A CONVENIENT METHOD FOR DETERMINATION OF LEVEL OF QUANTIFICATION (LoQ) FOR POLYBROMINATED DIPHENYL ETHERS IN BIOSOLIDS BY HR-SIR USING HRGC-HRMS

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Introduction

As an emerging class of chemicals of environmental concern, brominated flame retardants (BFRs) have received considerable attention from researchers since the mid nineties. A variety of these compounds are manufactured for use in diverse applications[1] to reduce the risk of fires. Of these, the additive flame retardants which are mixed with polymers and are hence not bound chemically to it, have a greater potential to leach out of their products[2] and have been found to be significant environmental contaminants. The polybrominated diphenyl ethers (PBDEs), polybrominated biphenyls (PBB) and hexabromocyclododecane (HBCD) belong to this category.

The PBDEs can exist as 209 possible congeners and are identified by the same IUPAC numbering as for PCBs[3]. The molecular weight range of congeners varies by almost an order of magnitude and the melting points[4] range from 0^oC to $>300^oC$. Besides, whereas lower substituted congeners are more volatile, the higher brominated homologues are photochemically and thermally labile. These features render the investigation of these compounds very challenging.

PBDEs are lypophylic, persistent and bioaccumulative and therefore, have been the focus of majority of investigations spanning environmental and toxicological concerns including transport dynamics and exposure risk assessment etc. Research has shown their presence in humans as well as biotic and abiotic matrices including sediments and their concentration is increasing over time. This has raised environmental[5] concerns around the world and these compounds have become the focus of monitoring by many regulatory agencies in different parts of the world including Canada. Under the Canadian Environmental Protection Act 1999, the main objective of the Toxic Substances Policy is virtual elimination of the substances on the Track I list. The initial step to achieve this goal is to set a benchmark value, which is the lowest concentration of a target compound that can be quantified confidently, defined as Limit of Quantification (LoQ).

In this study on biosolids, LoQ for selected PBDE congeners commonly found in Penta, Octa and Deca PBDE formulations, was determined. This method is based on previously established methods developed at the Environmental Technology Centre[6,7]. This is a congener specific method, where isotope dilution technique and HRGC/HRMS was used resulting in a robust, reliable and sensitive procedure using state of the art technology.

Materials and Methods

The Level of Quantification (LoQ) is defined as the lowest concentration that can be accurately measured using sensitive but routine sampling and analytical methods (CEPA section 65.1). The guideline of the American Chemical Society's Committee on Environmental Improvement[8] was adopted for the determination of LoQ. The American Society for Testing and Materials has adopted this guideline as a standard practice since 1998[9]. The calculation for LoQ is, LoQ = 10s, where s is estimated as *s*, the standard deviation (SD) of replicate measurements of an analyte at a concentration near the detection limit (DL).

In this study, dairy manure, a relatively uncontaminated matrix, was used as a representative for biosolids. The liquid dairy manure samples collected from a cattle farm were freeze dried and used as the matrix to determine LoQ for PBDEs.

Seven replicates of the sample each containing 1.5g of dried manure matrix were used for analysis and each was

spiked with MBDE-MIX, a solution of eleven ^{13}C labeled BDE congeners containing at least one congener for each level of bromination (Wellington Laboratories, ON, Canada). The sample was extracted with toluene for 18-20 hours using soxhlet extraction assembly. The toluene extract was concentrated to 2 mL, taken in 100 mL of dichloromethane and successively washed with 10% aqueous NaOH (2x25mL), 5% saline solution (2x50mL), concentrated sulphuric acid (4x25mL), 5% saline solution (2x50mL) and dried. The organic layer was concentrated to 2 mL, treated with activated copper granules to remove sulphur and further concentrated to 0.5mL. The extract was fractionated by column chromatography on activated silica gel (60-200 mesh, 350^o C, 24 hrs) in glass column (17mm OD) with silica packing length of 35cm topped by 5cm of Na_2SO_4. Three fractions were collected; Fraction 1 (50mL,100% hexane), Fraction 2 (200mL, 2.5% DCM-97.5% hexane) and Fraction 3 (50mL, 15% DCM-85% hexane).

Each fraction was concentrated to 1.5mL on rotary evaporator and then to 50µl under a gentle N_2 stream. The solution was then transferred to a 150µl GC vial quantitatively along with 3x25µl solvent washings and concentrated to dryness under N_2 stream. The sample was reconstituted in 20µl of performance standard (Labeled BDE-138) and analyzed by HRGC-HRMS.

The analysis was performed on a Micromass AutoSpec-Ultima mass spectrometer coupled to HP6890 Gas Chromatograph operating at 10,000 resolution. A DB-5HT GC capillary column (30m x 0.245mm x 0.10µm, J&W Scientific) was used for separation of the PBDEs. The GC operating conditions were to ramp the oven from initial 100^{o}C to 320^{o}C at the rate of 5^{o}C/min with injector temperature at 275^{o}C. A constant flow of helium at 0.70mL/min was maintained during the run. Selective Ion Recording (SIR) in Electron Ionization Positive ion (EI^+) mode was used to identify the targeted compounds. The analysis was carried out by scanning ten Voltage SIR scan functions with each function corresponding to one specific level of bromination in the PBDE congeners. The M^+ ions were monitored for congeners up to hexabromo substitution and M^+ -2Br ions for heptabromo to decabromo congeners. High boiling PFK was used for instrument calibration and flowed in continuously to provide for internal lock mass ions during acquisition of each function. Identification of peaks was based on the presence of two isotopic peaks for each monitored congener in correct isotopic ion ratios and elution time with reference to the spiked ^{13}C congener for the function group. The amount of native congener present was determined by correlating the peak areas of the native congener and the spiked congener of known concentration using MassLynx and QuanLynx software of Micromass. Similarly, recoveries of spiked congeners were calculated with reference to the ^{13}C BDE-138, the labeled performance standard.

Results and Discussion

The following 27 PBDEs were monitored in the study: BDE 3, BDE 7, BDE 15, BDE 17, BDE 28, BDE 49, BDE 71, BDE 47, BDE 66, BDE 77, BDE 100, BDE 119, BDE 99, BDE 85, BDE 126, BDE 154, BDE 153, BDE 138, BDE 156, BDE 184, BDE 183, BDE 191, BDE 197, BDE 196, BDE 207, BDE 206, BDE 209.

Only 16 congeners were detected in the blank matrix sample. The amount of detected native PBDEs is shown in Figure 1. The average concentration was in the range of 0.02-0.09 ng/sample except BDE-47, BDE-99, BDE-100 and BDE-209 which were at much higher concentrations, 0.23-1.70ng. The standard deviation, s, ranged from 0.01-0.40. The average recovery of $^{13}C_{12}$ labeled surrogates was 65% and most of the congeners were within the range of 51-90% except BDE-3 which is well documented to be more volatile than all other congeners. Based on the data, the pooled standard deviation (Sp) for native PBDEs calculated by the relationship

$$S_{pooled} = \sqrt{ (\sum(df_i \times S_i^2) / \sum df_i)}$$

is 0.153 and LoQ is 1.53ng/sample or 1.02ng/g of dry wt. of sample. A plot of correlation between mean concentration and SD is shown in Figure 2.

Conclusion

Based on the study presented in this report the LoQ for PBDEs was determined as 1.02ng/g of the dry weight of the

matrix from cattle farm manure. The dry weight was 4.4% of the wet weight. This LoQ value includes all the detected congeners. A linear regression plot for the congener concentration and standard deviation is given which can also be used to determine LoQ. The value of LoQ determined is based on the pooled standard deviation and hence could be applicable to any single PBDE congener.

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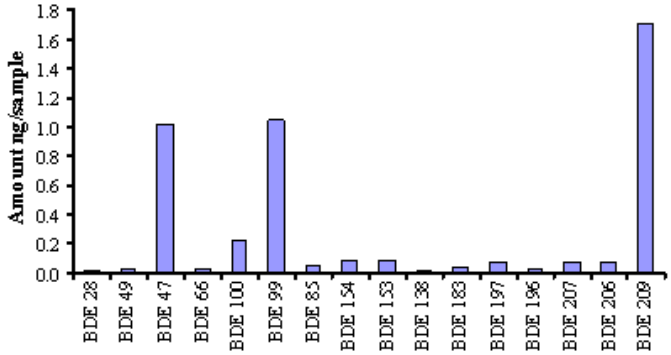

Figure 1 Mean Amount in ng/Sample of Native PBDE Congeners detected in Manure Background

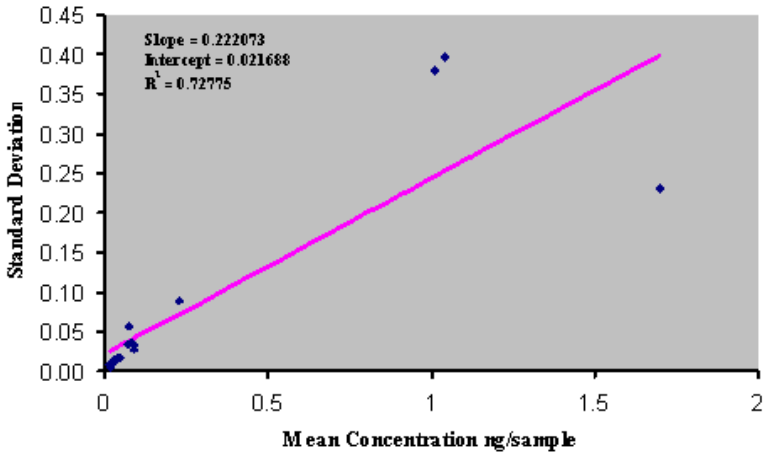

Figure 2 Regression Plot for SD vs. Mean Concentration of Congeners

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