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Analysis of polybrominated diphenyl ethers and emerging halogenated and organophosphate flame retardants in human hair and nails

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ABSTRACT

A method for the digestion, extraction, fractionation, and analysis of three classes of flame retardants, including 36 polybrominated diphenyl ethers (PBDEs), 9 halogenated alternative flame retardants (AFRs), and 12 organophosphate esters (OPEs) in human hair and nail samples was developed. The method employed HNO_3/H_2O_2 digestion, liquid–liquid extraction with (4:1 vol) hexane:dichloromethane, fractionation on a 6 g column of 2.5% water deactivated Florisil, and analysis by gas chromatographic mass spectrometry. The accuracy and precision of the method was validated using spiked samples of 6 replicates for both hair and nail samples. The method validation results showed good accuracy and precision for all PBDEs except BDE-209, all AFRs except hexabromobenzene (HBB), and all of the 12 OPEs, with average recovery efficiencies > 90% and relative standard deviations (RSDs) < 10%. The average recovery efficiencies for HBB were between 60% and 86%, with RSDs < 10%. BDE-209 had recovery efficiencies of 64% (RSD, 13%) for hair and 71% (RSD, 10%) for nail. This method was applied to analyze 5 human hair and 5 fingernail samples from the general student population at Indiana University Bloomington campus. BDE-47 and BDE-99 were the predominant PBDEs detected in both hair and nail samples, with a concentration range of 11–620 and 4.6–780 ng/g (dry weight) in hair and 7.3–43 and 2.1–11 ng/g in nails, respectively. Di-(2-ethylhexyl)-tetrabromophthalate (TBPH) and 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (TBB) were detected in all the samples, with concentrations of 20–240 and 11–350 ng/g in hair and <17–80 and <9.2–71 ng/g in nails, respectively. Among the 12 OPEs analyzed, *tris*(2-chloroethyl)phosphate (TCEP), *tris*(1-chloro-2-propyl)phosphate (TCIPP), *tris*(1,3-dichloro-2-propyl) phosphate (TDCIPP), and triphenyl phosphate (TPHP) were most often detected. The concentrations of these OPEs (summed together) were 1100–3900 and 380–18,000 ng/g in hair and nails, respectively. These levels exceed those of both the PBDEs and the AFRs.

1. Introduction

Flame retardants are added to numerous household and industrial products to enhance fire safety, but the widely used polybrominated diphenyl ethers (PBDEs) were taken off the market because of their environmental ubiquity and potential adverse health effects [1]. Alternative flame retardants (AFRs) have been introduced to replace PBDEs. For example, 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (TBB) and di-(2-ethylhexyl)-tetrabromophthalate (TBPH) are the two main components of Firemaster 550, which is now one of the most widely used commercial flame retardant mixtures. Other AFRs include 1,2-*bis*(2,4,6-tribromophenoxy)ethane (TBE), pentabromoethyl benzene (PBEB), hexabromobenzene (HBB), and pentabromobenzene (PBBZ) [2]. The highly chlorinated flame retardant, Dechlorane Plus (*syn*- and *anti*-DP) is also on the market. More recently, organophosphate esters (OPEs) are being increasingly used in response to the restrictions on PBDEs [3]. Halogenated as well as nonhalogenated aryl OPEs are used as flame retardants in various kinds of products including building materials, electronics, plastics, furniture, and textiles. Nonhalogenated alkyl OPEs tend to be used as plasticizers and antifoaming agents in hydraulic fluids, lacquers, and floor polishes [4].

Like PBDEs, AFRs and OPEs have been detected in various environmental media, including water [5], air [3], indoor dust [6,7], household products such as furniture [7], and biological

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samples [8]. Recent studies have suggested that the concentrations of replacement flame retardants in air have been increasing, and some of their concentrations have already surpassed those of PBDEs [3,9]. The analysis of brominated flame retardants (BFRs, including PBDEs and AFRs) and OPEs in atmospheric particle samples from a European arctic site demonstrated that the OPE concentrations were 1-2 orders of magnitude higher than those of BFRs [3]. People are continuously exposed to flame retardants, and thus, it is important to understand the details of human exposure to both older and newer flame retardants.

Biomonitoring is a useful tool to assess a population's exposure to flame retardants [10]. One of the most common complications of conventional biomonitoring, however, is the invasiveness of biological sampling, which most often includes sampling of human serum [11,12], adipose tissue [13], and milk [14]. Hair and nail samples are non-invasive biomonitors and obtaining such samples is simple and cost-effective [15,16]. In fact, hair and nails have been used in forensic and clinical studies for the analysis of toxic metals, pharmaceuticals, and illicit drugs [16]. More recently hair has been used to assess human exposure to persistent organic pollutants (POPs) [17]. These have included pesticides, polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins and dibenzofurans, Dechlorane Plus, and PBDEs [10,12,15,16,18]. A more recent study measured both PBDEs (by gas chromatographic mass spectrometry, GC–MS) and OPEs (by liquid chromatography coupled with tandem mass spectrometry) in human hair [19]; AFRs were not included in this study. The present study was designed to develop a method to simultaneously measure PBDEs, AFRs, and OPEs in hair and nail samples with GC-MS. The use of human nail samples in assessing human exposure to POPs is rare [20]. Only one study determined the concentrations of perfluorooctane sulfate (PFOS) and perfluorooctanoic acid (PFOAs) in human fingernails [20].

The main objective of this study was to develop and validate a GC-MS based method for the extraction, cleanup, and analysis of PBDEs, AFRs, and OPEs in both hair and nail samples. The accuracy and precision of the proposed method was determined using spiked replicate samples. Method applicability was tested for human hair and nail samples collected from the Indiana University Bloomington student population. Our study is the first to simultaneously measure PBDEs, AFRs, and OPEs in hair, finger- and toenail samples using GC-MS. This method development is a critical first step in being able to simultaneously analyze a large suite of restricted and alternative flame retardants in both hair and nail samples in order to facilitate their future use as human biomonitors.

2. Experimental

2.1. Chemicals and materials

A solution mixture BFR-PAR [consisting of BDE-7, 10, 15, 17, 28, 30, 47, 49, 66, 71, 77, 85, 99, 100, 119, 126, 138–140, 153, 154, 156, 169, 180, 183, 184, 191, 196, 197, 201, and 203–209, PBEB, HBB, and 1,2-*bis*(2,4,6-tribromophenoxy)ethane (TBE)] and individual non-PBDEs standards [including tetrabromo-*p*-xylene (pTBX), PBBZ, TBB, TBPH, *syn*- and *anti*-DP] were obtained from Wellington Laboratories (Guelph, ON). Individual OPE standards including *tri*-n-butylphosphate (TnBP), *tris*(2-chloroethyl)phosphate (TCEP), *tris*(1-chloro-2-propyl)phosphate (TCIPP), *tris*(1,3-dichloro-2-propyl)phosphate (TDCIPP), triphenyl phosphate (TPHP), 2-ethylhexyl-diphenyl-phosphate (EHDP), *tris*(2-ethylhexyl)phosphate (TEHP), *tri*-o-tolyl-phosphate (TOTP), tri-p-tolyl-phosphate (TPTP), *tris*(2-isopropylphenyl) phosphate (TIPPP), *tris*(3,5-dimethylphenyl)phosphate (TDMPP) were also purchased from Wellington Laboratories. *Tris*(4-tert-butylphenyl) phosphate (TBPP) was purchased from Sigma-Aldrich (St. Louis, MO).

BDE-77 and BDE-166 were purchased from AccuStandard (New Haven, CT), and $^{13}C_{12}$-BDE-209 was purchased from Wellington Laboratories. These compounds were used as surrogate recovery standards for the PBDEs and the AFRs. *Tris*(2-chloroethyl) phosphate-d_{12} from Sigma-Aldrich and $^{13}C_{18}$-triphenyl phosphate from Wellington Laboratories were employed as the surrogate recovery standards for OPEs analysis. BDE-118 from AccuStandard and BDE-181 and BB-209 from Wellington Laboratories were used as the internal quantitation standards for PBDEs and AFRs analysis. Deuterated PAH standards, anthracene-d_{10}, dibenz[*a*]anthracene-d_{12}, and perylene-d_{12} were purchased from Chem Service (West Chester, PA) and used as the internal quantitation standards for OPEs analyses. Florisil (Sigma-Aldrich, St. Louis) was baked at 300 °C overnight, cooled to room temperature, deactivated with 2.5% (by weight) of water, and stored in a desiccator overnight before use. Anhydrous sodium sulfate (Fisher Chemical, Fair Lawn, NJ) was baked at 500 °C overnight.

2.2. Hair and nail sampling

Hair and fingernail samples for method development and validation were from students, colleagues, and friends from Indiana University's Bloomington campus. Their ages ranged from 19 to 38, and all were apparently healthy individuals. The sampling of human hair and nails was approved by the Indiana University Institutional Review Board, and all participants signed an informed consent form. Hair samples were cut close to the scalp with stainless-steel scissors pre-cleaned with ethyl alcohol and then wrapped in aluminum foil, sealed in a Ziploc bag, and stored at −20 °C until extraction. Fingernail samples were collected with ethyl alcohol sonicated stainless-steel nail clippers and stored the same as hair samples.

2.3. Sample pretreatment

Because no method was available to simultaneously measure PBDEs, AFRs, and OPEs in human hair or nails with GC-MS, the available methods relating to the analyses of persistent organic pollutants (POPs) in hair and nails in the literature [12,19–21] were integrated and modified according to the physicochemical properties of our target analytes. We did not use any sample washing before extraction because a recent study suggested that there is no available medium that could exclusively remove external contaminants from hair [22]. Our main criteria was to get good recoveries of the target compounds and clean fractions that would facilitate their instrumental analysis. In our case, we used the same procedures for both the hair and nail samples even though we generally had 2–5 times more mass of hair compared to nails.

The hair and the nail samples were analyzed separately. Approximately 100 mg hair or all of the ten fingernail samples was weighed in a 50 mL glass centrifuge tube. Upon spiking with a known amount of the surrogate recovery standards, the samples were digested with 2 mL HNO_3/H_2O_2 (1:1 vol) for 2 h in a 60 °C water bath. The resulting mixture was diluted by adding 10 mL of HPLC grade water. This diluted mixture was liquid-liquid extracted with 10 mL of hexane:dichloromethane (4:1 vol). The tubes were shaken vigorously for 5 min, centrifuged for 10 min at 3500 rpm, and the upper organic layer was transferred to a pear shape flask. This step was repeated twice, and all of the organic layers were combined. The extract was rotary evaporated to approximately 1 mL with one solvent change with 25 mL of hexane.

Samples were fractionated on a 1-cm dia. glass column packed with 6 g of 2.5% (by weight) water deactivated Florisil and 2 cm anhydrous sodium sulfate on top. Samples were loaded and