POLYBROMINATED DIPHENYL ETHER RESIDUE ANALYSIS METHOD FOR FISH TISSUES FROM REMOTE, HIGH ELEVATION ECOSYSTEMS

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Introduction

Polybrominated Diphenyl Ethers (PBDEs) are a class of compounds used as flame retardants in synthetic materials whose production has been increasing substantially in the last two decades[2] (Figure 1)[2,3]. Some PBDEs have shown persistent[2,4], bioaccumulative[2,5], and toxic properties[2,6] (PBT). These compounds have been measured in urban and rural areas around the globe at low, but increasing levels of concentration over the past 20 years[2]. Remote atmospheric measurements confirm that some PBDEs have been transported atmospherically to remote areas[7], but there are few measurements of PBDEs in remote abiotic matricies to date. The documentation of PBDEs transport and fate to date is limited. Given these PBT properties, potential exists for adverse effects in remote ecosystems from the buildup of deposited PBDEs within high trophic level organisms. Some high trophic level marine species sampled in remote locations have shown moderate levels of PBDEs[8]. However, there is little evidence of atmospheric deposition of PBDEs to remote ecosystems.

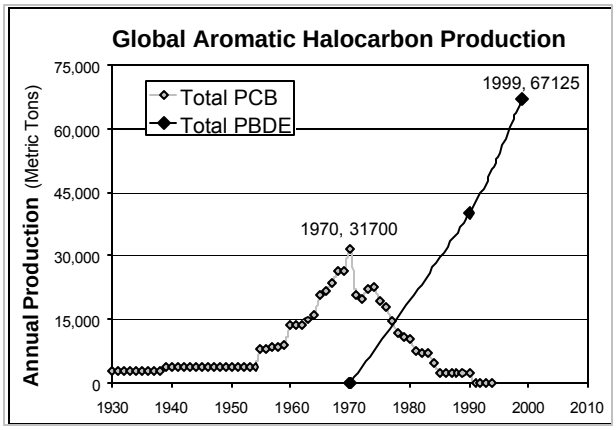

Figure 1 - Historic Annual PCB & PBDE Production

There has been some recent work documenting the atmospheric transport and deposition of similar, halogenated, persistent organic pollutants (POPs) to remote ecosystems[9,10]. These studies have shown that similar to latitudinal temperature trends that can cause global fractionation, altitude temperature trends can cause 'cold condensation' of these POPs in relatively remote mountain ecosystems. To date no studies have addressed the question of whether PBDEs

might be undergoing the same processes, or if any real potential exists for adverse effects in remote high elevation ecosystems.

This work represents initial efforts to test whether PBDEs are being atmospherically deposited in remote high elevation ecosystems, and if so whether or not they might pose a risk to these remote ecosystems. This is being accomplished as part of a 5 year, multi-agency sampling campaign in high elevation national park sites across the western continental United States and Alaska. The sampling, part of the US National Park Service's Western Airborne Contaminant Assessment Project (WACAP), is sampling fish, sediment, lake water, snow and ecosystem characteristics from 15 isolated high altitude lake ecosystems to test for evidence of accumulation of airborne contaminants (including PBDEs) and any potential ecological threat. To date, we developed a new method for sensitive isotope dilution analysis of PBDEs in environmental extracts using less expensive low resolution GC-MS instruments, and coupled it with whole fish tissue sample preparation methods to achieve a single method for the analysis of 38 PBDE congeners and at least 40 other semi-volatile organic compounds (SVOCs) including PCBs, PAHs, and pesticides. This work will review the reasons for testing PBDEs atmospheric deposition in these ecosystems, the research plan, the methods developed to measure PBDEs in these ecosystems, and the initial results of such tests.

Materials & Methods

Our method development and analysis utilized native and 13C labeled PBDE standards (CIL, Andover, MA), Optima grade solvents (Fischer, Pittsburgh, PA), UHP Helium, Methane, and Nitrogen (BOC, Murray Hill, NJ), and pesticide grade Na_2SO_4 (JT Baker, Phillipsburg, NJ). All glassware, foil, and Na_2SO_4 were baked at 400°C for 12 hours and solvent rinsed prior to use. Fish (weighed, measured, identified by sex and species, and dissected for histology and pathology) were packed in foil, and sealed bags on dry ice or -20°C freezer until homogenization under liquid N_2. Fish homogenate was spiked with isotope labeled recovery surrogates, ground with Na_2SO_4, and solvent extracted under temperature and pressure. Percent lipid was determined, and the extract was purified using various techniques including size exclusion and silica chromatography. Final extracts were reduced under N_2 to ~300 µL before being spiked with isotope labeled internals standards. Analysis was conducted on Agilent 6890 gas chromatographs (GC) coupled with 5973N mass spectrometers (MS) using both electron impact (EI) and electron capture negative ionization (ECNI) in the selected ion monitoring (SIM) mode.

Experiments were designed to identify a low resolution GC-MS technique for isotope dilution analysis of PBDEs in environmental extracts and to develop a whole fish tissues sample preparation method allowing for low level determination of PBDEs and other SVOCs. They included varying MS source parameters of electron energy, emission current, source temperature and pressure, and ECNI buffer gas to optimize PBDE specific ion production for isotope dilution methods. Experiments also included fish method development by optimizing extraction efficiencies, extract handling and purification techniques. The techniques were tested on whole fish samples from lakes in Alaska.

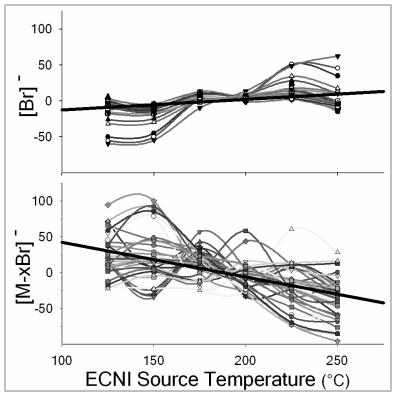

Figure 2 - Ion Abundance (% deviation from congener average) as a function of source temperature for 38 PBDE congeners.

Results

Optimization of GC-MS techniques yielded new optimum conditions previously unreported because previous work optimized the production of the non-specific bromide ion $[Br]^-$, whereas our work focused on PBDE specific molecular ion fragments $[M\text{-}xBr]^-$ (Figure 2). ECNI production of molecular fragment ions was found to be more variable than EI for each variable, while in GC-EI-MS analysis it was determined that electron energy was the most important variable. It was determined that optimizing both ECNI source temperature and pressure as well as electron energy was important in yielding a sensitive GC-MS PBDE analysis. The more sensitive ECNI technique was compared to previous optimizations and found to be significantly more sensitive (Figure 3).

Development of the fish method demonstrated that extraction and purification was most efficient using two DCM extractions and size exclusion (GPC) followed by silica chromatography for cleanup (Figure 4). The usefulness of a water back-extraction of the sample extract was evaluated to aid in the removal of mid weight fatty acid interferants. Method blanks did not

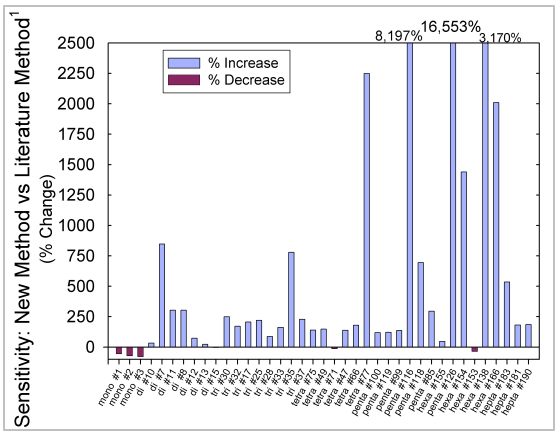

Figure 3 - Sensitivity increases relative to a previous GC-ECNI-MS method for 38

reveal any PBDE contamination.

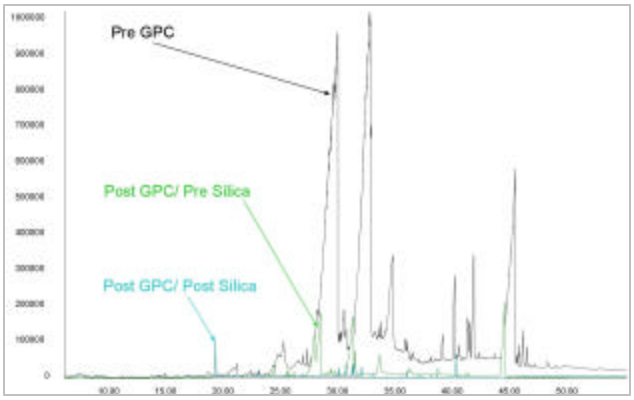

Figure 4 - Full Scan GC-EI-MS Chromatograms of whole fish extract throughout the purification

In addition, the method was evaluated for a list of SVOCs and it was determined that the method achieved good recoveries of 40 PAHs, PCBs, and pesticides. Finally the method was tested by evaluating landlocked fish gathered in 2002-2003 from lakes in remote Alaska. Some fish had SPBDE concentrations greater than 500 ng/g lipid (Figure 5).

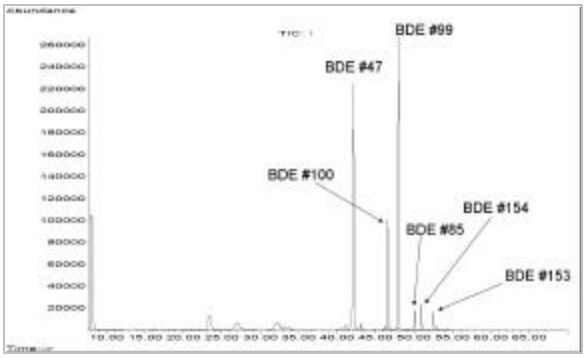

Figure 5 - GC-ECNI-MS Chromatogram of Arctic Greyling from Lake Matcharak, Gates of the Arctic National Preserve. AK. 2002.

Conclusions

PBDE analysis by GC-MS using isotope dilution can be achieved with benchtop ECNI-MS achieving substantial cost savings. Source temperature, pressure, and electron energy are important variables for molecular ion production. Fish analysis methods for PBDEs benefit from the sequential removal of smaller and smaller quantity interferants, and may be readily transferable to other SVOCs. Finally, high PBDE levels in some fish suggest that atmospheric deposition to remote North American lakes may occur and should be investigated.

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