Determination of polybrominated diphenyl ethers retardants in fish tissues samples from Vaal River, South Africa

Chokwe TB[1*], Okonkwo OJ[2], Krùger E[1], Hariram R[1]

[1]Scientific Services, Rand Water, 2 Barrage Road, Vereeniging, 1930, South Africa; [2]Department of Environmental, Water and Earth Sciences, Tshwane University of Technology, Nelson Mandela Drive, Pretoria, 0001, South Africa

tchokwe@randwater.co.za

INTRODUCTION

In recent years, polybrominated diphenyl ethers (PBDEs) have emerged as a subject of great concern because of their increasing levels in the human body, causing disturbance of the thyroid hormone homeostasis and chronic neurotoxicity (Alaee et al, 2003). PBDEs have been produced commercially in three major formulations: penta-BDE (consisting of 38-49% BDE47 and BDE99 each), octa-BDE (mixture of hexa-BDEs to octa-BDEs) and deca-BDE (up to 97% BDE209) (Abdallah et al, 2009). Since commercial PBDEs mixtures are incorporated into polymers and other substrates, they are more easily released into the environment during manufacture, incorporation into products, product disposal as well as during product repairs/ recycling (Li et al, 2011). The major components in the penta- and octa-commercial BDE products were listed as persistent organic pollutants (POPs) at the fourth meeting of the Conference of the Parties (COP4) of the Stockholm Convention in 2009 (Abdallah et al, 2009; Li et al, 2011).

One of the greatest challenges confronting global water resources is water pollution caused by numerous human activities. Freshwater systems, particularly the rivers which are receptacles for most urban sewage, industrial and agricultural discharges as well as highly contaminated wastes from informal settlements are most affected (Daso et al, 2011). Of serious concern is exposure of PBDEs to aquatic organisms (Lacorte *et al*, 2010). Additionally, aquatic organisms bio-accumulate PBDEs (de Wit *et al*, 2006); thus, interest has grown in determining these pollutants in various environmental matrices (desJardins Anderson & MacRae, 2006; Peng *et al*, 2007; Losada *et al*, 2009; Lacorte *et al*, 2010). Therefore, an important goal of the study was to report on the quantification of PBDEs in two fish species from sites selected on the Vaal River Catchment which is one of the largest river catchments in South Africa. It is hoped that the results obtained will serve as valuable references for future risk assessment and environmental management measures in the Vaal River Catchment.

MATERIALS AND METHODS

Standards and Reagents

The solvents acetone, methanol, dichloromethane and hexane used in the study were of GC grade and were used without further purification. Each certified standard solutions (1.2 mL of 20 mg/L) of seven

PBDEs congeners (BDE28, BDE47, BDE100, BDE99, BDE154, BDE153 and BDE183) were purchased from AccuStandard (USA). Anhydrous sodium sulphate (purity 99.9%), granular powder was purchased from Merck, sodium sulphate cartridges were purchased from Chemetrix, South Africa while Helium as He 5.5 pure was purchased from Air Products, Vereeniging, South Africa.

Fish Sample Collection

Sixteen (16) fishes (*Labeo umbratus*, n= 7 and *Carp*, n= 9) were collected from the Vaal River. The selected fishes were caught using a fishing rod. Fish selected for analysis were killed by a blow to the head and were individually wrapped in aluminum foil, placed in plastic bags packed with ice for transport to the laboratory where the samples were frozen pending preparation of the tissue samples. Fish tissue samples were prepared following the guidance in EPA (2000). Techniques to minimize potential for sample contamination were used. During sample preparation, non-talc nitrile gloves were worn and heavy-duty aluminum foil cutting board was used. The gloves and foil were changed between samples and the cutting board cleaned between samples. The fish were thawed enough to remove the foil wrapper and rinsed with tap water, then deionized water to remove any adhering debris. Before use, the skins were removed and the sample, muscle tissues, collected.

Muscle tissue sample preparation: Homogenization, Extraction and Clean-up

About 5 g of the tissue was weighed and mixed with 20 g anhydrous sodium sulphate and grounded to free flowing. The contents were extracted with 20 ml of hexane/acetone mixture (4:1) at 55°C for 45 min in two cycles. After the ultrasonic extraction, the extracts were combined and placed in separating funnel. Roughly 10 ml of concentrated sulphuric acid was added, the mixture shaken for 5 min and phase separated. The acid layer was washed once with 25 ml of hexane. The hexane extracts were combined and washed with 40% (v/v) sulphuric acid for further removal of residual lipids. The phases were separated and the organic phase evaporated to dryness using TurboVap II instrument. The residue was re-constituted with 2.5 ml of MeOH, diluted to 250 ml with MilliQ water and acidified to pH 3 with acetic acid. The mixture was then passed through an SPE cartridge conditioned with 6 ml of 30% MeOH in DCM, followed by 6 ml DCM. After passing the mixture through the cartridge, the cartridge was dried for 1 h and PBDEs eluted with DCM: hexane (4:1) mixture. The eluates were then concentrated under a gentle stream of nitrogen to dryness. It was reconstituted with 100 µl of hexane. The internal standards (PBB80, 20 µl of 2 ppm) was added into the extract, the volume made up to 200 µl and 1 µl of a mixture of extracts and internal standards injected into the GC-MS.

Instrumentation and GC/MS Conditions

An Agilent 6890 GC equipped with 5975 mass selective detector (MSD) was used for analysis. The GC was equipped with a Agilent autosampler A7693 and separation performed on a capillary column (Restek RTx-1614, film thickness 0.10 µm, 15m x 0.25mm I.D., (Chromspec cc South Africa)). The GC/MS conditions used for analysis were as follows: carrier gas He; linear velocity, 40 cm s^{-1}; injector temperature, 275 °C; transfer line temperature, 280 °C; ion source 150 °C. For analysis 1µl splitless injection were carried out by autosampler. The GC temperature program conditions were as follows: initial temperature 50°C, heated to 120 °C by a temperature ramp of 7.5 °C/min then 275 °C by a

temperature ramp of 15 °C/min then finally heated to 280 °C (held for 1 min) by a temperature ramp of 25°C/ min.

RESULTS AND DISCUSSION

Recovery test

Different methods (aminopropyl cartridge, destruction with concentrated sulphuric acid, silica gel stirring and/or their combinations) were tested for lipid removal (2.92% lipids, n= 16) and recovery of PBDEs from tissue samples. Concentrated sulphuric acid wash was used removed ~80% lipid. However, by washing the post-extract with concentrated acid, followed by a dilute sulphuric acid (40% (v/v)), ~97% lipids (n=16) was removed and the recovery after lipids removal and SPE clean up ranged between 60 ± 9.21 (BDE228) – 90.88 ± 6.32 (BDE47).

Concentration of PBDEs in fish samples

All target compounds, as presented in Figure 1, were quantified in both fish species with concentration from *Carp* higher than the detected concentration in *Labeo Umbratus* except for BDE28 which was found higher in *Labeo Umbratus*. Sum concentrations of PBDEs (∑PBDEs) were 18.68 ng/g ww and 11.60 ng/g ww in *Carp* and *Labeo Umbratus*; respectively with BDE99 being the most abundant PBDE congener. The profile of the detected PBDEs reflected the exposure to Penta-, Octa-PBDEs mixtures and to some extent Deca-PBDEs though it was not included in the study. The pattern of PBDEs detected in sediments from the Vaal River (Chokwe et al, 2016) were also dominated by BDE153 (component of octa-BDE) and BDE99 (component of penta-BDE).

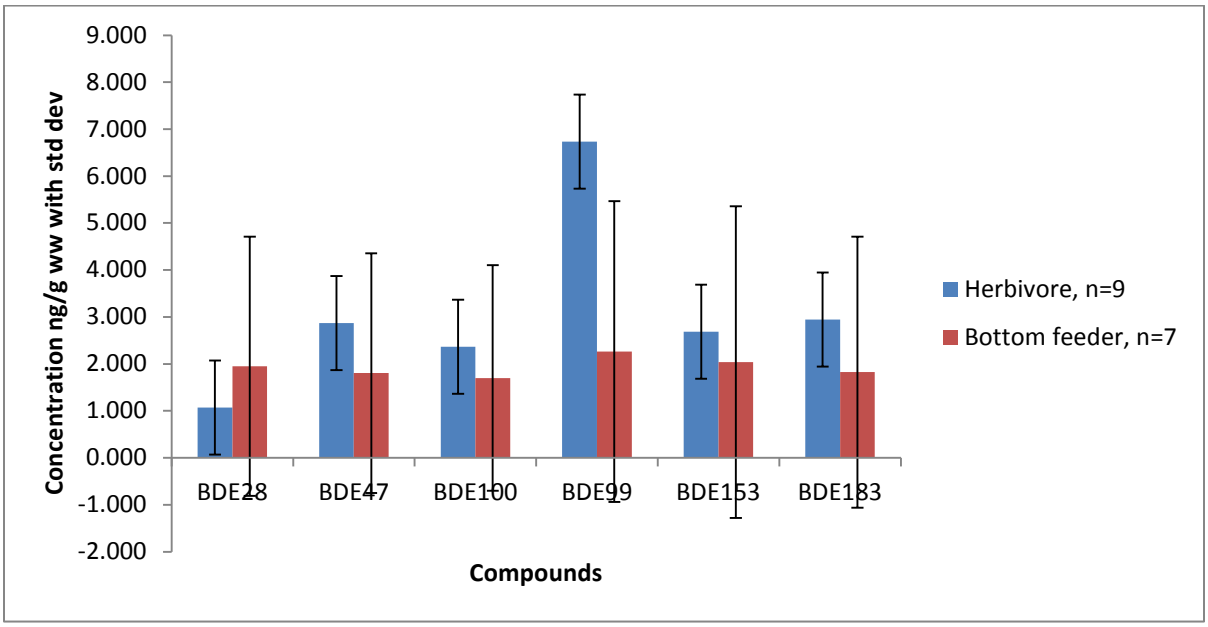

Fig. 1: Concentrations of PBDEs in two fish species

In conclusion, these results showed milder contamination of PBDEs in aquatic environment, however these pollutants should be monitored as commercial products containing BFRs, such as televisions, mobile phones and computers, may be the sources of BFRs exposure to South African environment.

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