Development of a sensitive magnetic particle immunoassay for polybrominated diphenyl ethers

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Introduction

Polybrominated diphenyl ether (PBDE) mixtures are manufactured as flame retardant additives for electronic equipment, plastics and textiles[1]. Three types of commercial PBDE mixtures find wide application[2], namely "pentaBDEs", "octaBDEs", and "decaBDEs". The penta formulation consists of a mixture of PBDE congeners that includes[3] BDE-47, BDE-99, BDE-100, BDE-153, and BDE-154. The octa formulation consists primarily of BDE-183, while the deca formulation consists primarily of BDE-209. North America accounts for approximately 98% of the global demand for the penta formulation[4].

PBDEs are ubiquitous environmental contaminants, their bioaccumulation has led to the detection of PBDEs in many species of wildlife[5], human blood plasma[6] and in human mother's milk[7]. PBDEs are structurally similar to polychlorinated biphenyls (PCBs), polychlorinated dibenzodioxins (PCDDs), and thyroid hormones, and therefore may interfere with thyroid hormone homeostasis[8]. Because of their potential health consequences it is desirable to have a rapid and high throughput assay to monitor PBDEs.

The quantification of PBDE samples is usually done by gas chromatography-mass spectrometry (GC-MS) or GC/ high resolution MS (GC/HRMS). While these methods are reliable, they are sophisticated and require extensive purification using large volumes of extraction solvents. The analysis of large numbers of samples using these techniques is not feasible. Therefore, a rapid, simple, and cost-effective method for the screening and quantitative analysis of PBDEs is required. Rapid, sensitive, accurate, and cost-effective enzyme immunoassays (ELISAs), have provided the analytical chemist an alternative tool to traditional instrumentation methods.

Magnetic particle-based ELISAs have previously been described and widely applied to the detection of pesticides and other environmental contaminants[9-11] in various sample matrices, including water, soil, produce, and fish tissue. The uniform dispersion of the particles throughout the reaction mixture allows for rapid reaction kinetics, precise addition of antibody and superior analytical sensitivity. This paper describes the assay performance of a PBDEs magnetic particle-based ELISA in ground water.

Materials and Methods

Carboxy terminated superparamagnetic particles of approximately 1 mm 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). Rabbit anti-PBDEs serum #122 was produced by immunizing the rabbit with 4-(2, 4-dibromo-5-(2, 4-dibromophenoxy)phenoxy)butyrate-BSA in according to Shelver et al.[12]. The PBDE ligand and horse radish peroxidase (HRP) were conjugated via NHS and EDAC reaction to yield PBDEs-HRP conjugate (Abraxis, Warminster, PA). TMB peroxidase substrate was purchased from BioFx (Randallstown, MD). PBDEs, PCBs, PCP and 2, 4-D were obtained either from Chem Service, West Chester, PA or AccuStandard, New Haven, CT).

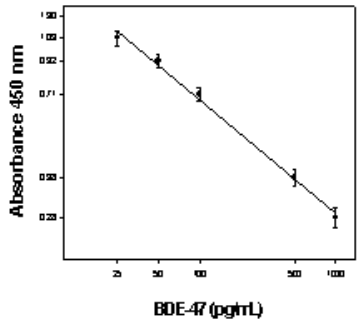
The anti-PBDEs coupled magnetic particles were prepared by NHS/EDAC activation, according to the procedure supplied by Seradyn. The unbound NHS/EDAC was removed from the particles by magnetic separation and washing two times with 50 mM of 2-(N-morpholino) ethane sulfonic acid (MES) buffer (pH 6.0). The PBDE antiserum and the activated particles were incubated overnight at room temperature with agitation. The reaction was then quenched with glycine buffer and the covalently coupled anti-PBDEs particles were washed and diluted with a Tris-saline/BSA

preserved buffer.

During the assay procedure, magnetic separation was performed using a magnetic separation rack (Abraxis, Warminster, PA). The device consists of a magnetic separation rack and a test tube holder, which fits over a magnetic separation rack containing permanent rare earth magnets. The two-piece design allows for up to sixty tubes to be set up, incubated and magnetically separated without removing the tubes from the tube holder.

Water samples were analyzed directly without any sample extraction or pre-concentration. After mixing 1:1 with methanol, 250 mL of the sample and anti-PBDE coupled magnetic particles (500 mL) were added to a disposable glass test tube and incubated for 20 minutes at room temperature. An aliquot of PBDE-HRP solution (250 mL) was added and the reaction was incubated for 20 minutes at room temperature. A magnetic field was applied to the magnetic solid-phase to facilitate washing and removal of unbound PBDE-HRP and to eliminate any potential interfering substances. The enzyme substrate and chromogen (peroxide/TMB) were then added (500 mL) and incubated for 20 minutes. The reaction is stopped with the addition of 2 N H_2SO_4 (500 mL) and the final color was read with analyzed using a photometer by determining the absorbance at 450 nm. The observed absorbance data were compared to a linear regression line using a log-log standard curve prepared from calibrators containing 0, 25, 50, 100, 500 and 1,000 parts per trillion (ppt) of BDE-47.

Results and Discussion



Dose Response Curve and Sensitivity. Figure 1 illustrates the mean standard curve for the BDE-47 calibrators collected over 24 assays, error bars represent one standard deviation (SD, n=24). The displacement at the 25 ppt level is significant (84% B/Bo, where B/Bo is the absorbance at 450 nm observed for a sample or standard divided by the absorbance at the zero standard). The assay sensitivity in water based on 90% B/Bo is 17 ppt.

Precision. Table 1 shows the results from a precision study in which surface and groundwater samples fortified with BDE-47 at 3 concentrations were each measured 5 times per assay and on five different days. The within and between day variation was estimated by the method of Bookbinder and Panosian[13]. Coefficients of variation below 5% were observed.

Table 1 Table 2

Precision of BDE-47 Measurements in Water BDE-47 Recovery in Water

Conc. of BDE-47 Added (ppt)	Recovery Mean (ppt)	S.D. (ppt)	%
62.5	56.4	2.9	90
125	131.5	5.3	105
250	270.6	12.8	108
500	510.4	28.3	102
Average			101.4

Control	1	2	3
Replicates	5	5	5
Days	5	5	5
n	25	25	25
Mean (ppt)	47	95	514
% CV (within assay)	3.2	2.2	2.0
% CV (between assay)	4.4	4.8	4.0

Accuracy. Known amounts of BDE-47 were added to four groundwater samples obtained from Warminster, PA. The samples included a municipal water source, a reservoir, a lake, a pond, and a creek. The accuracy (recovery) was assessed by analyzing the samples before and after the addition of BDE-47 and then subtracting the estimated concentration of PBDE before spiking. None of the samples had significant levels of PBDEs. Added amounts were accurately recovered (Table 2). An average assay recovery of 101% was obtained.

Table 3

Specificity (Cross-Reactivity)

Compound	LDD (ppb)	50% B/Bo (ppb)
BDE -47	0.017	0.135
BDE-99	0.02	0.15
BDE -28	0.045	0.9
BDE 100	0.055	5.5
BDE 153	0.075	10
BDE 154	3.5	580
BDE -183	13.5	2000
BDE -209	370	3000
Aroclor 1254	3	180
PCB-37	160	2000
PCB -77	>1000	>1000
PCP	3300	>10,000
2-4-D	>10,000	>10,000

Specificity. Table 3 summarizes the cross-reactivity data of the PBDE assay for various PBDE congeners as well as other environmental contaminants such as PCBs, PCP, and 2,4-D. The percent cross-reactivity was determined by estimating the amount of analogue required to displace 50% of the enzyme conjugate and comparing to the 50% displacement of the BDE-47 standard curve. The results showed that the antibody recognizes BDE-47 and BDE-99 equally well, the higher the brominate substitution the less cross-reactivity was observed. The antibodies have less than 0.1% cross-reactivity for Aroclor 1254.

Conclusions

This work describes the performance characteristics of a magnetic particle-based ELISA for the detection of PBDEs in groundwater samples. The assay is fast, and eliminates the need for expensive instrumentation and solvent disposal. The ELISA exhibits good precision and accuracy which can provide consistent and cost-effective monitoring of water samples. Using this ELISA, fifty results can be obtained in about one hour. Future efforts will be to extend these observations to the analysis of other type of matrices such as food, serum, milk, soil, and to perform comparison with other analytical methods.

Acknowledgements

The authors would like to thank Amy McGarvey for technical assistant.

References

1. IPCS (1994). Environmental Health Criteria 162: Brominated diphenyl ethers. International Programme on Chemical Safety, World Health Organization, Geneva.

2. Hardy, M.I. *Chemosphere* ***2002***, 46, 757-777.

3. deWitt, C.A. *Chemosphere* **2002**, 46, 583-624.

4. Boon, J.P.; Lewis, W.E.; Tjoen-A-Choy, M.R.; Allchin, C.B.; Law, R.J.; De Boer, J.; Hallers-Tjabbes, C.C.; Zegers, B.N.; *Environ. Sci. Technol.* ***2002***, 46, 697-707.

5. Anderson, O., and Blomkvist, G. *Chemosphere* ***1981***, 10, 1051-1060.

6. Klasson Wehler, E.; Hovander, L.; Bergman, A. *Organohalgen Compd.* **1997**, 33, 420-425.

7. Meiroyte, D.; Noren, K; Bergman, A. *J. Toxicol. Environ. Health.* ***1999***, 58, 329-341.

8. Brouwer, A.; Morse, D.C.; Lnas, M.C.; Schuur, A.G.; Murk, A.J.; Klasson-Wehler, E.; Bergman, A., and Visser, T.J. **1998**, *Toxicol. Ind. Health,* 14, 59-84.

9. Hottenstein, C.S.; Fleeker, J.R; Herzog, D.P.; Rubio, F.M.; Lawruk, T.S. J*ournal of Agricultural and Food Chemistry* ***1996***, *44:3576-358.*

10. Jourdan, S.W.; Scutellaro, A.M; Fleeker, J.R.; Herzog, D.P.; Rubio, F.M. *Journal of Agricultural and Food Chemistry***1995,** *43:2784-2788.*

11. Lawruk, T.S.; Gueco, A.M.; Jourdan, S.W.; Scutellaro, A.M.; Fleeker, J.R.; Herzog, D.P.; Rubio, F.M. *Journal of Wine Research* **1994,** *5:205-214.*

12. Shelver, W. L., Keum, Y. –S., Kim, H. –J., Rutherford, D., Hakk, H., Bergman, D., and Li, Q. X. *J. Agri. Food*

Chem. **2005** (web release: April 15, 2005)

13. Bookbinder, M.J.; Panosian, K.J. Correct and Incorrect Estimation of Within and Between-day Variation. *Clin. Chem.* **1986,** *32:1734-1737.*