined and concentrated to about 0.5 mL. After addition of 5 mL of hexane to each tube the sample was filtered through a high density polyethylene filter and loaded onto columns (an acid/neutral/basic silica column followed by an alumina column) for an automated cleanup procedure (Fluid Management System Waltham, MA). The isotope-dilution GC-MS method for PBDE congeners quantitation was similar to the EPA method 1613 as described by Huwe et al [6].

Results and Discussion

A substantial amount of pentaBDEs as flame retardants are found in polyurethane foam in furniture and car seats as well as various textiles while octa-BDEs are found in the polystyrenes used in televisions and computers [7]. The soils near a facility where furniture and other combustibles were burned for training firefighters was selected as the soil sampling site since BDE contamination could be expected. The top soil samples (0-15 cm) were collected in July 2005, and analyzed by both ELISA and GC-MS utilizing the separate sample purification approaches described above. The ELISA results in **Table 1** show that for locations down from the prevailing westerly wind the results decreased as the distance from the slab increased. The values to the NW showed a more random variation with distance from sites towards the prevailing wind where deposition would occur presumably on days with little or no wind. ELISA demonstrated soil contamination could be quickly and easily detected and the results showed the expected radial distribution. A high correlation between ELISA and GC-MS results was observed (**Figure 1**) although the results of the ELISA were much higher. This could be due to the fact that ELISA measures a sum of responses which include partially oxidized products (expected from a burn site) not measured by GC-MS since ELISA results for control sites and blank soils have below the limit of detection (20 ppt) PBDEs levels.

Table 1. ELISA results of PBDEs in soils (ng/g).

	Distance				
Direction	0 ft	3 ft	6 ft	9 ft	12 ft
SE	35	30	10	12	7
NW	43	NA[a]	54	NA	NA
NE	67	NA	12	NA	NA

[a] NA, not available

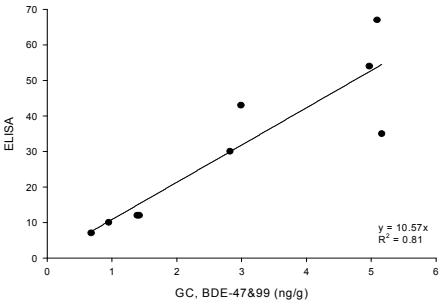

Figure 1. Comparison of ELISA and GC-MS results of burning site soils.

The GC-MS results showed a similar congener distribution pattern to that described by Hassanin et al [8] where the 5 major PBDEs are PBDE-47, -99, -100, -153, and -154, (BDE-209 was not considered to eliminate the additional contamination from the deca BDE formulation, which is currently becoming more important, but the flame retardants in burned articles of discarded furniture would most probably be derived from the pentaBDE formulation) (**Table 2**). These five congeners are included in the pentaBDEs formulation and Hassanin et al [8] claims that since the relative abundance found in soils is similar to the pentaBDEs formulation, this formulation is responsible for the

soil contamination. The abundance of BDE-47 to BDE-99 ratio was ca 1: 1.8; similar to our observation, (1: 1.6). The background soils found away from the burning site have more BDE-47 rather than BDE-99, so the trend is reversed from the BDE-burning sites, hence the origin of these PBDEs could not be attributed to the pentaBDE formula. The levels of PBDEs have a wide range in Hassanin's study while our levels were found to be 23 - 129 ppt for the three control sites and 1019 – 7796 ppt for the burning sites, clearly demonstrating the expected contamination did take place. The five major PBDEs represented 74% – 89% of the total PBDEs on the burning site soil (a rather narrow range), whereas for the control soils the ratio ranged from 50% - 100% and the levels were much lower indicating more random pollution. All 13C internal standards have recoveries greater than 25% but most often were found at near 100% with the exception of 13C-BDE-209 which was closer to 50%.

Although GC-MS provided great details in congeners quantitation information, a large quantity of organic solvent was used for sample cleanup (hexane 400 mL, methylene chloride 60 mL, toluene 14 mL, acetone 6 mL per sample). In comparison, ELISA used less than 6% of organic solvents (DMSO 1mL, methanol 25 mL, hexane 1.6 mL, acetone 0.4 mL). This factor alone makes ELISA a very environmentally friendly and cost effective screening method.

Acknowledgements
The authors wish to acknowledge Amy McGarvey for technical assistance; Kristin McDonald and Jean Picard for GC-MS soil sample cleanup; Margaret Lorentzsen for GC-MS analysis; and Drs. Gerald Larsen and Janice Huwe for reviewing the short paper. This research project was partially funded by USDA Trust Fund Cooperative Agreement number 58-5442-5-409 with Abraxis, LLC.

Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

References

1. Hottenstein CS, Fleeker JR, Herzog DP, Rubio FM, Lawruk TS J Agric Food Chem. 1996; 44:3576.
2. Jourdan SW, Scutellaro AM, Fleeker JR, Herzog DP, Rubio FM J Agric Food Chem. 1995; 43:2784.
3. Rubio F, Veldhuis LJ, Clegg BS, Fleeker JR, Hall JC J Agric Food Chem. 2003; 51: 691.
4. Shelver WL, Keum Y-S, Kim H-J, Rutherford D, Hakk HH, Bergman Å, Li QX J Agric Food Chem. 2005; 53:3840.
5. Shelver WL, Parrotta CD, Slaweck R, Li QX, Barcelo D, Lacorte S, Rubio FM Chemosphere. 2006; submitted.
6. Huwe JK, Larsen, GL Environ Sci Technol. 2005; 39: 5605.
7. Prevedouros K, Jones KC, Sweetman AJ Environ Sci Technol. 2004; 38: 3224.
8. Hassanin A, Breivik K, Meijer SN, Steinnes E, Thomas GO, Jones KC Environ Sci Technol. 2004; 38: 738.

Table 2. Summary of GC-MS results (pg/g) for major PBDE congeners and sum of 5 major congeners found in soil samples (after background subtraction).

PBDE	Sampling Sites											
	Con-1	Con-2	Con-3	SE-0	SE-3	SE-6	SE-9	SE-12	NE-0	NE-6	NW-0	NW-6
15	nd[a]	nd	nd	0.3	0.3	0.1	nd	0.1	0.3	0.5	0.5	0.3
17	nd	nd	0.2	23.6	8.4	3.7	4.1	2.1	16.2	5.4	9.1	16.0
25	nd	nd	nd	5.9	1.9	0.9	1.1	0.4	2.7	1.2	1.9	nd
28 & 33	nd	nd	0.7	61.6	27.5	12.8	15.8	7.8	55.7	20.7	35.0	58.5
37	nd	nd	nd	4.0	1.9	1.0	2.1	0.4	2.8	0.9	1.7	2.8
75	nd	nd	nd	16.9	6.3	2.1	3.3	1.3	11.7	3.5	7.3	12.5
49	nd	nd	0.2	140.6	63.9	25.8	33.2	17.0	126.6	43.7	80.7	137.1
47	30.2	10.1	35.1	1745	975.5	354.2	516.5	267.3	1825	558.3	1096	1890
66	nd	nd	nd	102.7	48.2	17.6	26.1	11.7	91.4	29.7	56.9	106.4
100	6.5	2.7	5.3	668.9	353.0	115.2	171.0	81.9	634.0	173.3	374.2	611.5
99	10.5	7.0	18.3	3392	1819	573.4	874.8	388.9	3242	806.2	1871	3059
154	1.1	nd	0.9	368.4	191.5	52.8	109.6	40.9	350.6	79.4	172.1	301.6
85	nd	nd	nd	185.9	110.8	29.4	57.8	24.9	238.2	44.7	111.6	186.4
153	5.2	1.9	4.2	520.4	270.6	68.1	162.3	55.8	437.0	94.1	236.2	381.2
183	0.6	nd	2.9	54.5	43.5	10.6	37.5	11.0	77.4	5.9	19.9	45.4
197	nd	nd	1.4	34.9	30.3	6.5	12.4	nd	104.6	nd	10.7	29.8
196	1.1	nd	1.5	51.9	24.6	10.2	18.0	nd	51.3	nd	nd	19.0
207	5.2	nd	16.9	176.4	118.6	91.5	66.5	36.2	257.6	50.6	50.9	133.1
106	19.3	0.9	41.2	242.1	173.2	205.5	171.8	71.1	197.4		77.6	225.1
∑major 5	53.3	22.6	63.8	6695	3610	1164	1834	835	6488	1711	3750	6243
∑all	79.7	22.6	128.8	7796	4269	1581	2284	1019	7722	1918	4213	7215

[a] nd, non-detectable after background subtraction.