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Development of an Analytical Method to Measure Polybrominated Diphenyl Ethers in Archived Extracts of Avian Eggs

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Abstract

olybrominated diphenyl ethers (PBDEs) are part of a group of brominated flame retardants used in a ide range of commercial and domestic applications to reduce product flammability. Large scale oduction of PBDEs started in the early 1970s with their presence in the environment first measured in 979. Production of PBDEs has increased steadily since then with global annual production in 2001 timated at 67,000 metric tons. In order to properly assess the level of risk associated with PBDEs, ccurate and sensitive methods are needed to measure these compounds. In this study we developed d validated an analytical procedure to measure 13 PBDE congeners in extracts of common (*Sterna irundo*) and roseate (*Sterna dougalli*) tern eggs originally processed for polychlorinated biphenyl CB) analysis. Negative chemical ionization mass spectrometry (NCI-MS) was determined to have uch lower detection limits for PBDEs than standard electron impact ionization (EI). Results are esented for eight environmentally ubiquitous congeners (PBDEs 17, 28, 47, 66, 99, 100, 153, 154). ovisional PBDE values from a NIST standard reference material (SRM1974b) compared well against ur results, indicating excellent extraction recoveries and accurate analytical data. Reanalysis of lected samples for PCBs showed little change in concentrations over time, indicating that analyte loss uring archival was not a factor. In addition, PBDE levels in the samples compared well with literature lues, providing further evidence of the robustness of the methods used. Overall, results show that eviously archived samples can be used to measure other classes of contaminants provided that ethodologies and procedures are properly validated.

ntroduction

lybrominated diphenyl ethers are currently used as additives in plastics, foam, textile products and er materials to reduce their flammability. PBDEs enter the environment during manufacture, leach m products during their use and when products are either recycled or disposed of. Production of DEs started in the 1970s, with a steady gain in global usage. One of the results of the growing use PBDEs over time has been the presence and increasing levels of these compounds in environmental mples as well as both animal and human tissues.

DEs have physical and chemical properties similar to other persistent organic pollutants (POPs) g., PCB, DDT) and have been found to be bioaccumulative and toxic. 209 PBDE congeners exist and numbered using the IUPAC system applied to PCBs (Figure 1). PBDEs are extremely hydrophobic g K_{ow} 5-10) with solubility and vapor pressure decreasing as the gree of bromination increases. A number of technical mixtures (*e.g.*, omkal) have been produced, ranging in the extent of bromination. mmercial production of PBDEs was limited to penta, octa and deca xtures. The composition of brominated diphenyl ethers (BDEs) in mulations varies by manufacturer, with congeners from BDE-17 to E-153 present in penta-BDEs, BDE-153 to BDE 209 for octa BDEs while Deca-BDE is composed BDE-209 exclusively (Figure 2a-b, Table 1).

Figure 1

his study, analytical methods were developed to measure concentrations of PBDEs in avian eggs m two tern species, common (*Sterna hirundo)* and roseate (*Sterna dougalli*). These samples had been iginally extracted for determination of PCBs by gas chromatography using electron capture (GC/ECD).

s chromatography mass spectrometry (GC/MS) using negative chemical ionization techniques was d to measure the PBDEs. The method was validated using provisional PBDE values from tissue ndard reference materials (SRMs) reported by Zhu and Hites (2003). This project is part of an growing ort to measure emerging contaminants of concern such as PBDEs in wildlife species and better derstand their environmental fate.

ure 2a

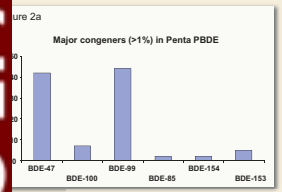


Figure 2b

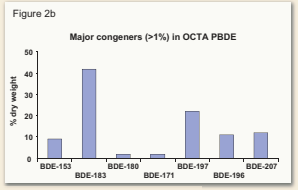


Table 1

Congener Abundance %

PBDE Product	Tri-BDE	Tetra-BDE	Penta-BDE	Hexa-BDE	Hepta-BDE	Octa-BDE	Nona-BDE	Deca-BDE
Penta-BDE	<1	25-39	50-60	4-8				
Octa-BDE				10-12	43-45	31-35	10-11	<1
Deca-BDE							<3	97

Overview of Mass Spectrometry Theory

- Using standard electron ionization (EI) techniques high energy electrons collide with analyte molecules to form positive ions.

$$M + e^- \rightarrow M^+ + 2e^-$$

- These molecules fragment in predictable patterns making identification and quantification possible.

$$M^+ \rightarrow M^+ + M_F$$

- In negative chemical ionization mode, electrons react with a reagent gas (*e.g.*, CH_4) to produce reagent ions.

$$CH_4 + e^- \rightarrow CH_4^+ + 2e^- \text{ (thermal)}$$

- These thermal electrons collide with analyte molecules resulting in a softer fragmentation of the parent molecule. In the case of PBDEs, this technique yields large quantities of bromine ions m/z = 79, 81 for quantitation, enabling very low detection limits

$$.MX + e^- \rightarrow MX^-$$

Methods and Materials (Avian sample prep)

- Tern eggs were initially extracted for analysis of select PCB congeners (*e.g.*, NIST 18). 4-5 grams (wet) of sample were extracted with acetonitrile, sonicated and back extracted into pentane. Concentrated sulfuric acid was added to destroy hydrocarbons and polar organic compounds. Carbon column cleanup was performed to isolate toxic congeners. Analysis of PCBs was conducted using an Agilent 6890 GC with electron capture detector (ECD) fitted with a 30m DB-5 x 250µm capillary column
- For PBDEs, sample extracts were analyzed using an Agilent 6890 Gas chromatograph coupled to an Agilent 5973 mass spectrometer (GC/MS) in either negative chemical ionization or electron impact ionization mode. All data was acquired in select ion monitoring mode (SIM). A 30m x 250µ with 25µ film thickness DB-5-ms column was used to separate the compounds of interest. Helium was used as the carrier gas at 1.5 ml/min. Injected sample volume was 1µl. Methane was used as the ionization gas when in NCI mode. In electron impact mode, molecular and high abundance fragment ions were used to identify and quantify the individual PBDE congeners. In NCI mode, bromide ions (m/z 79 & 81) were used for quantitation.
- Working PBDE standards were obtained from Cambridge Isotope Laboratories (Cambridge, MA) which included a total of 13 PBDE congeners (BDEs, 17, 28, 47, 66, 71, 85, 99, 100, 138, 153, 154, 183, 190). A four point calibration was constructed for each congener at 5.0, 10, 20, 40 & 80 pg/µl. Each curve was regressed using a linear equation resulting in r^2 values of 0.99 or greater. A PCB congener CB-198 was used as the internal standard for this study as it was used for the previous PCB analysis.

Results and Discussion

- The primary objective of this project was to develop a analytical method for the measurement of PBDEs. The method was created specifically for samples that had already been previously processed and analyzed for a different class of compounds (*e.g.*, PCBs). A number of steps were taken in order to insure high quality data.
- Significant time had passed since the original PCB analysis had been conducted (~ 1 year), and reanalysis of a subset of the total samples was performed to assess changes during storage. Results of the original data from 2006 are presented with the reanalysis results from 2007 (Figure 3). For this subset very little change in PCB levels has occurred, indicating that the extracts have not degraded or otherwise been adversely impacted during storage.

Figure 3

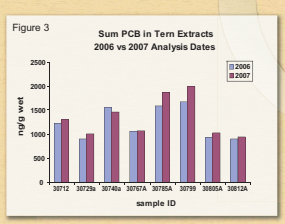
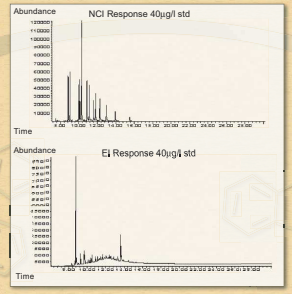
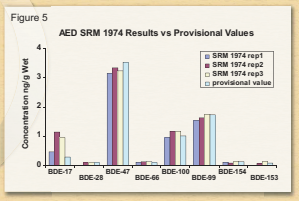


Figure 4



- Another step in the methods development procedure was to measure instrumental sensitivities to PBDEs in both EI and NCI modes. PBDE standards were prepared in the anticipated operating range of the study (0-100 pg/µl). In EI mode, very low responses were registered for the analytes at 40 pg/µl (Figure 4). In contrast, NCI mode showed high sensitivity with repeatable responses for the analytes at and below 1.0 pg/µl. In order to compensate for any changes in the extract over time, the internal standard (IS) from the PCB study, CB-198 was also used as the IS for the PBDEs. Detection limits for the study were estimated with the instrument in NCI mode. Limits of detection (LOD) increased as the degree of bromination increased. For this study, our method detection limit is 1.0 ng/g, based on the highest LOD calculated.
- To confirm that the PBDEs were extracted from the egg samples with reasonable recoveries, a reference material with known PBDE values was needed. SRMs used in the study were used to assess the recovery efficiency of PCBs were also used to assess PBDE recovery. Zhu and Hites (2003) published provisional PBDE reference values for the SRM (1974) used in the PCB study. A comparison of their PBDE values to ones we measured are presented in Figure 5. Results were very good overall, with five of the eight reported PBDE congeners within 10% of the recorded provisional values. One exception is PBDE-17, which had values an average of 30% higher than the provisional values. Other than the high recovery of PBDE-17, the extraction and analytical methods appear to have produced accurate, reliable results.

Figure 5



- In summary, we were able to accurately measure an emerging contaminant (PBDEs) in existing archived extracts using NCI-MS. The ability to use archived extracts allowed rapid examination of samples no longer available and provides a more complete picture of the classes of contaminants present in different environmental compartments. Currently work is underway refining the current method to accommodate carbon labeled PBDE standards $^{13}C_{12}$ which will improve method limits and accuracy.