Alkylphenol ethoxylates and brominated flame retardants in fish tissue samples from Vaal Barrage, South Africa

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INTRODUCTION

Large cities are known to act as major sources of pollution for aquatic systems. This is due in part to city sewage systems receiving effluents of the many industries located within their territories, along with domestic wastewater. All these effluents contain different xenobiotic and toxic substances[1]. Among the chemicals potentially associated with urban wastewater, alkylphenol ethoxylates (APEs) and brominated flame retardants (BFRs) have been identified as an important toxic group and a cause for concern due to their extended use in several consumer and personal-care products and as flame retardants. These products, as well as their degradation products, are reported to be persistent, bio-accumulating and highly toxic, with potential for estrogenic activity[2-3].

Alkylphenol ethoxylate compounds find their way into the environment in their original form and as natural degradation products of the parent alkylphenol ethoxylates that are popularly used in commerce for their surfactant properties[4]. Different PBDE congeners, like decabrominated diphenyl ether (BDE209), can be photo-chemically degraded (via either UV or sunlight) in organic and aqueous solvent systems and in soil and sediments. Degradation results in the formation of less brominated PBDE that are more persistent, bio-accumulative and toxic[5].

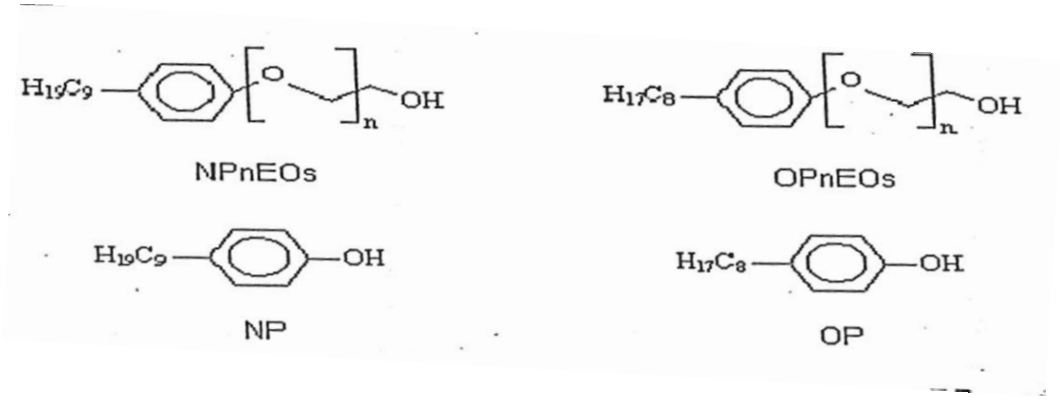

Figure 1: Structures of common APEs (nonyl- and octyl- phenol ethoxylates) and their metabolites

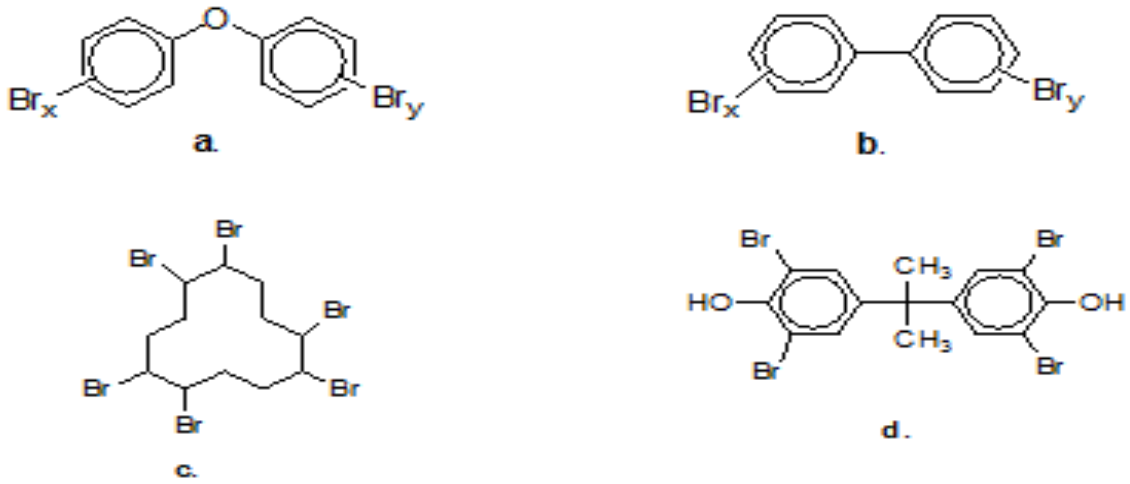

Figure 2: Structure of (a) PBDEs; (b) PBBs; (c) HBCD and (d) TBBPA

Studies conducted to date in some South African environmental samples (waters and sediments) have shown the presence of organochlorinated[6], alkylphenol ethoxylates[7] and PBDEs[8]. With the exception of recent study by Polder *et al*[9] who reported the presence of HBCD in bird egg in South Africa, the authors are not aware of any report on the determination of APEs and BFRs from any South African fish samples.

This study describes a method for the quantification of alkylphenol ethoxylates and brominated flame retardants in fish matrices using ultrasonic extraction followed by Aminopropyl cartridges combined with silica gel for removal of lipids; heptafluorobutyric anhydride derivatization and gas chromatography-mass spectrometry.

MATERIALS AND METHODS

MATERIALS

Standards and Reagents

Derivatizing agents (heptafluorobutyric anhydride (HFBA) was of analytical grade purchased from Sigma-Aldrich, South Africa. The solvents acetone and hexane used in the study were of GC grade and were used without further purification. The APEs and PBBs were purchased from Laboratories Dr Ehrenstorfer-Schäfers, Augsburg, Germany. Only the NPE, NPPE and OPPE were of technical grade and the remaining APEs, PBBs and PBDEs were of analytical grade. Tetrabromobisphenol A of technical grade as Firemaster BP4A and hexabromocyclododecane of technical grade were purchased from AccuStandard, USA. Helium as He 5.5 pure was purchased from Air Product South Africa, Vereeniging.

METHODS

Derivatization using HFBA

Into a vial, 0.15mL of; APs (1 mg L^{-1}), APEs (5 mg L^{-1}), PBBs (1 mg L^{-1}), PBDEs (1 mg L^{-1}), HBCD (5 mg L^{-1}) and TBBPA (4 mg L^{-1}); 0.1mL hexane; 40 µL of 0.1M triethylamine and 4 µL HFBA were added and the content heated

to 50°C for 30 min. Thereafter, the contents were cooled, washed with 0.5mL aqueous K_2CO_3 (5%). The organic phase was then drawn off, internal standards (Chrysene and PBB80) added and the volume made up to 1mL. Thereafter, 1 µL was injected into the gas chromatography-mass spectrometry analysis.

Instrumentation and GC/MS Conditions

An Agilent 6890 GC equipped with 5973 mass selective detector (MSD) was used for GC/MS analysis. The GC was equipped with a Gerstel autosampler. The GC separation was 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^{-1}.

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

12.5 g of the tissue was weighed and mixed with 50 g anhydrous sodium sulfate. The contents were extracted with 50 ml of hexane/acetone mixture (4:1) amended with 0.25% acetic acid at 55°C for 30 min twice. After the ultrasonic extraction, the extracts were concentrated to about 3 mL using TurboVap II instrument. Aminopropyl cartridges (APS, 500 mg, 3 mL LC-NH_2) and silica gel were used to remove lipids from the extract. The concentrated extract was first passed through pre-conditioned (Aminopropyl) cartridges at a rate of 5 mL/min. The cartridges were conditioned with 3* 3 mL acetone then 1* 3 mL of DCM and finally 3* 3 mL of hexane. The BFRs were collected first while the APEs were eluted from the cartridges with 7 mL of hexane: 2-propanol (9:1) solution. The hexane: 2-propanol was then treated with acidic silica column clean-up. This column contained 2.5 g of silica gel and 1.5 g of anhydrous sodium sulphate. The column was pre-eluted with hexane, and the extract was placed on the column and eluted with 40 mL of hexane: 2-propanol mixture. Residual fats, lipids and other material were retained on the column while the APEs and BFRs eluate was collected. The eluates were concentrated under a gentle stream of nitrogen to dryness and placed under derivatization conditions as mentioned above

RESULTS AND DISCUSSION

Four bottom feeder Labeo umbratus were collected at the Vaal Barrage, were extracted, purified and analyzed by GC-MS. These samples (as presented in Figure 3) indicated that nonylphenol ethoxylates isomers are major pollutant of the APEs while PBB101, BDE (3, 28, 99, 100 and 183) and HBCD were major pollutants for the BFRs. The concentration of these analytes ranged from 0.061 (BDE3) to 4.6 ng/g (di-NPE1). The method as developed can be extended to determine the mentioned analytes from a range of fish samples around the Vaal River catchment.

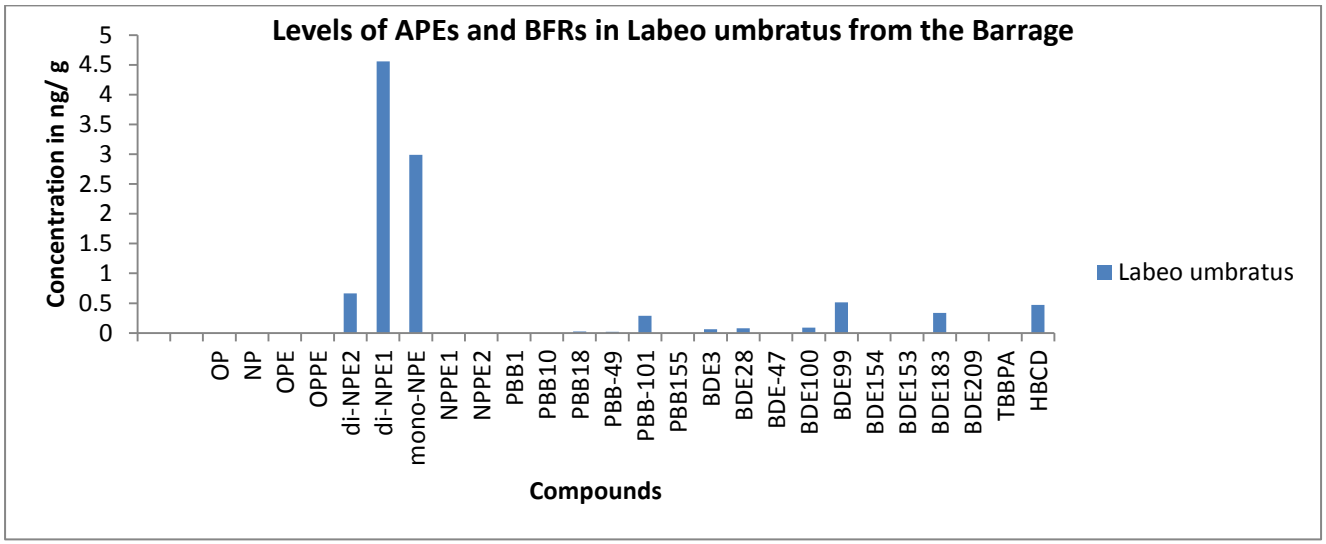

Figure 3: Levels of APEs and BFRs in Labeo umbratus, a sediment feeder, from the Barrage

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