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Identification, source and fate of flame-retardants in the environment: Past, present and future concerns

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Student Name:	**Mark Joseph La Guardia**		
Department:	**Earth & Environmental Sciences**	**First Supervisor:**	**Prof. Graham Mills**
Start Date: (or progression date for Prof Doc students)			

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Time:	

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Identification, source and fate of flame-retardants in the environment: Past, present and future concerns

A Thesis
Presented to
University of Portsmouth

In partial fulfillment
of the requirements of the Degree of
Doctor of Philosophy
by Publication

by
Mark J. La Guardia
2019

This work is dedicated to my wife, Terumi, our children,
Cherise, Kristin and Simone, and to their growing families, with
my gratitude for the love and support they have all given me
throughout my endeavors.

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Acknowledgements

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Declaration

Whilst registered as a candidate for the above degree, I have not been registered for any other research award. The results and collusions embodied in this thesis are the work of the named candidate and have not been submitted for any other academic award.

Identification, source and fate of flame-retardants in the environment: Past, present and future concerns

Mark J. La Guardia

Abstract

Over the past 20 years as a senior scientist at the Virginia Institute of Marine Science, I have first-authored 10 and coauthored 18 scientific publications with collaborators from academia, government and the private sector. The focus of these studies included: analytical method development, source identification, bioavailability, biomagnification, degradation and environmental and human health exposure to anthropogenic contaminants known as flame-retardants (FRs). Prior to my Master's dissertation I conducted scientific investigations that resulted in two first-authored publications and six co-authorships; this included a 2001 communication published in the journal *Nature* that highlighted land application of FR-laden sewage sludge on agricultural fields as a route of environmental release and possible path for human exposure. My dissertation research produced several publications, including a detailed analysis of the congener composition of the widely used FR, polybrominated diphenyl ether (PBDE) technical mixtures, cited over 1,000 times to date (Google Scholar, date accessed 220419). I followed with several publications on analytical advances, establishing the identities of PBDE degradation products and additional novel-FRs in environmental matrices. From these advances I observed *in situ* debromination of the PBDE decabromodiphenyl ether (BDE-209) and published on how this process increased BDE-209's toxicity. I then explored FR distributions in remote areas, co-authoring two publications that identified contributions from Antarctic research bases to that pristine environment and first-authored one of the first papers describing FRs in the continent of Africa. These publications supported the case for restricting the FRs, PBDEs and hexabromocyclododecane in U.S. and European commerce and their later listing as Persistent Organic Pollutants (POPs) by the United Nation's Stockholm Convention, restricting their usage globally.

I next studied the extent of human exposure from FR treated consumer products, focusing on preadolescent females and those of childbearing years. These individuals have a greater risk of attendant adverse health consequences. Here, I first-authored a paper indicating that, despite the removal of PBDEs from commerce ten years earlier, human exposure continued due to older treated products that remained in use. I then investigated FR exposures in the workplace, which included three co-authorships identifying several workplace exposure scenarios that exceeded those of the general population. In addition, I examined indoor air exposure and first-authored a publication on the contribution of airborne FR-laden particulate size to human exposure.

In conclusion, these contributions improved our knowledge on the sources, fate and exposure to flame-retardants.

IDENTIFICATION, SOURCE AND FATE OF FLAME-RETARDANTS IN THE ENVIRONMENT: PAST, PRESENT AND FUTURE CONCERNS

Chapter One - Introduction: An overview on usage, regulations, dissemination and health effects of flame retardants

Flame-retardants (FRs) are chemical additives that are incorporated into materials to impede the ignition and spread of fire. In this discussion the material focused on are polymers. Since the mid-1970s, FRs have been added to consumer, transportation, electrical/electronic and building products to meet regulations governing fire safety (e.g., California, USA 1975 home furniture flammability standards, Technical Bulletin 117 (DCA, 2000) and The Furniture and Furnishings (Fire) (Safety) Regulations 1980 (UK, 1988)). By interfering with the combustion process, FRs interrupt the radical gas phase and suppress flammability or produce an endothermic reaction releasing water vapor that cool the polymer and interrupt the combustion process or create a char layer that shields the material from oxygen and a flame or other heat source, limiting its ability to act as a fuel source. FRs exists in many different chemical compositions; they may contain halogens (bromine and chlorine), phosphorus, nitrogen, aluminum, magnesium, boron, antimony, molybdenum, or recently developed nano-fillers. In 2015, worldwide consumption of FRs reached 2.5 million tons valued at $5.8 billion (USD) with an estimated annual growth rate of 4.9%. Consumption is expected to double to $12 billion or 4.0 million tons by 2025 (Addit. Poly., 2017).

The construction industry is the largest consumer of FRs and accounts for about 30% of the global demand. The Asia-Pacific market was the largest electronic market for FRs in 2015. It accounted for approximately 48% of the global electronics-associated FR demand, followed by North America (23%) and Western Europe (19%). The European market consumed more than 600,000 tons of FRs in 2015, due to its strong fire safety regulations, widespread industrialization, and broad awareness of fire safety — an awareness further heightened after London’s catastrophic Grenfell Tower fire in 2017. Non-halogenated chemicals were the leading FR chemical class in 2015. They accounted for 58% of the total market volume, exceeding 1.4 million tons. Brominated flame retardants (BFRs) were in second position. These are widely used in electronic and electrical products, as well as insulating and polyurethane foams. BFRs had a market share of 28% in Asia, 6.4% and 12% in Western Europe and in North America respectively. However, environmental and health concerns regarding halogenated FRs has increased the demand for non-halogenated organophosphates and inorganic FRs (Addit. Poly., 2011).

Concern over the environmental and human health effects of halogenated FRs is not new. Polybrominated biphenyls (PBBs), the main ingredient of FireMaster BP-6 (Michigan Chemical Corp., St. Louis, Michigan, USA), used in thermoplastics electrical casing (e.g., computer monitors, televisions) coatings, lacquers and in polyurethane foam, was discontinued by the manufacturer following the accidental contamination of livestock feed in 1973. This accident necessitated the destruction of millions of dollars' worth of Michigan livestock and agricultural products, such as eggs, to reduce potential human exposure to PBBs (Di Carlo et al., 1978). This prompted the 1976 U.S. PBB production ban, occurring after the surfacing of reports of contaminated air, water, sediments, and biota near PBB manufacturing facilities. In 2010 PBBs were added to Appendix A of the United Nation's Stockholm Convention, identifying chemicals with bioaccumulative and toxicity concerns, listing them as Persistent Organic Pollutants (POPs) and restricting their global production and usage (Stockholm Convention, 2018). Shortly after the Michigan PBB incident, samples collected near U.S. facilities that manufactured or used other brominated-FRs were found to contain polybrominated diphenyl ethers or PBDEs (DeCarlo, 1979; Erickson et al., 1980). Thereafter in Europe, Andersson and Blomkvist (1981) reported finding PBDEs in fish collected from southern Sweden's Viskan-Klosterfjorden water system. Andersson and Blomkvist reported that the source(s) of these PBDEs were not determined at that time or were they detected here in previous samplings. In 1983 Stafford (1983) observed trophic transfers of PBDEs in tissues and eggs of piscivorous birds collected along the U.S. eastern seaboard, the Gulf of Mexico, and the Great Lakes. The earliest known occurrence of PBDEs was in a Michigan (USA) bald eagle (*Haliaeetus leucocephalus*) egg collected in 1969 (Stafford, 1983).

Nonetheless, rising concerns over property damage, injuries and loss of life caused by fires eclipsed concerns over the environmental dissemination of FRs. During the 1970s, U.S. residential fires caused thousands of deaths and injuries each year, damaging over a million structures and costing billions in property damage (Evarts, 2018). Smoking materials (i.e. lighted tobacco products) were a major source of unintentional fires. The primary path was smoldering smoking materials igniting trash, mattresses and upholstered furniture. In 1973 the U.S. Department of Commerce enacted a mandatory U.S. Standard for the Flammability of Mattresses and Mattress Pads, Title 16 CFR 1632 (US, 2011). The furniture industry introduced and adopted a similar measure soon after, with a voluntary standard by the Upholstered Furniture Action Council in 1978. This was judged substantial enough to remove the need for mandatory federal action at that time (Hall, 2013). However, in 1975 California's Technical Bill 117 (TB-117) was signed into law and established the 12-second flammability standard for products sold in the state of California (USA) (DCA, 2013). TB-117 required that exposed polyurethane foam used in consumer products such as furniture and bedding be capable of withstanding an open flame for 12 seconds without ignition. To comply, manufactures incorporated FRs, primarily PBDEs, into their products. Since California represents a large share of the U.S. market for furniture, local and global manufacturers applied this standard to all the furniture they made. The UK followed California with the signing of The Furniture and Furnishings (Fire) (Safety) Regulations 1980 (UK 1988), setting the standard for Europe. These fire safety standards and those enacted for the electronic, construction, and transportation industries fueled a global demand for FRs.

From the mid-1980s through the 1990s reports confirmed the global distribution of PBDEs within the environment and their presence within food webs. For example, Jansson and Asplund (1987) detected PBDEs in seals and pelagic fish-eating birds collected from the Baltic Sea, the North Sea, and the Arctic Ocean. PBDE concentrations in pilot whale blubber caught off of the North Atlantic's Faroe Island exceeded previous reports, with concentrations increasing from 1994 to 1996 (Lindström et al., 1999). In North America, beluga whale blubber collected from Canada's Cumberland Sound showed PBDE concentrations that increased 6.5-fold from 1982 to 1997 (Stern, 2000). Alaee et al. (1999) observed PBDEs in lake trout collected from the Great Lakes, Canada. In the U.S., Kuehl et al. (1991) reported PBDEs in dolphins collected from 1987 to 1988 along the southern Atlantic coast, and in the Mid-Atlantic region. **Hale et al. (2001a)**[1] observed PBDEs in freshwater fish tissue collected in 1998 and 1999 from the Roanoke and Dan River basins. In that study, carp tissue concentrations from the Hyco R., a tributary of the Dan R., exceeded the highest fish tissue values previously reported in the world. In the Pacific, PBDEs were detected in both seal blubber and their prey collected between 1989 and 1998 in San Francisco Bay, USA (She et al, 2000); in Japan, Watanabe et al. (1987) found similar results

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[1] First authored and coauthored publications are identified by bold type citations.

in marine fish, shellfish, and river and marine sediments. Approximately 30 years after their introduction in commerce, PBDEs were detected in sperm whale blubber, indicating their arrival in deep ocean waters (de Boer et al., 1998). In these samples concentrations were approximately 50-fold higher than PBBs.

Point sources (i.e., chemical manufacturing and usage sites), along with intentional (e.g., pesticides) and accidental releases were historically considered primary routes for anthropogenic contaminants entering the environment and food web. In the case of human exposure to FRs, however, secondary exposure — for example, from consumer products — appears to be of greater concern. Bergman et al. (1997) first reported PBDEs and other FRs in indoor air samples collected from offices containing electrical equipment such as computers. Rudel et al. (2003) detected Penta-BDE formulation related congeners in house-dust samples collected from 120 homes on Cape Cod, Massachusetts, USA. *(Note: PBDEs are manufactured as three technical mixtures: Penta-, Octa- and Deca-BDE at three levels of bromination: nominally penta-BDE, octa-BDE and deca-BDE. To differentiate PBDE technical mixtures from homologue grouping the first letter of the technical mixture is capitalized i.e. Penta-, Octa- and Deca-BDE. Lower case e.g. penta-BDE, is used when referring to the number of bromines of the homologue. The Penta-formulation, primarily added to polyurethane foam, contains tetra- through hexa-BDEs; specifically 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) (~40% of formulation weight) and 2,2',4,4',5-pentabromodiphenyl ether (BDE-99) (~47%). The Octa- formulation used in thermoplastics includes hexa- through deca-BDEs, mostly the hepta-BDE 2, 2', 3, 4, 4', 5', 6-heptabromodiphenyl ether (BDE-183) at 42% by weight. The Deca-formulation contains > 91% by weight decabromodiphenyl ether (BDE-209). It is used mostly to flame-retard textiles and electronic housings, e.g., televisions and computers (****La Guardia et al. 2006****).)* The authors attributed their detection in house dust to releases from consumer products containing treated polyurethane foam (e.g., furniture padding), a TB-117 requirement. These releases then accumulate in indoor dust, eventually entering wastewater streams. As these streams are treated, the FRs within are largely sequestered in the solids, i.e. sewage sludge. **Hale et al. (2001b)** detected Penta-BDE formulation constituents in U.S. sewage sludge at concentrations exceeding European sludge by 10- to 100-fold. This was proportionate to the greater U.S. Penta-BDE demand, which accounted for 49% (33,100 metric tonnes (MT) of the 2001 global demand, followed by Asia at 37% (24,650 MT) and Europe, 12% (8,360 MT) (Law et al., 2006). Releases from building, consumer, and scientific products accompanying researchers were also considered the source of PBDEs (**Hale et al., 2008**) and the FR 1, 2, 5, 6, 9, 10-hexabromocyclododecane (**HBCDD** or HBCD) (added to polystyrene building insulation) (**Chen et al., 2015**) detected in biota collected near wastewater discharge sites from the Antarctic U.S. McMurdo and New Zealand-operated Scott research bases. FR patterns observed in associated indoor dust and wastewater sludge supported this hypothesis. **La Guardia et al. (2010)** detected these and several additional FRs in U.S. sewage sludge, presumably also arising from consumer products. These included: HBCDD, PBDEs and PBDE alternatives (2-ethylhexyl 2, 3, 4, 5-tetrabromobenzoate (**EH-TBB** or TBB), bis (2-ethylhexyl) tetrabromophthalate (**BEH-TEBP** or TBPH), 1, 2-bis (2, 4, 6-tribromophenoxy) ethane (BTBPE) and decabromodiphenyl ethane (DBDPE) and chlorinated-FRs; *syn*- and *anti*-dechlorane (used in electrical wires and cables, computer connectors, and plastic roofing materials).

Release from consumer products into the indoor and eventually the outdoor environment presents a pathway for human exposure. Norén et al. (2000) reported that in breast milk collected from Swedish women, PBDE concentrations had doubled every five years between 1972 and 1997. Akutsu et al. (2003) observed similar concentrations and time trends in Japanese breast milk. In California, PBDE breast milk concentrations collected between 2003 and 2005 were more than an order of magnitude higher than those reported from Asia and Europe (Park et al., 2011), again proportional to FR consumption rates in the U.S. **Wu et al. (2007)** also observed a positive association between Penta-BDE concentrations in house dust and the breast milk of occupants. Blood serum levels collected from 12 couples living in Boston, MA (USA) strongly correlated with PBDE concentrations of the Penta-formulation detected in their house dust (Johnson et al., 2010).

Health concerns increased as documentation of exposure mounted. PBDEs were discovered to be potential thyroid disrupters and developmental neurotoxicants (Costa and Giordano, 2007), and to cross the placenta into the fetal vascular circulation (Gómara et al., 2007). Pre- or post-natal exposure of mice or rats to the Penta-formulation has been shown to cause long-lasting changes in spontaneous motor activity and disrupt performance in learning and memory tests (Eriksson et al., 2001; Branchi et al., 2003). PBDE-exposed rats from gestation to postnatal stages (Zhou et al., 2002), along with adult mice (Fowles et al., 1994), exhibited decreased serum thyroxine (T_4) levels. Thyroxine plays a key role in early brain and nervous system development. It should be noted that impacts on neurodevelopment have been reported in children of mothers with suppressed T_4 levels (Haddow et al., 1999). Maternal transfer is not the only path for infant contamination; infants and young children are vulnerable to inhalation and ingestion of FR-laden house dust by their proximity to the floor, i.e. crawling, and dust ingestion, i.e. hand to mouth contact (Stapleton et al., 2012). These health concerns, along with the growing reports of global PBDE dissemination, enjoined U.S. regulators to broker a voluntary cessation in the manufacture of two of the three PBDE formulations (Penta- and Octa-BDEs) starting in December 2004. This followed a European Union PBDE ban earlier that year (Renner, 2004). The Deca-formulation was banned in electronics and electrical applications by the EU, July 1, 2008 (Joined cases C-14/06 and C-295/06) and phased out in the U.S. at the end of 2013. In 2009, PBDEs containing four-to-seven bromines were added to Annex A of the Stockholm Convention. The amendment entered into force in 2010, eliminating the production and usage of the Penta- and Octa-mixtures in signatory countries. The fully brominated PBDE BDE-209 was later added to Annex A in May of 2017 (Stockholm Convention, 2018). However, the production and usage of BDE-209 still continues as a high production volume chemical in China (Ni et al., 2013 and Ji et al., 2017).

To fill the void projected by these restrictions, FR manufactories introduced alternative FRs into the marketplace. Unfortunately, these replacements lacked comprehensive evaluations which, have lead to their detection in the environment soon after their introduction (Covaci et al., 2011, Guigueno and Fernie, 2017). Several of these Penta-, Octa-, and Deca-BDE replacements products, including EH-TBB, BEH-TEBP, BTBPE, and DBDPE, respectively, have been detected in U.S. sewage sludge prior to and during their transition (**La Guardia et al., 2010**). These "newer" or novel-BFRs, including chlorinated organophosphate-FRs (Cl-OPFRs), have also been detected in house dust. These along with the previously restricted-FRs, released from older in-use products, are creating additional health concerns (**La Guardia et al., 2015**; Zheng et al., 2017). Both EH-TBB and BEH-TEBP have been shown to exhibit endocrine disruption potentials (Saunders et al., 2013). Adverse biological effects were observed by **Nacci et al. (2018)** in BEH-TEBP dietary exposure Atlantic killifish (*Fundulus heteroclitus*). Cl-OPFRs, tris(1-chloro-2-propyl)phosphate (**TCIPP** or TCPP) and tris(1,3-dichloro-2-propyl)phosphate (**TDCIPP** or TDCPP) have been banned for some uses due to health concerns (EU, 2014), but are now/still being used in non-restricted products (**Schreder et al., 2016**). TDCIPP, an analogue of TCIPP, was used as an FR in children's sleepwear until manufacturers voluntarily withdrew it in 1977 due to mutagenicity concerns (Gold et al., 1978).

As health concerns rise over replacement FRs and the controversy continues over regulations requiring their usage, realignment of the existing paradigm is needed. Fire safety advocates have often claimed that FRs contributed to the observed 55% decline in U.S. fire-related deaths between 1977 and 2017 (Roe and Callahan, 2012). However, these advocates neglect to acknowledge new regulation requiring smoke detectors, a decline in cigarette consumption, and the implementation of "fire safe" cigarettes that reduced their propensity to burn when left unattended. "Fire safe" cigarettes are believed to be a principal reason for the 30% decline in U.S. fire deaths from 2003 to 2011 (Hall, 2013). McKenna et al. (2018) also showed that while flame-retardant sofa beds burnt somewhat slower than non–flame-retardant sofa beds, they produced significantly greater quantities of fire's main toxicants, carbon monoxide and hydrogen cyanide. This bears out the findings of the open-flame test (TB-117), which dictated that FRs, designed to suppress ignition, have little effect on fire progression. Smoke inhalation, not burns, is the largest cause of fire-related deaths (Stec, 2017). To address these concerns, in 2013 California revised its 1975 flammability standard TB-117, now TB-117-2013, substituting the open-flame test with smolder-resistant covers, fabrics, barrier materials, and resilient filling materials (DCA 2013). This created the opportunity for choosing not to use FRs. Soon after the bill's passing, the first federal flammability standard was introduced on October 4, 2018. Titled U.S. Senate Bill 3551 "Safer Occupancy Furniture Flammability Act," it required the U.S. Consumer Product Safety Commission to adopt California TB-117-2013. However, the impact of California's 2013 revised flammability standard remains uncertain and, if the federal standard is implemented, furniture manufacturers can still choose to use FRs and be in compliance with the law. And, this standard applies to only a single class of FRs, those designed for use in polyurethane furniture padding. It does not address FRs used in other applications such as electronics, transportation and building materials. Without a holistic approach to both fire safety and chemical FR usage, FR disposal, health implementation, and dissemination will continue. At the same time, the introduction of inadequately understood replacement FRs will add to and create new health concerns, while potentially falling short of reducing losses caused by fires.

My contributions to these complex discussions are highlighted in the following chapters. Areas of research include the development of analytical techniques, identification of chemical composition of FRs in technical mixtures and complex environmental matrices, delineation of FR point sources and distributions in remote areas, demonstration of FR degradation products in real-world settings and uptake within ecosystems, and human exposure and inhalation of FR-laden airborne particulates. These contributions include 10 first-authored and 18 coauthored scientific publications representing my interactions and evaluations to promote greater understanding of this complex issue. Despite these efforts, it remains clear that future work is required. Scientists, academics, and regulators must continue to question and evaluate FR usage with the goal of developing safer products, protective of environmental and public health.

Chapter Two – Analytical advances in flame retardant analysis

Like polychlorinated biphenyls (PCBs), there are a possible 209 polybrominated diphenyl ether (PBDE) congeners. However, PBDE technical formulations are much simpler than those of PCBs. PBDEs are produced commercially at three levels of bromination: nominally penta-BDE, octa-BDE and deca-BDE. These formulations contain primarily 39 individual PBDE congeners (**La Guardia et al., 2006**). This is due to the directing influence of the diphenyl ether's oxygen atom and the steric hindrance of the bromine atoms. The first gas chromatogram/mass spectroscopy (GC/MS) investigations of the European Penta-BDE formulation, Bromkal 70-5DE (Chemische Fabrik Kalk, Köin, Germany), identified three major PBDE compounds, representing 93% of the mixture by weight. Two compounds were further isolated from the technical mixture and analyzed using nuclear magnetic resonance (NMR); these compounds were identified as 2,2',4,4',-tetrabromodiphenyl ether (BDE-47) (the same IUPAC naming scheme for PCBs is used for PBDEs) and 2,2',4,4',5-pentabromodiphenyl ether (BDE-99) at 41% and 45%, by weight, respectively (Sundström and Hutzinger, 1976). With the aid of commercially available analytical standards and GC/MS, Sjödin et al. (1998) identified an additional seven PBDEs within the Bromkal 70-5DE mixture used primarily in Europe. Sjödin's team confirmed BDE-47 and -99 as the two major constituents at 37 and 35% by weight, respectively, followed by two penta-BDEs; 2,2',4,4',6-pentabromodiphenyl ether (BDE-100) and 2,2',3,4,4'-pentabromodiphenyl ether (BDE-85) at 6.8 and 1.6% by weight and three hexa-BDEs; 2,2',4,4',5,5'-hexabromodiphenyl ether (BDE-153), 2,2',4,4',5',6-hexabromodiphenyl ether (BDE-154) and 2,2',3,4,4',5-hexabromodiphenyl ether (BDE-138) at 3.9, 2.5, and 0.41% by weight followed by an additional three minor PBDEs: two tri-BDEs; 2,4,4'-tribromodiphenyl ether (BDE-28) and 2,2',4-tribromodiphenyl ether (BDE-17) and 2,3,4,4'-tetrabromodiphenyl ether (BDE-66) totaling 0.35% by weight. Later Sjödin (2000) identified additional PBDE congeners by homologue grouping of several European (Chemische Fabrik Kalk Köin, Germany), Middle Eastern (Dead Sea Bromine Group, Beer Sheve, Israel) and U.S. (Great Lakes Chemical Corporation, West Lafayette, USA) Penta-, Octa- and Deca-PBDE commercial mixtures.

By the early 2000s, PBDEs had been widely used to flame-retard products common in homes and in the workplace. They also became widely dispersed in the environment. Detailed compositional knowledge of these complex PBDE mixtures beyond homologue grouping was thus crucial for a fuller understanding of their toxicological potencies and environmental fate due to selective congener biomagnification, degradation, and transport. Adding to uncertainty, some also had observed PBDEs might be natural products biosynthesized by marine sponges, adding to the debate over their environmental point of origin (Gribble et al., 1999; Renner, 2000). Using recent technical enhancements and the availability of a wider range of commercially available standards, we expanded on Sjödin's work by developing a GC/MS method capable of analyzing a larger suite of congener-specific mono- through deca-BDEs. Sixty-four commercially available PBDEs standards were chromatographed using two non-polar GC columns with different stationary phases, 100% dimethylpolysiloxane and 5% diphenyl/95% dimethylpolysiloxane. By altering the stationary phases, two relative retention indexes (RRI) were determined for each commercial PBDE standard, reducing the influence of co-eluting compounds. The creation of PBDE debromination products as a result of prolonged exposure to high GC injector temperatures of approximately 300 ^{o}C, common in split/splitless injector protocols (Covaci et al., 2003), was avoided by using a low temperature on-column injector. The initial on-column injector, set at 65 ^{o}C, was programed to follow the GC column oven. This minimized bromine loss from the PBDE through thermal degradation, avoiding misidentification of the parent PBDE. Structural conformations for each standard were then based on fragmentation patterns and molecular ions established by GC/MS, first operating in the electron-capture negative ionization (ECNI) mode and then electron ionization (EI) mode. These ion fragmentation patterns, isotope intensities and structural conformations were recorded and a PBDE reference library of their RRIs, ECNI and EI spectra was established. Using this library we then characterized the congener composition of six common Penta-, Octa- and Deca-technical mixtures. These included those from Chemische Fabrik Kalk and Great Lakes Corporation and the Deca-product Saytex-102E by Albemarle Corporation, Louisiana, USA. This work was published in *Environmental Science and Technology* (*ES&T*) and titled ***"Detailed polybrominated diphenyl ether (PBDE) congener composition of the widely used Penta-, Octa-, and Deca-PBDE technical flame-retardant mixtures,"*** which outlined the identification of 39 PBDEs in these technical mixtures, 29 at concentrations > 0.02% by weight (**La Guardia et al.,**

2006). Of these, 12 previously unreported PBDE congeners were confirmed as commercial mixture components. Five additional PBDE compounds were tentatively identified based on their molecular ion and ECNI fragmentation in the absence of corresponding analytical standards.

Additional observations originating from this research were that the softer ECNI ionization technique produced spectra with unique PBDE structure information, compared with the more commonly used EI ionization approach. For the higher brominated PBDEs, ECNI spectra provided ion fragments indicating bromine benzene ring substitution, molecular ion, and other PBDE characteristic ions. Of the possible 39 PBDEs identified in the three common technical mixtures, additional PBDEs have been reported in biotic and abiotic matrices. These occurred presumably via thermal, photolytic or biological debromination of PBDEs from the technical mixtures (**Chen et al., 2008; Hale et al., 2012**). However, specific compound identification of these debromination products has been under-reported due both to the cost of acquiring the full body of 209 individual PBDE analytical standards and to the exclusive use of EI spectra for identification - that only permitted classification to the level of the homologue grouping. By utilizing the ECNI-produced PBDE spectra we were able to identify structural differences existing between PBDE congeners, as a function of bromine position on the phenyl rings. We thus suggested that using ECNI, analysts could elucidate congener structure and, at a minimum, eliminate many possibilities within the homologue grouping. Published in a special edition of *Mass Spectrometry*, "Current Trends in Mass Spectrometry" and titled, ***"Use of electron-capture negative ion mass spectra to establish the identities of polybrominated diphenyl ether flame retardants and their degradation products"***; this technique demonstrated cleaving of the PBDE's ether bond by ECNI, producing ion clusters indicative of a bromine substituted benzene ring. It also showed that the bromine positioning (ortho, meta, and para) on the ring influenced the observed ion fragmentation patterns (**La Guardia, 2008**). It was apparent that in ECNI, opposing bromines in the ortho (6, 6') position contribute stress to the oxygen bond, causing the PBDE to be cleaved at the ether bond. Alternately, bromines in the para (4, 4') positions have a stabilizing effect on the ortho-position bromines, allowing for molecular ion identification; if the bromine shifts from the para-position to the meta-position (5') the molecular ion is not observed. Identification by ECNI spectra analysis was then assignable to PBDEs containing greater than five bromines and a searchable ECNI spectra library of 64 PBDEs was assembled (**La Guardia, 2008**).

Next, to show the ability of ECNI to analyze complex matrices for both known and unknown (non-targeted) halogenated organic compounds, we analyzed several types of stabilized land-applied sewage sludges, i.e., biosolids. Biosolids are increasingly land-applied as a soil amendment, but the compositional complexity of this matrix has hampered understanding the risks of land application. Compound-specific analytical approaches may also underestimate environmental impact of land application by overlooking additional contaminants contained within biosolids. However, by utilizing an alternative analytical approach based on compound functional group (i.e., alkyl halides) and ECNI spectra interpretation we observed 49 organohalogens by GC/ECNI-MS in biosolids; 23 identified as FRs which included 17 PBDEs, their replacement products (EH-TBB, BEH-TEBP, BTBPE and DBDPE) and HBCDD and Dechloran Plus. An additional 16 compounds were tentatively identified as triclosan (an antibacterial and antifungal agent), chlorinated pesticides, hexachlorobiphenyl (PCBs), EH-TBB degradation products, brominated furans, and two nonabromochlorodiphenyl ether (NBCDE). These finding were published in *ES&T* under the title ***"Flame-retardants and other organohalogens detected in sewage sludge by electron capture negative ion mass spectrometry,"*** and emphasized that this screening and quantitation approach can cost-effectively facilitate the identification of constituents present in complex matrices that may have detrimental human and environmental health consequences (**La Guardia et al., 2010**).

This study also assisted in determining a potential source of the novel brominated/chlorinated diphenyl ether (NBCDEs) identified by others in urban and e-waste recycling air and soil samples from Guangzhou China (Yu et al., 2011a). Mixed brominated/chlorinated dibenzo-*p*-dioxins and dibenzofurans have been previously detected at sites associated with anthropogenic combustion (Weber et al., 2003). Therefore, Yu et al. (2011a) proposed that these novel NBCDEs were thermolytic transformation products of PBDEs, formed during the open burning of e-waste. They detected two NBCDEs (5' Cl-BDE-207 and 4' Cl-BDE-208) in thermoplastics at 5–8% by weight of BDE-209, contributing by their inadvertent formation during the manufacturing of PBDEs and chlorine-containing products. However, we previously hypothesized that these products were constituents of an alternative Deca-BDE formulation by Albemarle Corporation (**La Guardia et al., 2010**). The Albemarle Corp. patent describes the co-feeding of diphenyl oxide, or partially brominated diphenyl oxide and bromine chloride with bromine or chlorine, into a refluxing reaction mixture of bromine and a Lewis acid bromination catalyst to produce > 96% BDE-209 and up to 4% by weight two NBCDEs (WIPO, 2008). Similar BDE-209 and NBCDE ratios were observed in thermoplastics that Yu et al. analyzed. Moreover, Albemarle Corp. is a worldwide manufacturer and supplier of FRs, including the manufacturing facilities in Guangzhou, China where Yu et al. collected their samples. Our observations were published in an *ES&T* article titled ***"Comment on: Identification of monochloro-nonabromodiphenyl ethers in the air and soil from South China,"*** which offered an alternative explanation for the source of NBCDE contamination in air and soil samples from Guangzhou, China (**La Guardia et al., 2011**). These comments were also the topic of several additional *ES&T* communications: authors Yu et al. acknowledged our findings and further explored their position (Yu et al., 2011b), and Albemarle Corp. explained their active research and developmental programme and confirmed registering the patent, but stated that they never manufactured Deca-BDE as described in their patent (Hardy et al., 2011). Regardless of whether NBCDEs were formed during the manufacture of thermoplastics, or during unregulated e-waste recycling, or introduced as trace additives, our detection of them in the environment validates the need for non-target chemical analysis, as demonstrated by GC/ECNI-MS, to further our understanding of the environmental occurrence and fate of potentially harmful anthropogenic contaminants.

Chapter Three - Detection of flame retardants and their degradation products in abiotic and biotic environments

The FRs BDE-209 and tetrabromobisphenol A (**TBBPA** or TBBP-A), the latter used to flame-retard polycarbonates used in electrical casings and printed circuit boards, were first observed in air, soil, sediments and human hair in areas that bordered industrial manufacturing or otherwise using these compounds in the mid-1970s (DeCarlo, 1979). Dissemination of an array of PBDEs in fish tissues from the Viskan R., Sweden was reported shortly after by Andersson and Blomkvist (1981) in areas where no manufacturing facilities were known and later in sperm whales by de Boer et al. (1998) which normally stay and feed in deep ocean waters. Trophic transfers were reported in avian tissues and eggs of fish-eating birds (Stafford, 1983). Extreme PBDE levels were later observed in carp (*Cyprinus carpio*) tissues collected from the rural freshwater Hyco R. USA (**Hale et al., 2001a**).

Once considered non-dispersive chemicals, polymer products that contain FRs can break down, releasing FRs into the environment. **Hale et al. (2002)** observed that after four weeks of exposure to ambient outdoor conditions, the surface of PBDE treated polyurethane foam became brittle and began to disintegrate. The fragments generated are then easily transported, entering waste streams within the outdoor and indoor environments (Renner, 2000). Partitioning of waste particulates and associated hydrophobic chemicals and their subsequent removal as sewage sludge is an integral step in the wastewater treatment process, as this process decreases the levels of pollutants discharged to receiving streams. However, in a 2001 *Nature* communication we reported the detection of several PBDEs in land-applied sewage sludge (biosolids) and its role in facilitating the dissemination of PBDEs and other chemicals sequestered in biosolids (**Hale et al., 2001b**). To further investigate additional chemical constituents within biosolids, we expanded our analysis to include several other contaminants of concern: 1) alkylphenol polyethoxylate (APEO) degradates (e.g., nonylphenol (NP)) formed from detergent additives (surfactants) which, are acute toxicants to aquatic organisms; 2) polycyclic musks used as fragrances, reported to accumulate in fish tissues and human breast milk; and 3) the anti-bacterial additive triclosan, an aquatic toxicant which may contribute to antibiotic resistance. The detection of these additional contaminants was confirmed along with PBDEs in biosolids obtained from multiple U.S. states. These observations were published in the *Journal of Residuals Science & Technology* article titled, ***"Organic contaminants of emerging concern in land-applied sewage sludge (Biosolids)"*** (**La Guardia et al., 2004**). This publication supported the U.S. National Academy of Science's 2002 request to update the 1990 National Sewage Sludge Survey and the corresponding risk assessment. We then explored ecosystem migration of PBDEs by analyzing soil, soil invertebrates, and vegetation collected from an agricultural field that had received biosolid applications since the mid-1980s. PBDEs with three to seven bromines were detected in the sludge-amended soil and soil invertebrates (earthworms and detritivores, e.g. woodlice and June beetles). PBDEs were below quantitation limits in vegetation from the sludge-amended site, and in soils, vegetation and soil invertebrates collected from the control site. These findings were reported in a coauthored paper (**Gaylor et al., 2014**), indicating that sewage-sludge amended lands are a point source for the environmental introduction of PBDEs and other toxic constituents contained within biosolids.

Contaminants that enter the wastewater treatment process and are not completely eliminated or removed by the sludge collection process have the ability to also enter receiving streams. It has been estimated that > 90% of the PBDEs that enter a wastewater treatment plants (WWTPs) will ultimately reside in sludge, with the remainder being released to wastewater-receiving streams (North et al., 2004; Song et al., 2006). As a follow-up to our previous report of high concentrations of PBDEs observed in fish tissue collected from the Hyco R., we sought to locate the source. According to the U.S. Environmental Protection Agency's (U.S. EPA) Toxic Release Inventory (TRI), which lists the U.S. industries that report releases (e.g., air emissions and surface water discharge) and transfers (e.g., landfills and WWTPs) of high production volume chemicals (chemicals produced or imported into the U.S. in quantities of 500 tons per year), a local textile manufacturer reported substantial transfers of the then-unrestricted BDE-209 to a WWTP for treatment. This WWTP releases its treated wastewater to Marlowe Creek, a tributary of the Hyco R. This WWTP's outfall is located approximately 11 km upstream from the Hyco R. and approximately 45 km from the initial site where we had previously observed exceptionally high concentrations of BDE-47 and -99 in carp tissue (**Hale et al., 2001a**). We hypothesized that once in the environment, BDE-209 may encounter conditions favoring debromination, forming congeners with greater toxicity, bioaccumulation potential, and persistence. However, at the time this had only been shown under laboratory conditions (Stapleton et al., 2004; Stapleton et al., 2006). To determine whether the Marlowe Creek WWTP outfall contributed to PBDEs detected in the Hyco R. and to examine if PBDE debromination was likely in the field, we analyzed sewage sludge for PBDEs and tracked PBDE congener profiles from the WWTPs' receiving stream sediments and associated aquatic biota. These results were published in the *ES&T* article titled, ***"Evidence of debromination of decabromodiphenyl ether (BDE-209) in biota from a wastewater receiving stream"*** (**La Guardia et al., 2007**). BDE-209 and 23 additional PBDEs were detected entering the aquatic ecosystem at this site. Sludge congener profiles resembled the commercial Penta- and Deca- formulations, suggesting minimal BDE-209 debromination during wastewater treatment. Similar profiles were observed in surficial sediments near the outfall and downstream. However, sunfish (*Lepomis gibbosus*), creek chub (*Semotilus atromaculatus*), and crayfish (*Cambarus puncticambarus sp. c*) collected near the outfall contained tri- through deca-PBDEs, including PBDE congeners not detected in the commercial PBDE mixtures, sludge or sediments. This suggested debromination. A previous *in vivo* laboratory study by Stapleton et al. (2004) identified these same congeners we detected as BDE-209 metabolic debromination products. Our research supported the hypothesis that metabolic debromination of BDE-209 does occur in aquatic environments under realistic conditions. This suggested that previous assessments that assume that BDE-209 does not debrominate likely underestimate associated bioaccumulation and toxicity attributable to the less brominated congeners produced.

In a later study of the Hyco R., we also detected HBCDD and PBDEs in several Hyco river fish species (**Chen et al., 2011**). In this publication we observed that, while PBDE concentrations had decreased from 2002 to 2007, HBCDD concentrations had increased from 13 ng to 4,640 ng g^{-1} (lipid weight basis). HBCDD concentrations in fish from the Hyco R. also exceeded those from rivers less influenced by manufacturing outfalls, an indication of the manufacturer's efforts to reduce PBDE environmental burdens by switching to an alternative FR. By the end of 2010, manufacture of Penta- and Octa-BDE formulations was discontinued globally and the mixtures added to the Stockholm Convention's Persistent Organic Pollutants list in May 2009 (Stockholm Convention, 2018). In July 2008, the third and most widely used PBDE formulation, Deca-BDE, was banned in electrical equipment in the EU under their Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) programme (Kemmlein et al., 2009) and phased out of

U.S. production by the end of 2013 (USEPA, 2017). After this removal from the marketplace, the flame-retardant industry quickly replaced these with alternative FR formulations. However, the environmental fate of these replacement products remained inadequately investigated (Covaci et al., 2011). Several of these alternatives, including HBCDD, were previously detected in sewage sludge (**La Guardia et al., 2010**). Like PBDEs, their removal by wastewater treatment may be incomplete, and they might still enter the aquatic ecosystems through wastewater discharge and ultimately land application of biosolids.

According to the U.S. EPA's TRI, an on-site textile wastewater treatment facility released 6000 kg of BDE-209 to surface waters of the Yadkin R. (North Carolina, USA) between 2000 and 2004. We hypothesized that replacement BFRs may also be used and released to the Yadkin R. by this facility. We then collected sediments, locally available bivalves (*Corbicula fluminea*), and gastropods (*Elimia proxima*) at the outfall, upstream of the outfall, and at several locations up to 44.6 km downstream. We then analyzed them for PBDEs, alternative-PBDEs; EH-TBB, BEH-TEBP, BTBPE and DBDPE, and HBCDD. We also evaluated these BFRs' bioavailabilities, which are influenced by the organisms' ecology (i.e., route of uptake) and *in situ* environmental factors. This research was published by *ES&T* in an article titled **"*In situ accumulation of HBCDD, PBDEs, and several alternative flame-retardants in bivalve (*Corbicula fluminea*) and gastropod (*Elimia proxima*)*** (**La Guardia et al., 2012**). The research confirmed that PBDEs, HBCDD, and the alternative-PBDEs were bioavailable to the filter-feeding bivalves and grazing gastropods. Tissue concentrations of HBCDD and PBDEs collected at the outfall are among the highest reported worldwide. HBCDD, PBDEs, and the alternative-PBDEs were detected in sediments at the outfall. FR sediment concentrations decreased as distance increased downstream from the outfall. However, FRs were still detected in sediments as far as 44.6 km from the WWTP's outfall, indicating that this outfall was likely a significant aquatic point source for these FRs. BDE-209 was the dominant BFR detected in the sediments. Despite its presumed modest bioavailability, due to its large molecular size, it was the dominant PBDE observed in both bivalve and gastropod tissues, probably caused by sediment particles present the gastrointestinal tract. This may have been avoided if the samples were depurated prior to chemical analysis. However, the percent contribution of BDE-209 to total PBDEs in these tissues was lower (37 to 67%) than in sediments (> 94%). This likely relates to its strong association with particulates and attendant lower bioavailability. In contrast, BDE-47, -99 and -100 contributed 18 to 59% of the total PBDEs in these mollusks, while their contributions in sediments ranged from only 0.5 to 3.6%, likely due to their greater bioaccumulation potentials. Probable BDE-209 metabolic debromination products were also detected, e.g. BDE-201. Bioaccumulation factors were similar between HBCDD and PBDEs with 4 to 6 bromines. Factors for EH-TBB, BEH-TEBP and BTBPE were lower, following the equilibrium partitioning theory. Despite different feeding strategies, the bivalves (filter feeders) and gastropods (grazers) showed similar BFR water and sediment accumulation factors. These observations indicated that the alternative-PBDEs have the same affinity to enter and accumulate in the environment as the FRs they replaced, and may contribute to any observed toxicological effects.

Shortly after the removal of PBDEs from the marketplace — or, according to some accounts, prior to that removal — the environmental dissemination of PBDEs replacement products had become widespread (Covaci et al., 2011). Technology and the consumption of tech-related products (e.g., computers, mobile phones) that contain FRs fueled this process. Challenges to address proper disposal of waste electrical and electronic equipment (WEEE) persists. Hicks et al, (2005) reported that nearly 80% of all U.S.WEEE generated in the early 2000s was exported to developing nations for metal salvaging (e.g., copper), recycling, and disposal. However, the crude electronic-waste (e-waste) recycling and disposal techniques that were practiced in poor underdeveloped regions exposed workers and the environment to complex mixtures of hazardous materials including FRs.

Prior to 1995, Guiyu, China was a poor, rural, rice-growing community. It subsequently became one of the largest e-waste "recycling" centers in the world, handling 1.6 million tons of imported e-waste annually (Bloomberg, 2015). To study the environmental impact of poorly regulated e-waste recycling we analyzed FRs in surface sediments collected along the Lian R. The Lian R. flows through the Guiyu e-waste recycling zone. Our analysis detected that Guiyu sediments contained some of the highest concentrations ever reported of Penta-BDE, BTBPE, TBBPA and triphenyl phosphate (**TPHP** or TPP), a plasticizer with FR properties used in electronic equipment (**Li et al., 2019**). This study also observed hazard quotients (HQ) > 1 for several FRs at Lian R. sample sites both within the Guiyu recycling zone and downstream (~ 30 km). Some of these sites were heavily contaminated, exhibiting HQs > 100. *(HQs were derived by the European Commission's Technical Guidance Document (TGD) to characterize ecological risk to FR exposure. A HQ > 1 indicates potential risk meriting further investigation/remediation.)*

The densely populated urban centers, urban sprawl and desire for new technologies in the northern hemisphere are believed to result in greater FR burdens than in the less populated southern hemisphere. However, electronics use as well as WEEE exportation from developed to developing nations is occurring within the southern hemisphere on the African continent. According to a 2011 United Nations Environmental Program (UNEP) report, it is estimated that 1.3 million MT of WEEE is disposed of annually in sub-Saharan African countries. To better understand the extent of FR environmental dissemination in a southern African urban community, we conducted analysis for BFRs in inland and coastal sediments from the eThekwini metropolitan municipality in South Africa. This municipality is located in the KwaZulu-Natal Province on the country's northeast coast and includes South Africa's third largest city, Durban, population 3.5 million. South Africa is the world's 28th richest country and Africa's leading economy. The results of this evaluation were published in an *ES&T* article titled ***"Brominated flame-retardants in sub-Saharan Africa; Burdens in inland and costal sediments in the eThekwini metropolitan municipality, South Africa"*** (**La Guardia et al., 2013**). This survey included 45 sediments collected from Durban Bay and 13 other rivers of the municipality. Most of the natural river systems within the urbanized area have been canalized to receive runoff and to facilitate drainage, preventing inland flooding during storm events. These systems have also become catchments for trash and illegal dumping. Several wastewater treatment plants also discharge into these rivers; all utilize only primary treatment, i.e., screening and grit removal. BFRs were detected in all samples. BDE-209 was detected in 93% of the samples, followed by the PBDE-replacement EH-TBB at a 91% detection rate. Other PBDE replacement products included BEH-TEBP, BTBPE, and DBDPE, at concentrations of up to 13,900 ng g^{-1}, total organic carbon (TOC). HBCDD was detected in 69% of the samples, up to 27,500 ng g^{-1}, TOC. Durban Bay, which is strongly influenced by urban runoff and tidal hydrology that traps sediments within the bay, exhibited PBDE concentrations that rival those in the heavily impacted Pearl River Delta (PRD), China. The PRD is located in the world's most densely urbanized region (population 120 million) and a major electronic manufacturing center. Our survey traversed the municipality from north to south and indicated that BFR concentrations increased near the coastal urban center, exceeding the mean total BFR concentration by several fold. Several highly contaminated sites were noted along rivers that flowed past industrial parks. One such site drained a large industrial area where automotive manufacturing and salvaging took place. These finding suggest that, if the demand for polymer products and electronics escalates and if the area remains a center for WEEE salvaging, BFR burdens will increase in sub-Saharan Africa’s urban centers.

These reports underscore the need for further investigation into environmental burdens of historic- and current-use BFRs, their degradation products and risks associated with wastewater discharge, WEEE disposal, and salvaging. They also highlight the need for developed nations to manage their WEEE within their own boundaries, and not to depend on safe disposal in developing nations that lack adequate environmental and human health regulations.

Chapter Four – Bioavailability of and human exposure to flame retardants

Many FRs are persistent toxicants with the ability to bio-magnify. Accumulated maternal body burdens may be transferred to their offspring during prenatal development, placing the fetus at greater risk. If unchecked, burdens will continue to accumulate in the newborn and throughout adulthood, transferring higher burdens onto the next generation. Kajiwara et al. (2008) estimated that melon-headed whales' (*Peponocephala electra*) maternal transfers of PBDEs to offspring at 85% of the mother's body load. Maternal FR burdens may also alter human prenatal development. Chao et al (2007) observed low birth weights associated with elevated maternal PBDE levels within a Taiwanese population. Transfers of lipophilic FRs also occur during breastfeeding, placing additional stress on early development. A California (USA) study of 82 first-time mothers determined that 60% of their breastfed infants had daily intake of BDE-47 exceeding the U.S. EPA's Reference Dose (R*f*D) for neurodevelopmental toxicity (Park et al., 2011). Since the 1970s, human blood, milk, and tissue PBDE levels have increased by a factor of 100 times (Hites, 2004). Steps to control exposures to legacy bioaccumulative contaminants (e.g., PBBs and polychlorinated biphenyls (PCBs)) have focused on restricting usage and their removal from the "food-basket." However, along with diet, direct exposure through inhalation and ingestion of indoor dust has been suggested as an equally or even more important route for FR exposure (Bramwell et al., 2016).

Norén and Meironyté (2000) observed a steep increase in Swedish PBDE breast milk concentrations between 1972 and 1997, with a doubling time of 5 years. Infant PBDE burdens increased 60 times during that period. However, with no known production or manufacturing of PBDEs in Sweden and no known exposure reflected in diet, the authors assumed that household goods containing FRs were responsible for the exposure. PBDEs, TBBPA, and nine organophosphate esters, including the Cl-OPFRs tris (2-chloroethyl) phosphate (TCEP), TCIPP, and TDCIPP, were first reported in indoor air collected from workplaces occupied by computers and other electronics (Bergman et al., 1997). Rudel et al. (2003) and Sjödin et al. (2008) later reported PBDEs in house dust and air samples, and proposed a potential route of human exposure. To further explore the potential of indoor exposure, we analyzed breast milk, house dust, and the diets of 46 mothers from the greater Boston, MA (USA) area to identify key routes of PBDE exposures (**Wu et al., 2007**). We found statistically significant, positive associations between PBDE concentrations in breast milk and house dust ($r = 0.79$, $p = 0.003$)—though not with BDE-209, due to low detection frequencies—and similar associations with reported dietary habits, particularly the consumption of dairy products ($r = 0.41$, $p = 0.005$) and meat ($r = 0.37$, $p = 0.01$) for tetra- through hexa-BDEs present in the Penta-formulation. These findings supported the hypothesis that along with diet, indoor dust contributes to PBDE exposure.

We then expanded our analysis to additional FRs in residential dust collected from the state of Washington (USA). Here we reported the detection of 21 compounds which included PBDEs of the Penta-formulation, BDE-209, alternative-PBDEs (EH-TBB, BEH-TEBP, DBDPE and BTBPE), TBBPA, TCEP, TCIPP and TDCIPP) and HBCDD (**Schreder and La Guardia, 2014**). The Penta-BDE concentrations were about double those of BDE-209 and 10 times higher than the alt-BFRs, but were comparable to TCIPP concentrations. We also concluded that FRs in household dust and laundry wastewater were significant sources to municipal wastewater treatment facilities. This was supported by observations of FR concentrations in household laundry wastewater and those of wastewater entering the treatment plant. We identified several FRs that were poorly removed during the treatment process. Specifically, the Cl-OPFRs had a < 16% removal rate versus a > 86% for other FRs (e.g., PBDEs). We thus estimated that 2% of the annual U.S. usage, 174,000 kg of TCIPP and 402,000 kg of TDCIPP, were discharged to the aquatic environment. This was also observed in a Swedish study with approximately 5% of their annual usage of TCIPP and TDCIPP being discharged (Marklund et al., 2005). We later reported that inhalation exposure for Cl-OPFRs exceeded dust ingestion rates (**Schreder et al., 2016**). This was an important observation as it was widely accepted that ingestion by hand-to-mouth contact was the primary route of exposure for children (Stapleton et al., 2012). In **Schreder et al. (2016)** we analyzed inhalation exposure rates of participants from the state of Washington (USA) using personal air samplers. These samplers collected both respirable (air particulates < 4 µm, nominal) and inhalable (> 4 µm, nominal) particulate fractions, measuring the likelihood of particles penetrating deep into the gas exchange region of the lungs (particulates < 4 µm). Cl-OPFR exposure via inhalation for children was estimated to be 9.8 times that from dust ingestion and 31.3 times for adults.

In addition to measuring FR exposure within the indoor environment, it is also important to understand the nature of products and interactions within these environments that can elevate exposure. For example, young children spend considerable time in contact with the floor (e.g., crawling). This makes floor dust a significant exposure point. Older children and adults spend more time near elevated surfaces (e.g., sitting on a sofa or using a computer); making dust on elevated surfaces an important route of exposure. It has been suggested that FR concentrations can vary widely across microenvironments (Harrad et al., 2009), and the presence of electronics may affect FR dust levels (de Wit et al., 2012; Harrad et al., 2004). To address this, we analyzed dust samples for FRs collected from floors and elevated surfaces from various rooms (e.g., apartments, classrooms, laboratories, and buses) containing various amounts of electronics on a college campus in California (USA). We reported our findings in the journal *Environmental Research* (**Allgood et al., 2017**). Results showed that exposure to FRs, i.e., the penta-formulation, EH-TBB, BEH-TEBP, and TDCIPP on elevated surfaces, were statistically higher than concentrations on floor surfaces. Secondly, elevated surfaces had higher FR levels in environments categorized as having a high density of electronics, compared to elevated surfaces categorized as having a low presence of electronics. This implied that human-exposure estimates based on the analysis of floor surfaces or on a combination of floor and elevated surfaces may underestimate true exposure, especially when there is a high density of electronic devices in the study area.

Activities such as occupations may also adversely expose certain sub-populations, i.e., those employed in polymer, textile, electronics manufacturing and dismantling of electronic waste (Besis and Samara, 2012), as well as workers such as aircraft personnel who frequent environments laden with FRs (Strid et al., 2014). Lifestyle, including sports or recreation may also alter exposure, e.g. collegiate gymnast (Carignan et al., 2013) were shown to have elevated PBDE serum levels compared to the general population. To further explore exposures through recreational activities we then studied exposures within gymnastic studios by analyzing FRs in indoor dust and size-fractionated respirable and inhalable air particulates. These were collected at four studios and values compared to air and dust samples collected from the homes of coaches employed at these facilities. Polyurethane foam blocks, which are used in safety pits, were also analyzed to characterize potential FR sources. These findings were published in the journal

Environmental International in an article titled, ***"Halogenated flame-retardants concentrations in settled dust, respirable and inhalable particulates and polyurethane foam at gymnastic training facilities and residences"*** (**La Guardia et al., 2015**). It was observed that the pit foam used in these studios contained multiple FRs at cumulative concentrations of 12,100 to 25,800 $\mu g\ g^{-1}$ or 1.2% to 2.6% by weight. EH-TBB and BEH-TEBP were the most abundant FRs detected, followed by TDCIPP and several PBDEs. The mean total FR dust burden detected at the gym was 8.6-fold higher than that observed in the house dust samples. The polyurethane additives EH-TBB and TDCIPP exhibited significantly greater levels ($p < 0.05$) in the gym than house dust. Mean concentrations of BDE-99, -100, -153, -209 and TDCIPP were also higher in gym respirable particulates than the homes. BDE-47, EH-TBB, BEH-TEBP and DBDPE were higher at the homes than the gyms; but these differences were not significant ($p > 0.05$). Several additional FRs were detected in inhalable particulates: mean levels of BDE-66, -206 and TCIPP were higher in the homes and BDE-47, -85, -99, -100, -153, -209, EH-TBB, BEH-TEBP and TDCIPP were higher at the gyms. BDE-100, EH-TBB and TDCIPP were significantly greater ($p < 0.05$) in the gym inhalable particulates than the homes. Concentrations of FRs used in polyurethane foam were higher in gym air and in dust compared to homes, particularly EH-TBB and TDCIPP. These were also the primary FRs detected in the gym's pit-foam samples.

Firefighters, too, have exhibited evidence of exposure to elevated levels of FRs, and their personal safety equipment has shown to be a sink for FRs released during a fire. This equipment can be a source of exposure later if not properly cleaned. We explored this phenomenon with colleagues from the U.S. National Institute for Occupational Safety and Health (NIOSH) at the Centers for Disease Control and Prevention (CDC). By collecting protective hood samples (a cloth garment worn around the face and neck, under the helmet, respirator and coat) previously exposed in a controlled burn exercise and then washed, we sought to determine the efficiency of laundering to remove fire contaminants (e.g., FRs and polycyclic aromatic hydrocarbons (PAHs)) from personal protective gear (**Mayer et al., 2018**). We also conducted a field evaluation with NIOSH CDC using sequential hand wipes. Such wipes are commonly used to evaluate dermal exposure. In this study, published by *Chemosphere* we used three sequential hand wipes collected from 12 employees at a U.S. electronics recycling facility immediately at the end of their shift and analyzed them for a range of FRs (**Beaucham et al., 2019**). Results showed that, although the first wipe removed the highest median percentage of the total of the three wipes for most FRs, there was a wide variation for individual FRs. Generally those FRs with low log K_{ow} and high water solubility (e.g., TCIPP) were mostly efficiently removed by the first wipe. Those with high log K_{ow} and low water solubility, e.g., BEH-TEBP were retained on the hand and mostly removed by later wipes (>50 % of the total by the third wipe). This suggests that a single wipe may not be sufficient to characterize the extent of dermal contamination.

As a follow-up to our earlier gymnastic studio FR exposure study, and with collaborators from NIOSH CDC, we evaluated FR exposure at gymnastics studios before, during, and after the replacement of existing pit foam blocks with PBDE-free foam blocks (**Ceballos et al., 2018**). This evaluation included hand wipes of coaches to measure levels of FRs on their skin before and after the work shift, as well as before and after foam replacement. Analysis was conducted on FR concentrations in dust and on window glass in the gymnastic areas and office areas, and in new and old foam blocks used throughout the studios. We found statistically higher concentrations of nine FRs, those of the Penta-formulation, EH-TBB, BEH-TEBP and TDCIPP, and found higher concentrations on employees' hands after work than we found before work, and this difference was reduced to four analytes (i.e. BDE-153, EH-TBB, BEH-TEBP and TDCIPP) after the foam replacement. Windows in the gymnastic areas still had higher concentrations of four, i.e. BDE-99, EH-TBB, BEH-TEBP and TDCIPP, of the 13 FRs analyzed than windows outside the gymnastic areas. However, concentrations were significantly reduced after replacing the pit foam. Analysis of the new replacement blocks did agree with their third-party certificate of FR analysis, i.e., that they did not contain PBDEs. But they did contain EH-TBB and BEH-TEBP, up to 3.4% by weight. EH-TBB and BEH-TEBP were not included in the third-party's certificate of analysis. We concluded that replacing the pit foam blocks eliminated a source of PBDEs, but not all FRs (e.g., EH-TBB and BEH-TEBP). Additional recommendations were provided to further minimize employee exposure to FRs at work and acknowledge the challenges consumers have in identifying chemical contents of products purchased.

Exposures along with bioavailability are key issues when determining health risks associated with FRs. Strong correlations between dust FR concentrations and body burdens have been previously observed, but there is little scientific consensus on what constitutes a dust sample.

Most studies have include dust particles > 100 µm. These larger particulates are less likely to be inhaled and interact deep inside the alveolar region of the lung, which may lead to an overestimation of exposure. Ingestion of these larger dust particles would likely be the primary route of exposure, however, FR bioavailability (i.e., desorption from particles and uptake by the digestive tract) has been suggested to be < 100% (Fang and Stapleton, 2014), and thereby only partial uptake via the vascular system would occur. It has also been suggested that once FRs are released, > 80% will remain in the particle and not volatilize into the gas phase (Cetin et al., 2008). Rauert and Harrad (2015) did not observe detectable transfers from source materials (i.e., FR-treated television plastics) to dust by volatilization at 22 ^{0}C and 60 ^{0}C, but they did detect transfers as fragments abraded following a motion that mimicked repeated wiping/moving/bumping. An electron microscopy examination of large dust particles approximately 30 µm in diameter, conducted by Webster et al, (2009), revealed that BDE-209 was highly associated (1000 ppm or 0.1% by weight) with polymer/organic matrix clusters, suggesting fragments of FR-treated products were transferred to dust. To further investigate the bioavailability of airborne FRs, we collected respirable air (airborne particles < 4 µm that can enter and interact with vascular membranes within the alveolar region of the lung) and inhalable air (airborne particles > 4 µm which are trapped on the mucosal lining of the respiratory tract, expelled or swallowed entering the digestive tract) and analyzed them for FRs and their bioavailability estimations. This first-authored examination titled, ***"Human indoor exposure to airborne halogenated flame retardants: Influence of airborne particle size"*** (**La Guardia et al., 2017**), was published in the *International Journal of Environmental Research and Public Health*. We observed that the larger, inhalable air particulates carried the bulk (> 92%) of the FRs: BDE-47, -85, -100, -99 and -153, EH-TBB, BEH-TEBP, TCEP, TCIPP and TDCIPP. These findings suggested that estimates relying on a single exposure route, such as alveolar gas exchange at a 100% adsorption rate, may not accurately estimate FR internal dosage because they ignore additional contributions from larger inhalable particulates that enter via the digestive tract. Consideration of the fate and bioavailability of these larger particulates resulted in higher dosage estimates for FRs with log K_{oa} < 12 (i.e., Penta-BDEs and Cl-OPFRs) and lower estimates for those with log K_{oa} > 12 (i.e., EH-TBB and BEH-TEBP) when compared to the alveolar route exposure alone. Of those FRs examined, the most significant effect was the 41% lower estimate for BEH-TEBP.

These contributions have supported other human exposure studies of legacy and replacement FRs and support the need for future work on exposure, uptake and health effects. These reports also support the need to continue to monitor discontinued FRs. The need for this monitoring is seen as new products comprised of recycled materials containing discontinued FRs (e.g., carpet underlayment (rebond) can contain PBDEs up to 0.1% by weight (http://carpetcushion.org/Bonded-Cushion-and-FRs.cfm)) continue to enter the marketplace and as in-use products complete their end-of-life cycle. These new sources and products will add to releases from still-in-use products, contributing additional indoor and outdoor burdens. Unfortunately, this will continue until proper disposal methods are established to remove from commerce those discontinued-FRs and replacement-FRs that are of concern, and until safer FR alternatives are identified.

Chapter Five – Concluding remarks

The work and associated publications presented in this thesis has supported the global manufacturing and usage restrictions of several FRs (e.g., PBDEs and HBCDD) and the decisions of regulators across the globe to enact and evaluate improved fire-prevention and safety legislation (e.g., California's (USA) TB-117 2013). These studies identified FR point sources to populated areas (e.g., land applied sewage sludge) as well as remote sites (e.g., Antarctic research bases). We observed novel-FRs (e.g., PBDE degradation and replacement products) and other FRs entering the environment and their accumulation within the ecosystem. We also observed that alternative-PBDEs are released into the environment by similar processes as the toxic FRs they replaced, raising additional health concerns. These studies have clearly shown that the chemical industry's *modus operandi* of replacing one FR with another has often been flawed, and complicated our understanding of adverse health consequences. Legislation by the United Nation's Stockholm Convention on POPs has reduced potential environmental and human health effects by removing several toxic FRs from commerce. However, these actions often take place after detrimental health impacts have occurred. Movements like the EU's REACH programme to identify and curb the dissemination of potentially hazardous chemicals before harm escalates, appears to be a better approach. However, such regulations need to be adopted globally before viable changes can take place. This work has also identified several research gaps covering analytical aspects, environmental issues (e.g., source, persistence, and degradation products), routes of exposure and bioavailability measured in relevant matrices (e.g., bound to polymers or un-bound/dissociated chemical interactions with gut fluids) and the potential risk associated within their environmental presence (e.g., one's occupation or recreational activities) and subsequent environmental and human health exposure. All of these areas of study need continued examination.

Finally, this thesis is a culmination of 20 years of research using a multifaceted approach to improve our knowledge on the state of FRs. It incorporated analytical method development, abiotic and biotic environmental analysis, and human exposure evaluations. This endeavor integrated interdisciplinary collaborations with professionals in toxicology, occupational hygiene, epidemiology, and other disciplines. Efforts were also greatly enhanced by scores of volunteers who invited us into their homes and workplaces, completed our surveys, and allowed us to stick and prod them and their families. Their efforts have greatly contributed to the success of this work, which will continue to provide guidance for a healthier and safer environment for all, now and into the future. To those collaborators and volunteers I offer my most sincere gratitude. With your assistance, I truly believe we have contributed to the health of our environment.

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APPENDIX – FIRST AUTHORED PUBLICATIONS

Publication title and citation *(In the order as they appear in the commentary text.)* **page**

Original article

La Guardia, M. J.; Hale, R. C.; Harvey, E. (2006).
Detailed polybrominated diphenyl ether (PBDE) congener composition of the widely used Penta-, Octa- and Deca-PBDE technical flame-retardant mixtures.
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First Author and Principal Investigator
Project designer
Principal chemist, analyzer and data examiner
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Detailed Polybrominated Diphenyl Ether (PBDE) Congener Composition of the Widely Used Penta-, Octa-, and Deca-PBDE Technical Flame-retardant Mixtures

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Polybrominated diphenyl ethers (PBDEs) have been widely used to flame-retard products common in homes and the workplace, and subsequently, they have become widely dispersed in the environment. Detailed compositional knowledge of these complex PBDE mixtures is crucial to a fuller understanding of their toxicological potencies and environmental fate due to selective congener biomagnification, degradation, and transport. Utilizing recent technical enhancements and newly available commercial standards, we developed a method capable of analyzing a larger suite of mono- through deca-BDEs. We then characterized the congener composition of six common technical flame-retardant mixtures: two penta-BDE products (DE-71 and Bromkal 70-5DE) two octa-BDE products (DE-79 and Bromkal 79-8DE) and two deca-BDE products (Saytex 102E and Bromkal 82-0DE). PBDEs were analyzed by gas chromatography/mass spectrometry (GC/MS). Structural conformations based on fragmentation patterns and molecular ions were established by electron-capture negative ionization (ECNI) and electron ionization (EI). Sixty-four commercially available PBDE standards were chromatographed on two GC columns (DB-1HT and DB-5HT) and relative retention indexes (RRI) calculated. Thirty-nine PBDEs were identified in these products, 29 at concentrations >0.02% by weight. Of these, 12 previously unreported congeners have been confirmed as commercial mixture components. Four of these congeners were detected >0.02% w/w (BDE-144, -171, -180, and −201) and three (BDE-75, -184, and -194) at <0.02%. Five other congeners (four <0.02% by weight) were tentatively identified based on their molecular ion and ECNI fragmentation in the absence of corresponding analytical standards.

Introduction

Brominated flame-retardants (BFRs) are used in a large variety of flammable polymers. The demand for polymer-based products for electrical and electronic equipment, as well as automotive equipment, construction materials, and textiles has increased over the past decade. Accordingly, the demand for BFRs has doubled from an estimated 145 000 metric tonnes (MT) in 1990 to 310 000 MT in 2000 (*1*) and continues to be produced at high volumes (237 727 and 223 482 MT for 2002 and 2003, respectively) (http://www.bsef.com/bromine/our_industry/, ref *2*). Of the BFRs, polybrominated diphenyl ethers (PBDEs) were only surpassed by tetrabromobisphenol-A (TBBPA) in regards to production volume (2001 global demand 67 390 versus 119 700 MT respectively, ref *2*). PBDEs have been commonly used in furniture (polyurethane foam), wire and cable insulation (styrene copolymers), electronics, and computers (high-impact polystyrene). However, unlike reactive BFRs like TBBPA, PBDEs are simply blended with the polymers during its formation and thus may migrate from products more readily (*1*).

PBDEs were typically produced commercially at three different levels of bromination; nominally penta-, octa- and deca-BDE. The deca-PBDE formulation made up 83.3% of the 2001 PBDE global market demand, followed by penta-11.1%, and octa- 5.6%. However, due to growing environmental and human health concerns, the latter two formulations were banned in the European Union (summer 2004). In the United States (U.S.), their sole American manufacturer voluntarily halted production of these in December 2004 (*3*). These restrictions will not eliminate PBDE releases from products currently in-service or new products manufactured with recycled PBDE-containing material. It has also been hypothesized that the widely used, unregulated deca-BDE product may, to some extent, debrominate once released into the environment, resulting in a suite of less-brominated congeners with enhanced toxicity and ability to bioaccumulate relative to the parent. Finally, due to the persistence of PBDEs, contaminated sediments and soils will serve as long-term reservoirs for PBDE release.

It has been previously reported that the European penta-formulation (Bromkal 70-5DE) primarily consists of ten isomers (tri- through hexa-PBDEs) with BDE-47 and BDE-99 contributing >70% (*4*). In a 2005 study the U.S. penta-product (DE-71), was subjected to NMR and GC/MS analysis. The authors confirmed these 10 congeners and identified nine more minor constituents, totaling 1.4% w/w (*5*) (Table ST1, Supporting Information). The major components of the octa-formulation are BDE-183, constituting >40%, and four octa-BDEs making up >30% of the product (*6*). Two more recent studies identified additional minor congeners within the European octa-formulation (Bromkal 79-8DE). However, these reports did not detail the mass percent of the individual congeners (Table ST1, Supporting Information). Deca-BDE has been reported as >97% decabromodiphenyl ether (BDE-209) with trace contributions of the three nonabromodiphenyl ethers (*6*). Trace levels of octa-BDEs have also been reported as constituents of the deca-formulation (*7*).

In 2000, an interlaboratory comparison study compared results obtained from 18 laboratories for 14 individual PBDEs in a series of standards, biological, and sediment samples. Satisfactory agreement was only achieved for one PBDE (BDE-47). Results were inconsistent for the other commonly detected PBDE contaminants, most notably BDE-209 (*8*). There have been four additional tests on similar matrixes using a smaller suite of eight PBDEs, the last test concluded in July 2005 (http://www.quasimeme.org, ref *9*). Results from the latter studies were improved. However, laboratories still experience difficulty in analyzing BDE-209 in biota and sediments. In addition, results for the other PBDEs tested diverged as analytes approached their detection limits. The author(s) concluded that high laboratory background contamination and certain gas chromatographic techniques could cause erratic results (e.g., BDE-209 can degrade if

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exposed for long periods at elevated temperatures in the injector, column, and/or detector).

Previous studies have also reported the presence of a number of unidentified PBDE congeners in various matrixes. At best, these have only been characterized to the homologue level (e.g., penta-BDE). This ambiguity limits efforts to determine the sources of PBDEs detected in the environment. The less brominated diphenyl ethers (e.g., BDE-47, and -99) have become globally distributed, akin to other persistent organic pollutants (POPs) such as PCBs. These have been detected in fresh water fish (*12*), human breast milk (*13*), and blood from human populations inhabiting even remote areas (*14*). To date, researchers have reported on over 40 specific PBDEs, ranging from mono- through deca-BDEs in environmental and biological matrixes (Table ST2, Supporting Information). To our knowledge, about half of these have never been identified as components of the PBDE technical mixtures. Efforts have been made to eliminate the use of the penta- and octa-formulations due to the accumulation of their presumed constituents in biological matrixes such as breast milk, potential for eliciting toxic effects, long-range transport, and environmental persistence. In contrast, deca- (primarily BDE-209), the PBDE product in greatest demand (in 2003 globally 56 418 MT, ref *2*), has not been regulated. BDE-209 has primarily been reported from sites near points of initial release and levels in biota to date have been comparatively low. This has been attributed to its reportedly low bioavailability and tendency to strongly bind to sediment and soil. However, BDE-209 has been detected with increasing frequency as more laboratories improve their analytical methods. Watanabe and Tatsukawa (*15*) reported that BDE-209 debrominates when dissolved in certain solvents and subjected to UV irradiation. Major homologues observed were tri- through octa-PBDEs and a host of brominated dioxins, and furans. Eriksson (*16*) and Söderström (*17*) also observed photolytic debromination of BDE-209 dissolved in solvents and associated with artificial and natural sediment, soil, and sand. Both studies, observed an increase in the lower brominated (hexa- through nona-) PBDEs. However, the majority of the octa- through hexa-BDEs observed were not specifically identified. Söderström (*17*) identified BDE-47, -99, and -154 on silica gel originally amended with BDE-209. They also reported -209 dissolved in toluene and exposed to UV light, generated BDE-100, -99, -154, and -153.

There have been limited studies on the dietary uptake and biotransformation of BDE-209. Kierkegaard (*18*) detected BDE-47, -99, -153, and several nonspecified hexa- to nona-PBDEs in juvenile rainbow trout (*Oncorhynchus mykiss*) fed cod chips spiked with BDE-209. Preferential uptake of less-brominated congeners could not be ruled out because some of these congeners were present as minor constituents in the spiked solution. However, BDE-153, -154, and an unidentified octa-BDE were not detected in the original Deca-mixture, indicating possible biotransformation of BDE-209. More recently, juvenile carp (*Cyprinus carpio*) were fed BDE-209 (>98% purity) spiked food and apparent metabolic debromination of BDE-209, as evidenced by the bioaccumulation of seven less-brominated BDEs (penta- to octa-BDE), was reported. Only BDE-154 and -155 were positively identified (*19*).

In light of these studies, debromination of components of the deca-BDE product might be a potential source of less-brominated congeners observed in the environment. While presently this might be dwarfed by contributions tied to penta- usage, this picture might change due to deca-'s historical market dominance and continued usage. To date, the full inventories of individual congeners constituting the major technical formulations in use in Europe and North America have not been identified and quantified. These mixtures are the original source materials for all subsequent PBDE congeners in the environment, and hence, uncertainty as to their complete composition limits our ability to elucidate which congeners in environmental samples likely result from degradation processes. Here, we present an analytical approach which permits the quantitative measurement of mono- through deca-BDEs, including the major and minor constituents of six commercial PBDE mixtures, i.e., DE-71 and DE-79 (Great Lakes Chemical Corp., Indiana), Bromkal 70-5DE, Bromkal 79-8DE, Bromkal 82-0DE (Chemische Fabrik Kalk, Köln, Germany), and Saytex 102E (Albemarle Corp., Louisiana).

Experimental Section

Sixty-four individual PBDE standards (Table 1) were first analyzed by GC (6890N, Agilent Tech., Palo Alto, CA) with MS detection (JMS-GC Mate II, JEOL, Peabody, MA). Ion fragmentation patterns were produced by electron-capture negative ionization (ECNI) and electron ionization (EI). PBDE resolution capabilities of two different 30 m, 0.25 mm i.d., 0.1 μm film thickness columns (DB-1HT and DB-5HT, J&W Scientific, Agilent Tech.), were compared and a relative retention index (RRI) (Figure SF1, Supporting Information) was calculated for each congener on each column (Table 1). HT columns can withstand temperatures up to 400 °C without degrading, which is useful in the chromatographic elution of high boiling residuals associated with the analysis of the BDE-209. Pentachlorobenzene (PtClB), decachlorodiphenyl ether (DCDE) (Ultra Scientific, North Kingstown, RI), and C_{13}-BDE-209 (Cambridge Isotopes Labs., Andover, MA) were added to each sample as marker compounds for RRI calculations. If a sample contained BDE-209, C_{13}-BDE-209 was not added, as both compounds have identical elution times.

Standards (1-μL) were introduced into an on-column injector at an initial injector temperature of 65 °C. Temperature was then increased to 150 °C at 30 °C/minute, then 10 °C/minute to 300 °C, and held for 15 min. It was then increased to 350 °C at 30 °C/minute and held at 350 °C for 5 min, as a bake-out procedure. The GC column oven was programmed to follow the injector temperature ramp program. This low temperature on-column injection technique with column temperature tracking reduces the likelihood of PBDE debromination, reported as a result of prolonged exposure to high injector temperatures common with split/splitless injectors (*20*). The target analytes were first detected by ECNI, scan range 10−550 *m/z*. Electron energy of 62.1 eV was used for ionization of the reagent gas (methane). The GC/MS interface was maintained at 300 °C, filament delay 3 min, ion source temperature 300 °C, pressure 3.3 mPa, and scan time 1 s. The electron magnet and optical path were calibrated using perfluorokerosene (PFK) and 1,2,4-trichlorobenzene at a 1000:1 mixture ratio. Quantification curves were generated for each target compound by monitoring *m/z* 79 ($[^{79}Br]^-$) and 81 ($[^{81}Br]^-$). Mono-through nona-BDE concentrations ranged from 10 to 1000 pg and BDE-209 ranged from 50 to 5000 pg on-column. An internal standard, DCDE, was added to each sample (200 ng) and used for quantitation, monitoring 264 *m/z* from the $[C_6Cl_5O]^-$ ion by ECNI. The minimum r^2 value for acceptance of the five-point quantification curve was 0.998.

For each PBDE standard, fragmentation patterns, isotope intensities and structural conformation were observed by monitoring ECNI spectra (scan range 10−550 *m/z*) and EI spectra (scan range 50−1000 *m/z*, scan time 0.30 s., electron energy 70 eV). For this study, the previously stated GC conditions were used for both ECNI and EI analyses. In ECNI of PBDEs, the predominant ions generated are 79 and 81 *m/z*. Cleaving at the ether bond has also been observed for hepta, octa-, nona-, and deca-BDEs. These produced spectra with ion clusters centered around 328 and 330 *m/z* for

TABLE 1. PBDE Congener Analysis, Associated Relative Retention Indices (RRIs) and Major Fragmentation Ions in EI and ECNI

		DB-5HT		DB-1HT		EI (*m/z*)	ECNI (*m/z*)
compound	**IDs**	**r-time**	**RRI**	**r-time**	**RRI**	$[M]^+$, $[Br2\text{-}M]^+$	$[^{79}Br]^-$, $[^{81}Br]^-$, $[C_6Br_xH_yO]^-$
pentachlorobenzene[d]	PtClB	4.48	1000	4.25	1000	250	35, 37[b]
3-mono BDE[e]	BDE-3	5.58	1095	5.28	1092	248, 250	79, 81
2, 4-di BDE[e]	BDE-7	7.50	1261	7.07	1251	168, 328	79, 81
4,4′-di BDE[e]	BDE-15	8.07	1310	7.58	1297	168, 328	79, 81
2,4′, 6-tri BDE[f]	BDE-32	9.65	1447	9.08	1430	246, 248, 406, 408	79, 81
2,2′, 4-tri BDE[e]	BDE-17	9.83	1462	9.27	1447	246, 248, 406, 408	79, 81
2′, 3,4-tri BDE[e]	BDE-33	**10.13**	**1488**	**9.53**	**1470**	246, 248, 406, 408	79, 81
2, 4,4′-tri BDE[e]	BDE-28	**10.15**	**1490**	**9.53**	**1470**	246, 248, 406, 408	79, 81
3,3′, 4-tri BDE[f]	BDE-35	10.33	1506	9.73	1488	246, 248, 406, 408	79, 81
3, 4,4′-tri BDE[f]	BDE-37	10.55	1525	9.95	1508	246, 248, 406, 408	79, 81
	tetra-a	11.43	1601	10.75	1579	326, 486	79, 81
2, 4,4′, 6-tetra BDE[f]	BDE-75	11.68	1622	**11.05**	**1606**	326, 486	79, 81
2,2′, 4,6′-tetra BDE[c]	BDE-51	11.77	1630	**11.07**	**1607**	326, 486	79, 81
2,2′, 4,5′-tetra BDE[e]	BDE-49	11.82	1634	**11.23**	**1622**	326, 486	79, 81
2,3′, 4′, 6-tetra BDE[e]	BDE-71	**11.90**	**1641**	**11.23**	**1622**	326, 486	79, 81
2,2′, 4,5-tetra BDE[c]	BDE-48	**11.92**	**1643**	**11.25**	**1623**	326, 486	79, 81
2,2′, 3,4,4′, 5,6,6[d]-octachlorobiphenyl[d]	PCB-204	11.98	1648	11.47	1643	430	35, 37[b]
2,2′, 4,4′-tetra BDE[e]	BDE-47	12.13	1661	11.50	1646	326, 486	79, 81
2, 4,4′, 5-tetra BDE[c]	BDE-74	12.28	1674	11.65	1659	326, 486	79, 81
2,3′, 4,4′-tetra BDE[e]	BDE-66	**12.42**	**1686**	**11.77**	**1670**	326, 486	79, 81
2,2′, 3,4′-tetra BDE[c]	BDE-42	**12.45**	**1689**	**11.77**	**1670**	326, 486	79, 81
3,3′, 4,4′-tetra BDE[e]	BDE-77	12.87	1725	12.22	1710	326, 486	79, 81
	penta-a	13.10	1745	12.43	1728	404, 406, 564, 566	79, 81
2,2′, 4,5,6′-penta BDE[c]	BDE-102	13.52	1781	12.82	1763	404, 406, 564, 566	79, 81
2,2′, 4,4′, 6-penta BDE[e]	BDE-100	13.63	1791	12.97	1776	404, 406, 564, 566	79, 81
2,2′, 4, 5,5′-penta BDE[c]	BDE-101	13.72	1799	13.07	1785[a]	404, 406, 564, 566	79, 81
2, 3′, 4,4′, 5-penta, 2,3′, 4,5,5′-penta BDE[e]	BDE-119, -120	**13.77**	**1803**	**13.01**	**1780**[a]	404, 406, 564, 566	79, 81
2,2′, 4,4′, 5-penta BDE[e]	BDE-99	14.05	1827	13.37	1812	404, 406, 564, 566	79, 81
2,2′, 3′, 4, 5-penta 2,3′, 4,4′, 5-penta BDE[c]	BDE-97, -118	14.40	1857	13.72	1843[a]	404, 406, 564, 566	79, 81
2,3,4,5, 6-penta BDE[f]	BDE-116	14.45	1862	13.52	1825[a]	404, 406, 564, 566	79, 81
2,2′, 3, 4,4′-penta BDE[e]	BDE-85	14.80	1892	14.07	1874	404, 406, 564, 566	79, 81
3,3′, 4,4′, 5-penta[3], 2,2′, 4,4′, 6,6′-hexa BDE[f]	BDE-126, -155	**14.92**	**1902**	**14.22**	**1888**	404, 406, 564, 566, 484, 644	79, 81
2,2′, 4,4′, 5,6′-hexa BDE[e]	BDE-154	15.22	1928	14.52	1915	484, 644	79, 81
2,2′, 3, 4,5′, 6-hexa BDE[c]	BDE-144	15.43	1946	14.7	1931	484, 644	79, 81
	hexa-a	15.53	1955	14.83	1942	484, 644	79, 81
2,2′, 4,4′, 5,5′-hexa BDE[e]	BDE-153	15.77	1976	15.1	1966	484, 644	79, 81
2,2′, 3,4,4′, 6-hexa BDE[c]	BDE-139	16.02	1997	15.28	1982	484, 644	79, 81
decachlorodiphenyl ether[d]	DCDE	16.05	2000	15.48	2000[a]	444, 514	35, 37[b]
2,2′, 3,4,4′, 6′-hexa BDE[c]	BDE-140	16.17	2010	15.4	1993[a]	484, 644	79, 81
2,2′, 3,4,4′, 5′-hexa BDE[e]	BDE-138	16.55	2034	15.78	2027	484, 644	79, 81
2, 3, 4,4′, 5, 6-hexa BDE[f]	BDE-166	16.63	2040	15.83	2032	484, 644	79, 81
2, 3,3′, 4,4′, 5-hexa BDE[e]	BDE-156	16.80	2051	16.07	2053	484, 644	79, 81
2,2′, 3, 4,4′, 6,6′-hepta BDE[e]	BDE-184	17.18	2077	16.4	2083	562, 564, 722, 724	79, 81, 328, 330, 408
	hepta-a	**17.29**	**2085**	16.53	2095[a]	562, 564, 722, 724	79, 81
2,2′, 3,3′, 4,4′-hexa BDE[c]	BDE-128	**17.33**	**2087**	16.47	2089[a]	484, 644	79, 81
2,2′, 3,3′, 4,5′, 6-hepta BDE[c]	BDE-175	**17.40**	**2092**	16.63	2104	562, 564, 722, 724	79, 81, 328, 330, 408
2,2′, 3, 4,4′, 5′, 6-hepta BDE[e]	BDE-183	**17.40**	**2092**	16.68	2108	562, 564, 722, 724	79, 81, 328, 330, 408
2,2′, 3, 4,4′, 5,6′-hepta BDE[c]	BDE-182	17.55	2103	16.78	2117	562, 564, 722, 724	79, 81, 328, 330, 408
2,2′, 3, 4, 5,5′, 6-hepta BDE[c]	BDE-185	**17.63**	**2108**	16.83	2122	562, 564, 722, 724	79, 81
2, 3,3′, 4, 5,5′, 6-hepta BDE[c]	BDE-192	**17.68**	**2111**	16.92	2130	562, 564, 722, 724	79, 81
2, 3,3′, 4,4′, 5′, 6-hepta BDE[e]	BDE-191	17.83	2122	17.08	2144	562, 564, 722, 724	79, 81
2,2, 3, 4,4′, 5, 5′-hepta BDE[e]	BDE-180	18.08	2139	17.32	2166	562, 564, 722, 724	79, 81, 328, 330, 408
2,2′, 3, 4,4′, 5, 6-hepta BDE[e]	BDE-181	18.28	2152	17.48	2180	562, 564, 722, 724	79, 81
2,2′, 3,3′, 4, 5, 6-hepta BDE[c] 2,3,3′, 4,4′, 5, 6-hepta BDE[c]	BDE-173, -190	**18.43**	**2163**	**17.58**	**2189**[a]	562, 564, 722, 724	79, 81
2, 2′, 3,3′, 4,4′, 6-hepta BDE[e]	BDE-171	**18.46**	**2165**	**17.57**	**2188**[a]	562, 564, 722, 724	79, 81, 328, 330, 408
2,2′, 3,3′, 4,5′, 6,6′-octa BDE[e]	BDE-201	19.37	2227	18.35	2259	642, 802	79, 81, 408
2,2′, 3, 4,4′, 5, 6,6′-octa BDE[c]	BDE-204	**19.53**	**2238**	18.47	2269	642, 802	79, 81, 328, 330, 408, 486, 488
2,2′, 3,3′, 4,4′, 6,6′-octa BDE[e]	BDE-197	**19.57**	**2241**	18.52	2274	642, 802	79, 81, 408
2,2′, 3,3′, 4, 5,5′, 6-octa BDE[c]	BDE-198	**19.87**	**2261**	18.75	2295	642, 802	79, 81, 328, 330, 408, 486, 488
2,2′, 3, 4,4′, 5,5′, 6-octa BDE[c]	BDE-203	**19.88**	**2262**	18.82	2301	642, 802	79, 81, 328, 330, 408, 486, 488
2,2′, 3,3′, 4,4′, 5,6′-octa BDE[e]	BDE-196	20.07	2275	18.92	2310	642, 802	79, 81, 408
2, 3,3′, 4,4′, 5,5′, 6-octa BDE[c]	BDE-205	20.58	2310	19.28	2342	642, 802	79, 81, 328, 330, 408, 486, 488

TABLE 1. (Continued)

compound	IDs	DB-5HT r-time	DB-5HT RRI	DB-1HT r-time	DB-1HT RRI	EI (m/z) $[M]^+$, $[Br2\text{-}M]^+$	ECNI (m/z) $[^{79}Br]^-$, $[^{81}Br]^-$, $[C_6Br_xH_yO]^-$
2,2′, 3,3′, 4,4′, 5,5′-octa BDE^{3}	BDE-194	21.12	2347	19.78	2387	642, 802	79, 81, 408
	octa-a	21.70	2386	20.1	2416	642, 802	79, 81, 328, 330, 408, 486, 488
2,2′, 3,3′, 4, 5,5′, 6,6′-nona BDE^{3}	BDE-208	23.12	2483	21.17	2513	720, 722, 880, 882	79, 81, 328, 330, 408, 486, 488
2,2′, 3,3′, 4,4′, 5, 6,6′-nona BDE^{3}	BDE-207	23.45	2506	21.45	2538	720, 722, 880, 882	79, 81, 328, 330, 408, 486, 488
2,2′, 3,3′, 4,4′, 5,5′, 6-nona BDEe	BDE-206	24.33	2566	22.2	2605	720, 722, 880, 882	79, 81, 328, 330, 408, 486, 488
2,2′, 3,3′, 4,4′, 5,5′, 6,6′-deca BDE[e]	BDE-209	30.68	3000	26.58	3000	800, 960	79, 81, 328, 330, 408, 486, 488
2,2′, 3,3′, 4,4′, 5,5′, 6,6′-deca BDE[c] (C^{13})[f]	BDE-209 (C^{13})	30.68	3000	26.58	3000	812, 972	79, 81, 334, 336, 414, 492, 494

[a] Retention times reversed compared to DB-5HT. [b] $[^{35}Cl]^-$, $[^{37}Cl]^-$. [c] AccuStandard, Inc. New Haven, CT. [d] Ultra Scientific, North Kingstown, RI. [e] Wellington Laboratories, Ontario, Canada. [f] Cambridge Isotope Laboratories, Andover, MA. Bold = *r*-time and RRi include coeluting compounds.

$[C_6Br_3H_2O]^-$, 408 m/z for $[C_6Br_4HO]^-$, and 486 and 488 m/z for $[C_6Br_5O]^-$ (*21*). When analyzing PBDEs by EI, others have observed dominant ion clusters centered on the molecular ion $[M]^+$ and the loss of two bromines $[M\text{-}Br_2]^+$ (*22*).

Serial dilutions of each of the technical PBDE formulations were prepared in hexane and all solutions were stored in amber glass. To permit accurate quantitation of both major and minor constituents of each commercial mixture, three different concentrations (0.5, 5, 100 ng/μL) of each product were prepared and analyzed. The quantitation limit (QL) for the minor constituents was estimated to be 0.02% w/w within the technical formulations. Each formulation was first analyzed by GC/MS-ECNI and monitored for bromine ions (79 and 81 m/z). Monitoring ions m/z 35 ($[^{35}Cl]^-$) and 37 ($[^{37}Cl]^-$) established retention times for marker peaks PtClB and DCDE. These two marker compounds were assigned index values of 1000 and 2000, respectively. Indices of the other standards were calculated by interpolation. Tentative identification was established if a peak's RRI was within ± 3 of a previously determined PBDE RRI. PBDEs were then quantified based on peak area produced by summing the 79 and 81 m/z ions. Samples were reanalyzed by EI. Structural confirmation was established for all identified analytes in the commercial formulations above our QL, by observing both ECNI and EI spectra. Homologue identification was determined for compounds detected in the technical formulation that did not coincide with an RRI, by matching ion fragmentation patterns to those produced by the analytical standards. Each technical product was analyzed three times at each concentration and the relative standard deviation (RSD) of the resulting peak RRIs for all reported congeners present at concentrations >0.02% w/w, was <20%. PBDEs detected below our QL were also reported; however, their RSDs ranged from >20 to 60% and were, therefore, simply reported as <0.02%.

Results and Discussion

Analytical methods are needed that are capable of identifying and quantitating a greater number of the PBDEs which constitute the technical products in commercial use, as well as additional PBDEs that may appear in complex environmental matrixes as a result of transport, degradation, or concentration processes. In this study, 64 PBDEs were examined. These encompassed all the congeners previously reported present in these mixtures and the environment (Tables ST1 and ST2, Supporting Information). Two different analytical columns have historically been recommended for analyzing PBDEs: a short column (15 m or less) for thermally labile congeners (e.g., BDE-209) and a longer column to resolve the remaining brominated BDEs (*10*). This approach results in undesirable instrument downtime (e.g., for switching columns), analytical delays (run each sample twice), or the need for duplicate instrumentation and increased analytical costs. To determine if BDE-209 degraded as a result of extended exposure to high temperature, it and thermally stable decabromobiphenyl (PBB-209), (decomposition temperature >395 °C, ref *23*) were analyzed together on DB-5HT columns of different lengths (15 and 30 m), using identical GC techniques (see the Experimental Section). Chromatographic band broadening occurs co-incident with increasing retention time and may be misinterpreted as compound degradation. Co-injection of PBB-209 and BDE-209 onto both columns and comparison of the ratio of their peak areas normalizes peak integration complications associated with such band broadening using a 30 m column. Similar peak area ratios ($n = 3$) were observed 2.36 (SD = 0.13) and 2.28 (SD = 0.14) for the 15 and 30 m columns, respectively). This indicates a minimal difference in BDE-209 degradation between the two columns (Figure SF2, Supporting Information). Small amounts of nona-BDEs (BDE-206, -207) were also detected in both runs. Their total contributions were similar (1.8 and 1.5% for the 15 and 30 m columns, respectively), and are likely impurities within the original analytical standard (AccuStandards, Inc. New Haven, CT. BDE-209, stated purity 98.3%).

Resolution of all targeted PBDEs was compared on DB-1HT and DB-5HT GC columns. Using the ECNI GC/MS runs we calculated separate RRIs for each PBDE congener on each of the two columns (Table 1). Congeners eluted in the same order from these columns with the exception of BDE-116, -119/120, -128, -140, and -171. Elution from the DB-5HT required approximately 3 min longer than the DB-1HT column. This may be attributable to its slightly higher polarity (5% phenyl-methylpolysiloxane versus 100% dimethylpolysiloxane for the DB-1HT column). Korytár (*11*) also reported greater retention times with the DB-5 column. Compounds were considered to be unresolved if their RRIs differed <3 and they produced similar mass spectra. The DB-1HT produced six groups of coeluters: BDE-28/33, -49/71/48, -66/42, -75/51, -119/120, -126/155, and -173/190/171. Four additional coeluting pairs were noted on the DB-5HT: -175/183, -185/192, -204/197 and -198/203. Separation of -175/183, -204/197, and -198/203 was only slightly better (by 1−3 additional RRI) on the DB-1HT column. BDE-126 and -155 coeluted on both columns. However, as they possess differing degrees of bromination (BDE-126 is a penta- and -155 is a hexa-BDE) they can be separated based on mass spectral information, albeit not when simply monitoring -79 and -81

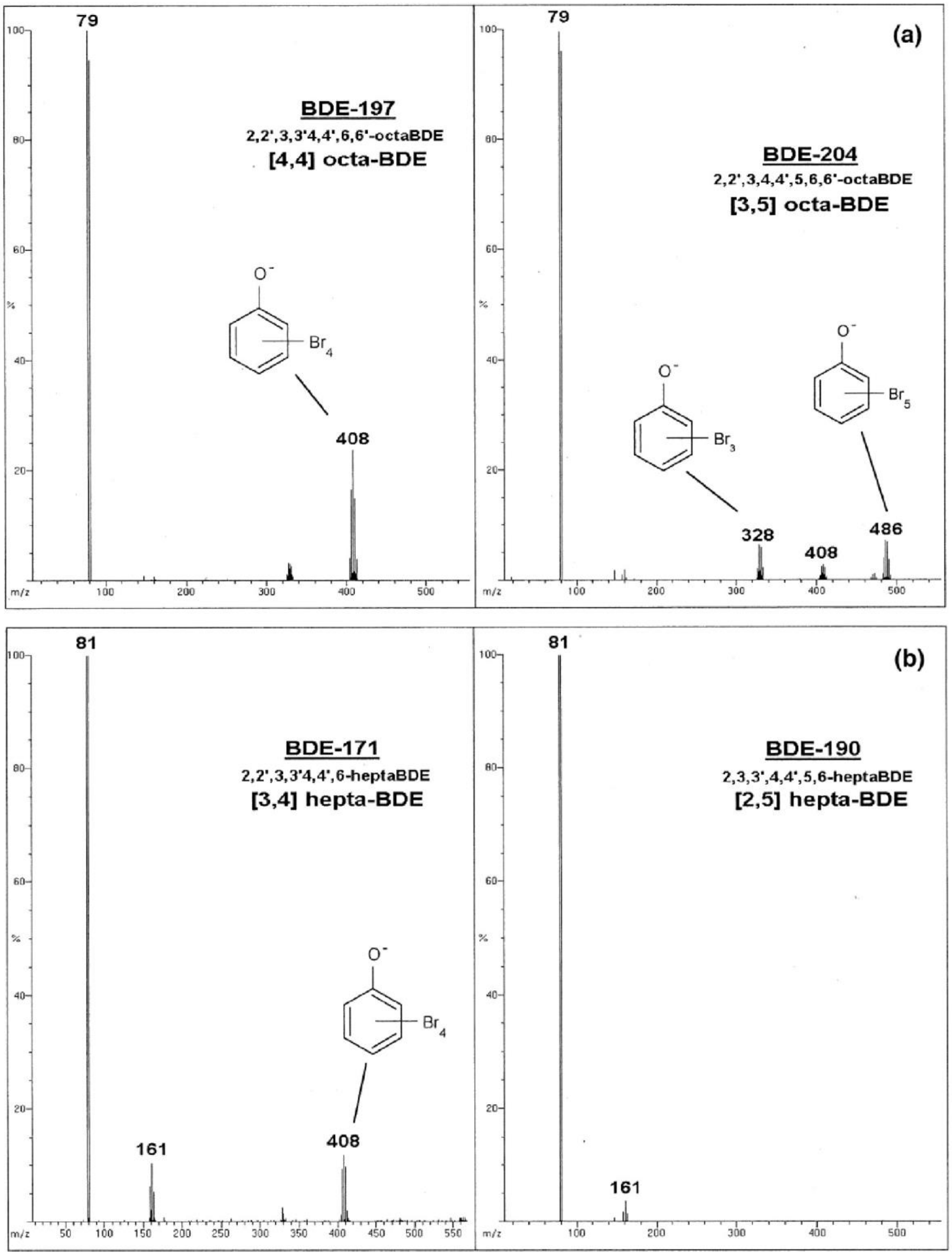

FIGURE 1. (a) ECNI spectra of the octa- substituted congeners, illustrating differences in spectra as a function of bromine position (BDE-197 and -204); and (b) spectra illustrating hepta- substituted congeners (BDE-171 and -190).

as is commonly done. The DB-5HT ultimately was chosen for the analysis of the PBDE technical products as it retained the congeners to a greater extent. This factor may provide for greater resolution as researchers encounter additional congeners not examined here. The 30 m DB-5HT column also produced BDE-209 peaks with characteristics comparable to those obtained with the shorter 15 m column.

GC/ECNI−MS of each of the targeted PBDE generated spectra with 79 and 81 *m*/*z* as the major ions. As mentioned above, Bjorklund (*21*) also observed that under ECNI PBDEs are also cleaved at the ether bond producing a strong signal corresponding to $[C_6Br_xH_yO]^-$. Deca- and nona-BDEs produced significant ion clusters centered at 486 and 488 *m*/*z* for the $[C_6Br_5O]^-$ and 408 *m*/*z* for the $[C_6Br_4HO]^-$ ions. Similar fragmentation was noted in this study for the hepta- and octa-BDEs as a function of the substitution pattern of the ring bromines. The predominant ions detected were: 486, 488, and 408 *m*/*z*, and 328 and 330 *m*/*z* for $[C_6Br_3H_2O]^-$.

TABLE 2. Concentrations (%, w/w) of PBDEs in Penta, Octa-, and Deca- Technical Products^{a}

	penta-PBDE		octa-PBDE		deca-PBDE			penta-PBDE		octa-PBDE		deca-PBDE	
IDs	DE-71	Bromkal 70-5DE	DE-79	Bromkal 79-8DE	Saytex 102E	Bromkal 82-0DE	IDs	DE-71	Bromkal 70-5DE	DE-79	Bromkal 79-8DE	Saytex 102E	Bromkal 82-0DE
-17	0.07	0.05	nd	nd	nd	nd	-139	0.8	0.38	nd	nd	nd	nd
-28/33	0.25	0.1	nd	nd	nd	nd	-140	0.17	0.1	<0.02	nd	nd	nd
tetra-a	<0.02	nd	nd	nd	nd	nd	-138	0.73	0.53	0.62	nd	nd	nd
-75	<0.02	nd	nd	nd	nd	nd	-184	<0.02	nd	<0.02	<0.02	nd	nd
-51	<0.02	nd	nd	nd	nd	nd	hepta-a	nd	nd	<0.02	nd	nd	nd
-49	0.74	0.36	nd	nd	nd	nd	-175/183^{b}	0.1	0.33	42	12.6	nd	nd
-48/71	<0.02	nd	nd	nd	nd	nd	-191	nd	nd	<0.02	nd	nd	nd
-47^{b}/74	38.2	42.8	nd	nd	nd	nd	-180	nd	nd	1.7	nd	nd	nd
-66/42	0.53	0.21	nd	nd	nd	nd	-171	nd	nd	1.81	0.17	nd	nd
penta-a	0.05	nd	nd	nd	nd	nd	-201	nd	nd	0.78	<0.02	nd	nd
-102	0.15	nd	nd	nd	nd	nd	-197	nd	nd	22.2	10.5	nd	0.03
-100	13.1	7.82	nd	nd	nd	nd	-203	nd	nd	4.4	8.14	nd	0.07
-99	48.6	44.8	nd	nd	nd	nd	-196	nd	nd	10.5	3.12	nd	0.46
-97/118	<0.02	0.12	nd	nd	nd	nd	-194	nd	nd	<0.02	nd	nd	nd
-85	2.96	2.16	nd	nd	nd	nd	octa-a	nd	nd	<0.02	nd	nd	nd
-126/155^{b}	0.21	0.67	nd	nd	nd	nd	-208	nd	nd	0.19	<0.02	0.06	0.07
-154	4.54	2.68	1.07	0.04	nd	nd	-207	nd	nd	11.5	11.2	0.24	4.1
-144	nd	nd	0.1	0.12	nd	nd	-206	nd	nd	1.38	7.66	2.19	5.13
hexa-a	nd	nd	<0.02	nd	nd	nd	-209	nd	nd	1.31	49.6	96.8	91.6
-153	5.44	5.32	8.66	0.15	nd	nd							

[a] nd = not detected. [b] major congener of the coeluting pair, BDE-3, -7, -15, -32, -35, -37, -77, -101, -119, -120, -116, -128, -156, -166, -173, -181, -182, -185,-190, -192,-198, -204, and -205 were not detected in these PBDE-technical formulations based on analysis of available standards.

Unlike the spectra produced by EI, which permits limited identification of PBDE congeners to the level of homologue group (e.g., octa- and hepta-BDEs) based on their molecular ions $[M]^+$ and reduced $[M\text{-}2Br]^+$ ion clusters for non-ortho bromine substituted (e.g., BDE-126) compared to ortho-substituted PBDEs (e.g., BDE-100), the ECNI spectra permit in, some cases, differentiation as to bromine positioning. For example, an octa- can have either three bromines on one ring $[C_6Br_3H_2O]^-$ and five on the second $[C_6Br_5O]^-$ (referred to as *(3, 5)* substituted, in this manuscript) or four bromines on each ring $[C_6Br_4HO]^-$ (referred to as *(4, 4)* substituted). Using the more informative ECNI spectra, two octa- coeluters (BDE-197 and -204) could be differentiated [Figure 1a]. Some hepta-BDEs could be differentiated in the same way. A hepta-BDE substituted with three bromines on one ring and four on the second (*(3, 4)* substituted) produces spectra indicating ion clusters for $[C_6Br_3H_2O]^-$ and $[C_6Br_4HO]^-$. However, a congener with a two and five bromine *(2, 5)* substitution on the two rings does not produce significant spectra for the $[C_6Br_2H_3O]^-$ and $[C_6Br_5O]^-$ ions. This difference can be observed for the coeluting hepta-BDEs (BDE-173, -190, and -171), where both -173 and -190 are *(2, 5)* substituted and -171 is *(3, 4)* substituted [Figure 2b]. Major ions (*m/z*) produced by ECNI for the PBDE congener standards examined are listed in Table 1. For additional compound identification, each target compound was reanalyzed by GC/MS−EI, and in these cases, the major ions produced [Table 1] were centered on the molecular $[M]^+$ and $[M\text{-}Br_2]^+$ ions.

Each of the six technical products was analyzed by both EI and ECNI for structural fragmentation identification, as previously established for the target compounds. Congeners were quantified using peak areas produced by ECNI for ions 79 and 81 *m/z*. From the 79 and 81 *m/z* plots for each of the products, it appears that the penta- and octa- European formulations contained fewer brominated components than the analogous U.S. products (Figure SF3a and SF3b, Supporting Information). However the converse is apparent for the deca- products (Figure SF3c, Supporting Information). A total of 34 PBDEs were positively identified (RRI and MS−EI, ECNI spectra matching) in the various technical products. Six congeners or congener pairs (BDE-48/71, -51, -75, -184, -191, and −194) were present at <0.02% by weight (Table 2). An additional five congeners (tetra-a, penta-a, hexa-a, hepta-a, and octa-a BDE) were categorized only to their degree of bromination.

DE-71 and Bromkal 70-5DE (Penta-BDE). Analysis of DE-71 revealed a total of 23 PBDE congeners. To our knowledge, six of these have not been previously reported as components of this formulation (BDE-75, -97/118, -140, -184, and one tetra-BDE and one penta-BDE that did not correspond to congeners in our RRI). These constituents were tentatively identified (tetra-a and penta-a) by their EI spectra and quantified assuming a detector response identical to their nearest retention time homologue. We failed to detect two PBDEs (BDE-91 and -119) previously reported to be components of DE-71 (Table ST1, Supporting Information). However BDE−91, which elutes between -99 and -100 (*5*) was not available to us at the time of this study and -119 was previously detected well below our quantitation limit. Korytár (*11*) recently identified 18 PBDE congeners in Bromkal 70-5DE, eight more than reported by Sjödin (*4*). Korytár (*11*) utilized GC-ECD, which provides limited structural information, and separated the congeners on a DB-5 (J&W Scientific) column using a slow temperature ramp rate (1.5 °C/min.). In our study we identified 16 congeners. We could not separate the previously identified BDE-74 and -101 using our faster rate of 10 °C/min. from two major coeluting 70-5DE components, BDE-47 (42.8%) and -100 (7.82%, w/w). It is interesting to note, that BDE-101, a penta-BDE, eluted slightly before -119 and an unspecified penta- congener, which was previously detected as a component of DE-71 (*5*). Both of the penta-formulations exhibited six major congeners: BDE-99 > -47 > -100 > -153 > -154 > -85, ranging from 48.6 to 2.96% w/w (Table 2).

Bromkal 79-8DE (Octa-BDE). Two previous studies examined the congener composition of Bromkal 79-8DE (Table ST1, Supporting Information). Björklund (*24*) identified eight congeners (BDE-209, -208, -207, -206, -203, -197, -196, and -183) with one unknown octa-BDE (octaBDE:1). Korytár (*11*) detected these eight, plus an additional four, and identified octaBDE:1 as BDE-204. We detected the original eight (BDE-209 > -175/183 > -207 > -197 > -203 > -206 > -196 ≫ -208), plus five additional congeners not previously reported, i.e., BDE-171 > -153 > -144 > -154 > -201, ranging from 0.17 to <0.02% w/w, respectively. Korytár (*11*) identified five additional congeners (BDE-205, -204, -191, -181, and

−173/190). However, we observed no peaks for BDE-205, -191, and -181 in the chromatogram produced from our most concentrated standard (100 μg/mL). For the BDE-173/190 group a peak with a RRI corresponding to BDE-173, -190, and -171 was confirmed as a hepta-BDE by its EI spectra. On further evaluation of the ECNI spectra, a strong signal was observed centered on 408 *m/z*, suggesting cleavage at the ether bond of a (*3, 4*) substituted hepta-BDE. This may correspond to BDE-171 rather than -173/190 (which are both (*2, 5*) substituted hepta-BDEs) (Figure 1b). The ECNI spectrum was also useful for identifying BDE-201 (RRI no. 2227), which produced ion clusters centered at 328, 330, and 408 *m/z*. These indicated a (*4, 4*) substituted octa-BDE, similar to BDE-197 (Figure 1a). This may have been previously identified as BDE-204 (*12, 24*), which elutes slightly later than -201 and is a (*3, 5*) substituted octa-BDE.

DE-79 (Octa-BDE). In the DE-79 mixture, 22 PBDEs were detected and 15 were above the quantitation limit (Table 2). The major congeners were: BDE-175/183 (42%), -197 (22.2%), -207 (11.5%), and -196 (10.5%). Of the seven PBDEs below the quantitation limit, three did not correspond to RRIs of available standards and were tentatively identified based on their EI spectra (hexa-a, hepta-a, and octa-a). The ECNI spectrum for hepta-a produced ion clusters centered on 328, 330, and 408 *m/z*, i.e., a (*3, 4*) pattern. There are a possible 24 hepta-substituted PBDE congeners; 18 of these are (*3, 4*) substituted. Four of these possibilities were eliminated by the analysis of standards in the ECNI mode. As for the octa-a unknown, the ECNI spectra produced ion clusters indicative of a (*3, 5*) substituted octa-BDE. For the octa-BDEs only 12 possible congeners exist and our target list included eight of these. Out of the remaining four, only two are (*3, 5*) substituted, indicating the octa-unknown is either BDE-195 or -200. The profiles of the major PBDE constituents (>0.02% w/w) in the two octa-formulations were similar. However, BDE-209 was the major congener (49.6%) in the 79-8DE formulation, while BDE-183 (42%) dominated in DE-79 (Table 2). (BDE-209 has previously been reported as a major constituent of the Bromkal 79-8DE product, refs *6, 10, 11*). This suggests differences in manufacturing conditions, although these products were marketed for similar purposes. Batch differences may also have contributed. Such variations may complicate interpretation of degradation processes.

Saytex 102E and Bromkal 82-0DE (Deca-BDE). The major congener detected in the two deca- formulations (Saytex 102E and Bromkal 82-0DE) was BDE-209, 96.8 and 91.6%, respectively (Table 2). Both formulations contained the three nona-BDE congeners (BDE-206 > -207 > -208), but the 82-0DE also contained three octa-BDEs (BDE-196, -203, and -197), total octa- contributions 0.56%, w/w. However, the latter product has not been manufactured for over a decade. To our knowledge this is the first time these three octa-BDEs have been identified and quantified as components of a deca-formulation. However the higher purity of the current use Saytex product may indicate improvements in manufacturing practices, fueled by growing concerns over products containing trace levels of lower brominated BDEs.

By utilizing technical improvements in the analysis of PBDEs we were able to develop a method capable of analyzing a larger suite of PBDEs. This improvement has led to the identification of 12 previously unreported PBDE congeners in the technical PBDE-formulations examined. A total of 34 PBDE congeners were positively identified by EI and ECNI mass spectral data and matched to RRIs of pure analytical standards. Five additional PBDEs present in the commercial mixtures did not match any of the 64 RRIs. These were classified according to their homologue grouping, as indicated by their EI-produced molecular ion. Two were further classified based on bromine substitution of the phenyl rings (e.g., hepta-a as (*3, 4*) hepta-BDE and octa-a as (*3, 5*) octa-BDE). Thus a total of 39 individual PBDEs were identified in six formulations tested. We failed to detect 13 PBDEs (BDE-7, -15, -32, -35, -37, -77, -119, -120, -166, -181, -190, -204, and -205), previously detected by others in various environmental and biological matrixes (Table ST2, Supporting Information), in the technical PBDE products tested in this study. These additional congeners may be the result of photolysis or metabolic debromination of the parent PBDEs originally contained in the technical product. Alternatively they may occur due to differences in manufacturing conditions (as seen between the U.S. and European formulations in this study) or biomagnification of PBDEs present in the technical products but below our QL. Understanding the congener composition within these "real world" source mixtures is also necessary for toxicological studies as individual congeners may exhibit varying potencies and interactions may occur between constituents within these mixtures.

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Supporting Information Available

Tables ST1, ST2, and Figures SF1, SF2, and SF3 (a,b,c). This material is available free of charge via the Internet at http://pubs.acs.org.

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Principal chemist, analyzer and data examiner
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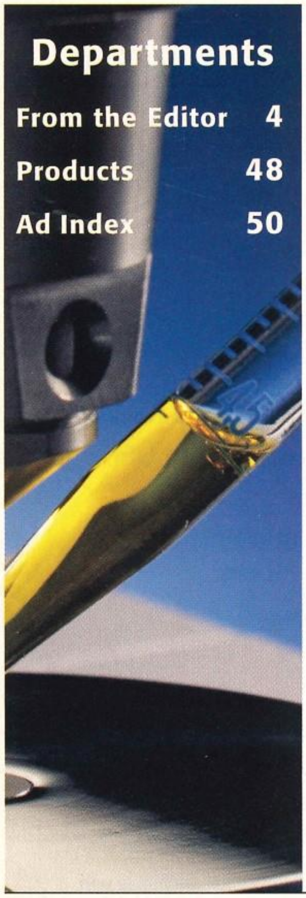

Departments

Articles

Use of Electron-Capture Negative Ion Mass Spectra to Establish the Identities of Polybrominated Diphenyl Ether Flame Retardants and Their Degradation Products

Detrimental health effects of a group of brominated flame retardants, polybrominated diphenyl ethers (PBDEs), have been recognized recently, but only after their wide usage and consequently, global dispersal. Of the possible 209 PBDE congeners, 39 (varying in degree of bromination from mono to deca) have been identified previously in the three common technical mixtures. Additional congeners, presumably debromination products of the fully brominated decabromodiphenyl ether (BDE-209), also have been reported in biotic and abiotic environments. However, costly analytical standards are needed to confirm their identification. In addition, the most widely used identification approach, electron ionization (EI) mass spectrometry (MS), primarily produces spectra indicating only homologue grouping (for example, hepta-BDE). Without specific compound identification, full assessment of toxicological consequences of PBDE burdens is impeded. It has been reported previously that electron-capture negative ionization (ECNI), a softer ionization technique, produces spectra with additional PBDE structure information (1). For the higher brominated PBDEs, ECNI spectra have provided ion fragments indicating bromine benzene ring substitution, molecular ion, and other PBDE characteristic ions. Using this softer ionization technique, a mass spectra library for 64 individual PBDEs, ranging from mono- to deca-BDEs was constructed. This library, along with unique ECNI spectra observations, was later used to confirm potential BDE-209 debromination products detected in biota from the aquatic environment.

Mark J. La Guardia

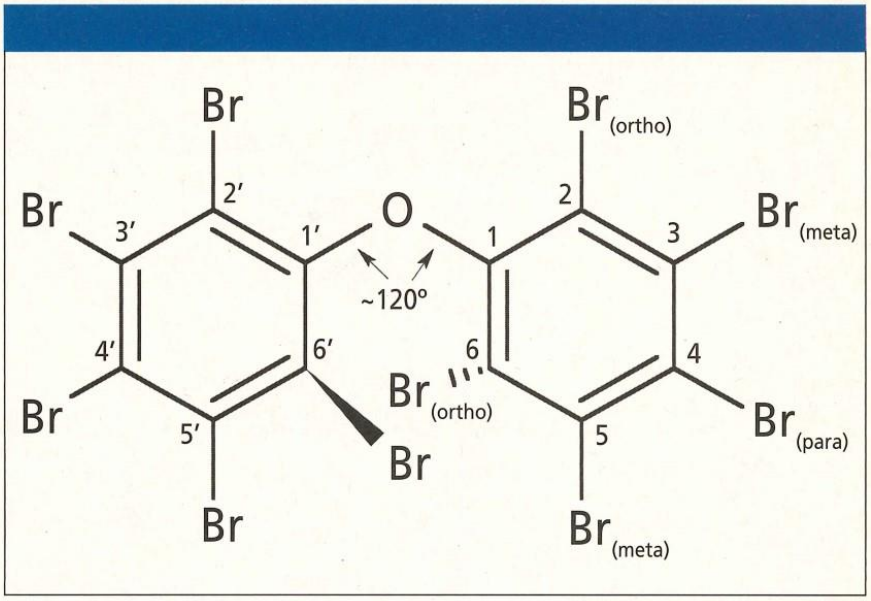

Figure 1: The structure of decabromodiphenyl ether (BDE-209), illustrating bromine numbering and the ether bond angle.

Polybrominated diphenyl ethers (PBDEs) are a class of brominated flame-retardant (BFR) additives, common in plastics (for example, electronic casings, computers, and televisions), circuit boards, polyurethane foam (furniture padding), and textiles. These widely used chemicals have been manufactured since the mid-1970s. However, following evidence that constituents of these mixtures bioaccumulate and disrupt biological processes such as the endocrine system, the manufacture of two of the three common formulations (penta- and octa-BDEs) was discontinued in the U.S. in 2004. This followed their ban by the European Union earlier that year (2). These two formulations, as well as the still-manufactured deca- (consisting of >97% by weight of BDE-209), are considered environmentally persistent and globally dispersed (3,4). Sources include industries producing or using PBDEs, as well as releases from finished products (5,6). The latter suggests that PBDEs will continue to be released into the environment long after the 2004 cessation in production. Even before termination of penta and octa production, deca- was the most widely used PBDE formulation [>83% 2001 global production (7)]. It is considered a lesser health concern because of its lower bioaccumulation potential, attributed to its extreme hydrophobicity and significant molecular mass (959 Da). However, reports have indicated that BDE-209 is bioavailable (8–10), although to a lower extent than the constituent congeners of the penta- and octa-formulations. Continued use of deca-BDE might be problematic because it has been reported to undergo abiotic [photolytic (11) and metal oxide reduction (12)] and biotic debromination (9,10), thus potentially adding to existing burdens of the lower brominated diphenyl ethers.

Like polychlorinated biphenyls (PCBs), 209 different PBDE congeners are feasible, and the same IUPAC scheme is used for their naming. In reality, the three commercial mixtures tend to be much simpler than those of PCBs, consisting primarily of 39 individual PBDE congeners (7). This is due to the directing influence of the oxygen and the large size of the bromine atoms (Figure 1). PBDEs are analyzed routinely by gas chromatography (GC) coupled with electron-capture detection (ECD), or more commonly, low resolution mass spectrometry (LRMS), operated either in electron ionization (EI) or electron-capture negative ionization (ECNI) modes. High resolution MS (HRMS) also has been used and has a number of advantages over LRMS (such as increased sensitivity and selectivity), but it requires more experienced users and is much more costly and labor intensive (13). Selective ion monitoring (SIM) ECNI (e.g., monitoring bromine ions ([^{79}Br]$^-$ and [^{81}Br]$^-$) has been shown to be a very sensitive technique and is used widely for the determination of low sub-parts-per-billion (ppb) levels. However, like ECD, this technique can result in misidentification (for example, 2,2′4,4′,5,6′-hexabromodiphenyl ether (BDE-154) is coeluted with 2,2′4,4′,5,5′-hexabromobiphenyl (BB-153) (14). Further spectral information can be obtained by scanning multiple ions, or an extended range of ions, at the loss of some sensitivity.

Ion fragment clusters centered on the molecular ion [M]$^+$ and the loss of two bromines [M-2Br] have been observed previously in PBDE EI spectra (13). This approach, in combination with retention-time data, has found utility for compound identification. However, acquisition of standards for all 209 PBDEs can cost from $20,000 to $30,000, and most searchable mass spectra libraries presently do not contain PBDEs (for example, NIST/EPA/NIH Mass Spectral Library with Search Program: NIST 05, Ver. 2.0d). So most laboratories focus on a subset of congeners previously identified in the three commercial mixtures. "Unknowns" typically are only characterized to the level of homologue (for example, hepta-BDE). Consequently, this lack of specificity limits specific congener identification, toxicological studies, and degradation pathways for these quasi-labeled PBDEs.

However, scanning the full range of ions in ECNI provides additional PBDE structural information, often permitting compound identification. For example, constituents of the penta-formulation were identified tentatively using GC–ECNI-MS spectra as penta- and hexa-BDEs by observing ion fragments representing the molecular ion [M]$^-$ and fragments indicating the loss of sequential bromine ions ([M-Br]$^-$, [M-2Br]$^-$, and [M-

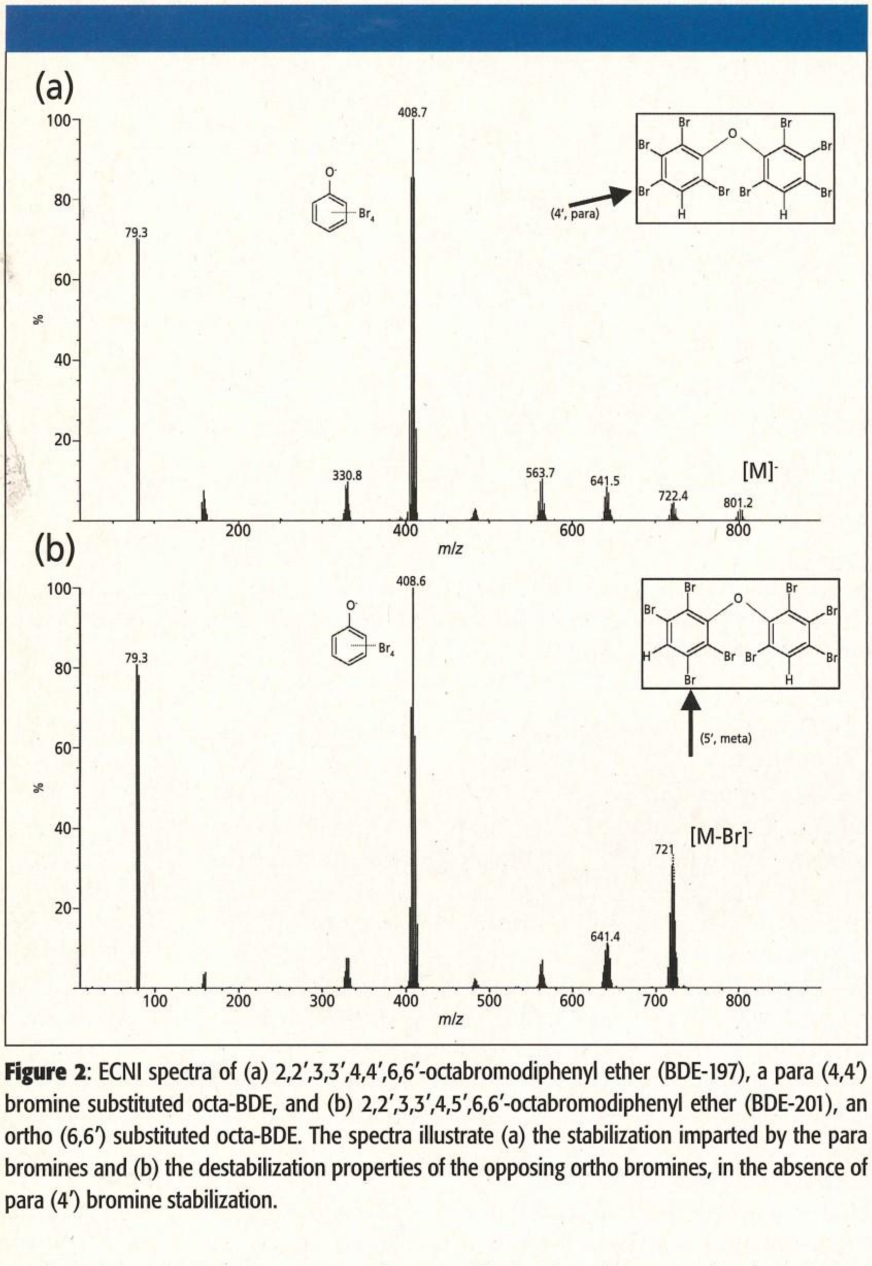

Figure 2: ECNI spectra of (a) 2,2′,3,3′,4,4′,6,6′-octabromodiphenyl ether (BDE-197), a para (4,4′) bromine substituted octa-BDE, and (b) 2,2′,3,3′,4,5′,6,6′-octabromodiphenyl ether (BDE-201), an ortho (6,6′) substituted octa-BDE. The spectra illustrate (a) the stabilization imparted by the para bromines and (b) the destabilization properties of the opposing ortho bromines, in the absence of para (4′) bromine stabilization.

3Br]⁻) (1). Similar fragments also were observed for a tetra-BDE in the mixture, but the molecular ion was not present. Stemmler and Hites (1) also reported that increasing the ion source temperature from 100 °C to 250 °C resulted in the production of an additional ion cluster in the hexa-BDE spectra, indicative of cleavage at the ether bond. Unlike EI, where molecules are ionized directly through interaction with high-energy electrons, in ECNI, an analyte interacts with a lower energy reagent gas, such as methane or isobutene. The reagent gas molecules, at about 1 torr pressure in the source, are excited by an electron beam (~200 eV). Analytes with an affinity for electrons (such as halogenated compounds) will form negative ions, which are stabilized by the higher pressures produced by the reagent gas. This mode often is referred to as soft ionization; ECNI imparts significantly less energy than EI, resulting in less fragmentation. Also, at lower internal energy, fragmentation or ion abundance is influenced greatly by the ion source conditions (temperature and pressure) (15). These parameters can then be modified, producing structural information in ECNI mode complementary to that of EI alone. As stated previously, EI spectra of PBDEs generally exhibit the molecular ion $[M]^+$ and fragments representing the loss of two bromines $[M\text{-}2Br]^+$. However, increasing the ECNI ion source temperature from 100 °C to 250 °C results in cleavage of the ether bond, producing ion clusters centered around 328 and 330 *m/z*, indicative of a benzene ring containing three bromines ($[C_6Br_3H_2O]^-$) (1). Additional GC–ECNI-MS studies also have observed PBDE cleaving with ions centered on 408 *m/z*, 486 *m/z*, and 488 *m/z*, diagnostic of a benzene ring containing four ($[C_6Br_4HO]^-$) and five ($[C_6Br_5O]^-$) bromines, respectively (7,16). Unlike EI spectra, which only permit classification to the level of homologue group, the ECNI spectra can vary as a function of bromine position on the phenyl rings. Consequently, analysts can elucidate the structure of some congeners using this technique, or at a minimum, eliminate some possibilities.

Experimental

Individual PBDE standards (Table I) were analyzed by GC (6890N, Agilent Technologies, Palo Alto, California) with MS detection (JMS-GC Mate II, JEOL, Peabody, Massachusetts) using ECNI and methane (99.99%) as the reagent gas. Samples were introduced (1-μL) into the split–splitless injector, equipped with a 1-mm i.d. glass liner, using a 15-m retention column (J&W Scientific, Agilent Technologies, DB-5HT, 15 m × 0.25 mm, 0.1-μm stationary phase) to separate analytes from their carrier solvent (hexane). The injector temperature was 300 °C, and initial carrier gas (helium) head pressure was 50 psi. This injection "pressure pulse split–splitless" technique has been reported to be suitable (minimum BDE-209 thermal degradation) for mono- through deca-PBDE analysis (7,14,17). The split vent was opened and pressure was reduced to 15.2 psi (flow 1.5 mL/min) after 4 min, following sample injection. Thereafter, column flow rate was kept constant throughout the remaining portion of the run by increasing carrier gas pressure to the head of the column, compensating for increasing oven temperatures, following the ideal gas law. Initial column oven temper-

Table I: PBDE congeners: Major fragmentation ions in ECNI (mono- through hexa-BDEs)

		ECNI, % Base								
IUPAC IDs	Compound	$[^{79,81}Br]^-$	$[Br_2]^-$	$[M-3Br]^-$	$[M-2Br]^-$	$[M-Br]^-$	$[M]^-$	$[C_6Br_3H_2O]^-$	$[C_6Br_4HO]$	$[C_6Br_5O]^-$
BDE-3	4-Mono BDE[3]	100	-	-	-	-	-	-	-	-
BDE-7	2, 4-Di BDE[3]	100	-	-	-	-	-	-	-	-
BDE-15	4,4′-Di BDE[3]	100	-	-	-	-	-	-	-	-
BDE-17	2,2′,4-Tri BDE[3]	100	20	-	-	3.0	-	-	-	-
BDE-28	2, 4,4′-Tri BDE[3]	100	12	-	-	7.4	-	-	-	-
BDE-32	2,4′,6-Tri BDE[4]	100	23	-	-	6.6	-	-	-	-
BDE-33	2′,3,4-Tri BDE[4]	100	8.4	-	-	-	-	-	-	-
BDE-35	3,3′,4-Tri BDE[4]	100	-	-	-	-	-	-	-	-
BDE-37	3,4,4′-Tri BDE[4]	100	-	-	-	-	-	-	-	-
BDE-42	2,2′,3,4′-Tetra BDE[1]	100	28	-	-	-	-	-	-	-
BDE-47	2,2′,4,4′-Tetra BDE[3]	100	42	-	6.8	2.7	-	-	-	-
BDE-48	2,2′,4,5-Tetra BDE[1]	100	34	-	-	-	-	-	-	-
BDE-49	2,2′,4,5′-Tetra BDE[3]	100	39	-	6.1	1.7	-	-	-	-
BDE-51	2,2′,4,6′-Tetra BDE[1]	100	36	-	3.4	2.0	-	-	-	-
BDE-66	2,3′,4,4′-Tetra BDE[3]	100	34	-	2.9	1.2	-	-	-	-
BDE-74	2,4,4′,5-Tetra BDE[1]	100	30	-	2.0	-	-	-	-	-
BDE-75	2,4,4′,6-Tetra BDE[4]	100	33	-	4.5	2.5	-	-	-	-
BDE-77	3,3′,4,4′-Tetra BDE[3]	100	-	-	-	-	-	-	-	-
BDE-85	2,2′,3,4,4′-Penta BDE[3]	100	26	3.3	8.4	-	-	-	-	-
BDE-97	2,2′,3′,4,5-Penta BDE[1]	100	24	-	-	-	-	-	-	-
BDE-99	2,2′,4,4′,5-Penta BDE[3]	100	29	2.3	7.8	-	-	-	-	-
BDE-100	2,2′,4,4′,6-Penta BDE[3]	100	29	4.3	30	1.3	-	-	-	-
BDE-101	2,2′,4,5,5′-Penta BDE[1]	100	18	2.4	3.9	1.7	1.3	-	-	-
BDE-102	2,2′,4,5,6′-Penta BDE[1]	100	20	4.3	8.3	2.1	-	-	-	-
BDE-116	2,3,4,5,6-Penta BDE[4]	100	24	1.5	1.5	1.3	5.5	-	-	-
BDE-118	2,3′,4,4′,5-Penta BDE[1]	100	20	1.3	-	-	-	-	-	-
BDE-119	2,3′,4,4′,6-Penta BDE[3]	100	29	1.8	7.5	-	-	-	-	-
BDE-120	2,3′,4,5,5′-Penta BDE[3]	100	24	-	4.3	2.1	1.5	5.0	-	-
BDE-126	3,3′,4,4′,5-Penta BDE[3]	100	-	-	-	2.6	1.7	-	-	-
BDE-128	2,2′,3,3′,4,4′-Hexa BDE[1]	100	17	1.4	4.9	1.1	-	-	-	-
BDE-138	2,2′,3,4,4′,5′-Hexa BDE[3]	100	43	2.7	3.8	3.7	-	-	-	-
BDE-144	2,2′,3,4,5′,6-Hexa BDE[1]	100	32	10	18	-	1.6	-	-	-
BDE-153	2,2′,4,4′,5,5′-Hexa BDE[3]	100	29	8.3	6.5	17	14	5.4	-	-
BDE-154	2,2′,4,4′,5,6′-Hexa BDE[3]	100	23	12	29	41	-	6.7	-	-
BDE-155	2,2′,4,4′,6,6′-Hexa BDE[4]	100	15	16	5.1	8.1	7.2	70	-	-
BDE-156	2,3,3′,4,4′,5-Hexa BDE[3]	100	29	3.3	1.2	4.1	-	-	-	-
BDE-166	2,3,4,4′,5,6-Hexa BDE[4]	100	16	7.6	5.7	14	8.3	-	-	-

Table I (continued): PBDE congeners: Major fragmentation ions in ECNI (hepta- through deca-BDEs)

		ECNI, % Base								
IUPAC IDs	Compound	$[^{79,81}Br]^-$	$[Br_2]^-$	$[M-3Br]^-$	$[M-2Br]^-$	$[M-Br]^-$	$[M]^-$	$[C_6Br_3H_2O]^-$	$[C_6Br_4HO]$	$[C_6Br_5O]^-$
BDE-171	2,2′,3,3′,4,4′,6-Hepta BDE[3]	100	8.8	3.1	5.4	10	-	-	-	-
BDE-173	2,2′,3,3′,4,5,6-Hepta BDE[1]	100	12	2.3	4.9	14	4.2	-	-	-
BDE-175	2,2′,3,3′,4,5′,6-Hepta BDE[1]	100	19	5.9	12	12	<1.0	3.2	10	-
BDE-179	2,2′,3,3′,5,6,6′-Hepta BDE[1]	100	5.2	8.9	17	78	-	25	115	-
BDE-180	2,2′,3, 4,4′,5, 5′-Hepta BDE[3]	100	7.3	5.0	13	26	-	4.2	8.0	-
BDE-181	2,2′,3, 4,4′,5,6-Hepta BDE[3]	100	11	7.4	13	23	20	-	-	-
BDE-182	2,2′,3, 4,4′,5,6′-Hepta BDE[1]	100	5.2	5.4	13	27	-	3.7	5.6	-
BDE-183	2,2′,3,4,4′,5′,6-Hepta BDE[3]	100	37	16	41	5.0	2.3	-	-	-
BDE-184	2,2′,3,4,4′,6,6′-Hepta BDE[3]	100	13	3.8	19	10	7.3	37	96	-
BDE-185	2,2′,3,4,5,5′,6-Hepta BDE[1]	100	25	7.2	2.8	1.7	11	-	-	-
BDE-188	2,2′,3,4′,5,6,6′-Hepta BDE[1]	100	3.8	3.7	13	31	1.8	53	70	-
BDE-190	2,3,3′,4,4′,5,6-Hepta BDE[1]	100	19	2.2	4.2	14	5.2	-	-	-
BDE-191	2,3,3′,4,4′,5′,6-Hepta BDE[3]	100	44	12	18	3.4	3.3	-	-	-
BDE-192	2,3,3′,4,5,5′,6-Hepta BDE[1]	100	20	3.4	2.0	2.1	11	-	-	-
BDE-194	2,2′,3,3′,4,4′,5,5′-Octa BDE[3]	100	16	8.6	7.5	9.3	3.1	-	3.3	-
BDE-196	2,2′,3,3′,4,4′,5,6′-Octa BDE[3]	100	16	21	23	26	<1.0	-	14	-
BDE-197	2,2′,3,3′,4,4′,6,6′-Octa BDE[3]	100	6.8	8.4	6.5	2.9	7.3	8.5	118	-
BDE-198	2,2′,3,3′,4,5,5′,6-Octa BDE[1]	100	14	11	8.5	8.4	7.1	2.6	4.8	6.5
BDE-201	2,2′,3,3′, 4,5′,6,6′-Octa BDE[3]	100	5.1	8.8	14	38	-	9.5	124	-
BDE-202	2,2′,3,3′,5,5′,6,6′-Octa BDE[1]	100	5.8	11	27	85	-	8.3	137	-
BDE-203	2,2′,3, 4,4′,5,5′,6-Octa BDE[1]	100	18	13	6.4	5.2	8.1	1.9	1.8	-
BDE-204	2,2′,3,4,4′,5,6,6′-Octa BDE[1]	100	6.3	12	13	6.3	5.4	24	30	62
BDE-205	2,3,3′, 4,4′,5,5′,6-Octa BDE[1]	100	16	7.6	4.4	3.8	10	-	-	-
BDE-206	2,2′,3,3′,4,4′,5,5′,6-Nona BDE[3]	100	19	35	35	40	11	2.2	14	26
BDE-207	2,2′,3,3′,4,4′,5,6,6′-Nona BDE[3]	100	5.9	13	11	2.9	<1.0	7.8	78	69
BDE-208	2,2′,3,3′,4,5,5′,6,6′-Nona BDE[3]	100	6.1	13	24	23	-	6.2	67	65
BDE-209	2,2′,3,3′,4,4′,5,5′,6,6′-Deca BDE[3]	100	3.3	15	5.7	1.8	<1.0	4.7	38	121

Standard suppliers: 1 = AccuStandard, Inc., New Haven, Connecticut; 2 = Ultra Scientific, North Kingstown, Rhode Island 3 = Wellington Laboratories, Ontario, Canada; 4 = Cambridge Isotope Laboratories, Andover, Massachusetts.

ature was 90 °C, held for 4 min, and then increased to 150 °C at 30 °C/min, then 10 °C/min to 300 °C, and held for 7 min. It was then increased to 350 °C at 30 °C/min and held at 350 °C for 5 min, as a bake-out procedure.

The mass spectrometer was tuned and calibrated using a 1000:1 mixture of perfluorokerosene (PFK) and 1,2,4-trichlorobenzene, using ion 331 *m/z*, resolution 1200. Mass calibration included 84 points, ranging from 35.0 to 998 *m/z*. Typical values for the ion source were: electron energy 200 eV, filament 300 μA. A source temperature of 200 °C was observed to be optimal to produce fragmentation characteristic of PBDE ether cleavage and molecular ions for the higher brominated compounds. ECNI mass spectra were collected in full scan (10 to 1000 *m/z*), at 1.0 scans/s. All reported spectra were identified at greater than one-half peak height. In addition to ionization mode, sample concentration can influence fragmentation patterns observed in the spectra (for example, percent abundance of minor ions). Therefore, for spectra to be relevant to environmental contaminant levels, PBDE standard concentrations used in this study were approximately 500 pg on-column.

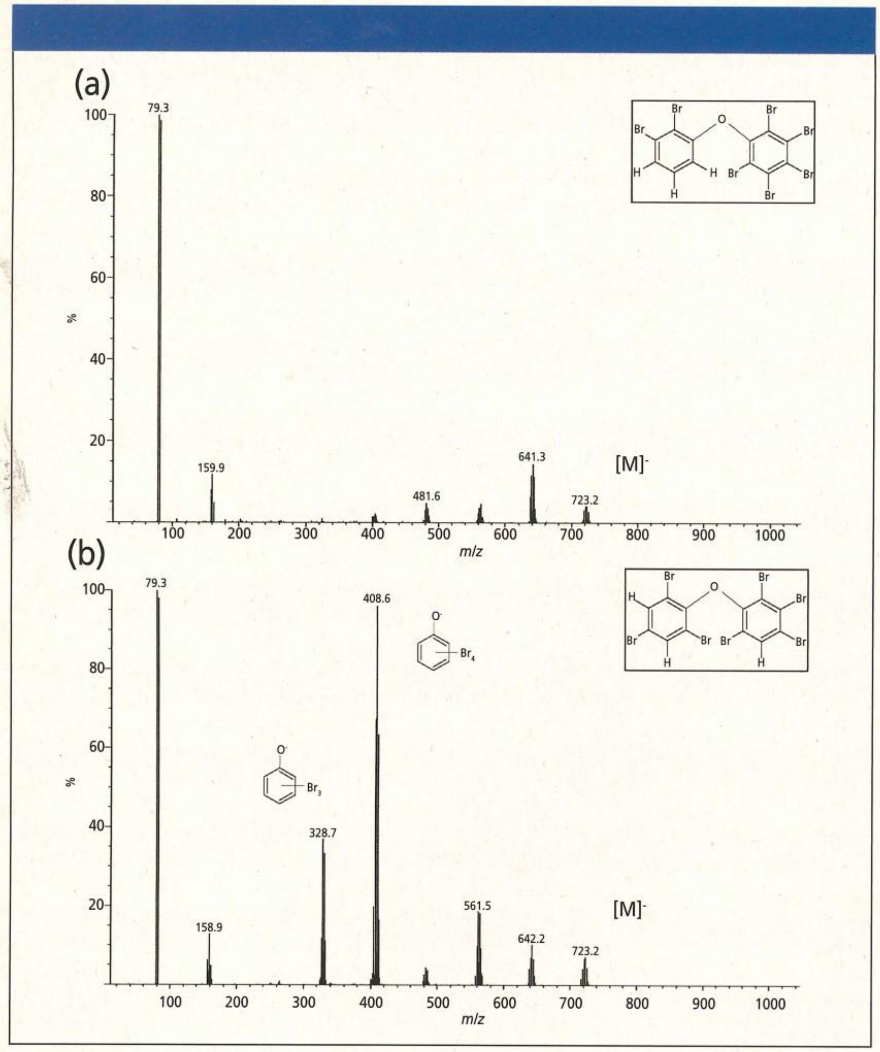

Figure 3: ECNI spectra of (a) 2,2′,3,3′,4,5,6-heptabromodiphenyl ether (BDE-173), a [5,2] bromine substituted hepta-BDE, and (b) 2,2′,3,4,4′,6,6′-heptabromodiphenyl ether (BDE-184), a [4,3] bromine substituted hepta-BDE. The spectra illustrate (a) the stabilization imparted by the presence of the fully brominated phenyl ring and nonopposing ortho bromines and (b) the cleavage at the ether bond.

Results and Discussion

The most abundant ion observed in 59 spectra of the 64 PBDE standards, analyzed by GC–ECNI-MS, was the bromine ion $[Br]^-$, 79 and 81 *m/z*. Spectra for the other five PBDEs contained major fragments corresponding to cleavage of the ether bond. These corresponded to 409 *m/z* ($[C_6Br_4HO]^-$) for BDE-179, -197, -201, -202, and ions 487 and 489 *m/z* ($[C_6Br_4HO]^-$) for BDE-209. Each spectrum was normalized to the bromine ion signal and % abundance calculated for each of the other major fragment ions detected (Table I). The spectra for all 64 PBDEs can be viewed at http://www.vims.edu/env/people/staff/laguardia.html. As mentioned previously, ECNI is a softer ionization technique than EI, generating PBDE spectra corresponding to $[C_6Br_xH_yO]^-$, cleaving at the ether bond. In this study, similar patterns also were observed primarily for PBDEs with greater than six bromines. The molecular ion $[M]^-$ followed by fragments derived from the loss of bromines ($[M\text{-}Br]^-$, $[M\text{-}2Br \ldots]^-$) also was detected, mostly for PBDEs containing greater than five bromines. It also was observed that bromine positioning (ortho, meta, and para) on the diphenyl rings influenced fragmentation patterns greatly. These observations indicate that acquisition of ECNI spectra is more powerful for PBDE identification than EI alone.

Unlike PCBs, in which nonortho-substituted rings can rotate more freely, PBDE rotation is hindered by the oxygen bond angle (~120°) between the rings (Figure 1). This influences bromine placement and compound stability. When subjected to ECNI, opposing bromines in the ortho (6,6′) position (Figure 1) contribute stress to the oxygen bond, causing the PBDE to be cleaved at the ether bond. This is indicated by an intense fragment corresponding to $[C_6Br_xH_yO]^-$, as observed in each PBDE spectrum containing bromines in the ortho (6,6′) position (that is, BDE-155, -179, -184, -188, -197, -201, -202, and -204) (Table I). It also was apparent that in ECNI, bromines in the para (4,4′) positions have a stabilizing effect on the ortho-position bromines, allowing for molecular ion identification. For example, the molecular ion centered at 802 *m/z* was detected for BDE-197 (Figure 2a); however, the molecular ion ($[M]^-$) was not observed for BDE-201 (Figure 2b). The difference was due to a shift of the para (4′ position) bromine in BDE-197 to the meta (5′ position) in BDE-201 (indicated by the arrows in Figures 2a and 2b).

Cleavage at the ether bond also produces spectra indicating the number of bromines per phenyl ring (Table I, columns $[C_6Br_3H_2O]^-$, $[C_6Br_4HO]^-$, and $[C_6Br_5O]^-$). Hepta-BDEs with five bromines on one ring and two on the other (referred to as a [5,2] bromine distribution) did not produce spectra indicating cleavage at the ether bond (Figure 3a). This might be an indication of the stability provided by the fully brominated phenyl ring or the lack of opposing bromines in the ortho (6,6′) position. However, the [4,3] distributed hepta-BDEs did produce ion clusters at 329, 331, and 409 *m/z* ($[C_6Br_3H_2O]^-$ and $[C_6Br_4HO]^-$, respectively), indicating cleavage at the ether bond (Figure 3b). However, three [4,3] hepta-BDEs (BDE-171, -183, and -191) did not produce this type of signal. These contain stabilizing para (4,4′) position bromines and lack destabilizing opposing bromines at the ortho (6,6′) position. Octa-BDEs also displayed a signal indicating phenyl ring bromine distribution, either [4,4] for BDE-194, -196, -197, -201, and –202 or [5,3] for BDE-198, -203, and -204. One octa-, BDE-205, did not demonstrate this, but again, this might be due to nonopposing ortho bromines and fully substituted para bromines (as seen in BDE-171, -183, and -191). The three nona-BDEs had strong signals indicating [5,4] bromine distribution, and the strongest ion cluster observed in the deca-BDE (BDE-209) spectra was produced by cleavage at the ether bond, $[C_6Br_5O]^-$ (Table I).

By interpreting ECNI spectra in the manner described earlier, it was possible to support the identification of previously suspected metabolic debromination products of BDE-209, the primary component of the deca-formulation. These PBDEs were apparent in biota collected downstream from the outfall of a wastewater treatment plant, which received BDE-209 from a plastic manufacturing facility (10). One octa-BDE could not be identified conclusively by EI. However, examination of the ECNI spectra revealed ether cleavage, a strong signal at 409 *m/z* with no observed molecular ion. This indicates an even distribution [4,4] of bromine on each phenyl ring, ortho (6,6′) substitution, and no para (4,4′) substitution. Of the 12 possible octa-BDEs, 7 are [4,4] bromine distributed and only one (BDE-202) does not contain para substituted bromines. This indicates that BDE-202 is a likely match for the unknown. Two unknown hepta-BDEs also were identified in the same manner. Their spectra indicated [4,3] bromine distributions, characteristic of strong ion signals centered on 409 *m/z* $[C_6Br_4HO]^-$ and 329, 331 *m/z* $[C_6Br_3H_2O]^-$. However, one spectrum did contain a slight signal for the molecular ion. Of the possible 24 hepta-BDEs, 18 are [4,3] distributed, but only 4 have opposing bromines on the (6,6′) ortho position (BDE-176, -179, -184, and -188), needed to produce cleavage at the ether bond. BDE-184 was eliminated as a possibility because of the stabilization properties of its bromines in the (4,4′) para positions. This produces a strong molecular ion signal, which was absent in the unknown spectrum. However, BDE-176 and -188 only contain single bromine in the para position; thus, these remain as possible candidates. The other peak was identified as BDE-179, because it does not produce a molecular ion signal, characteristic of the lack of para (4,4′) bromines.

Conclusion

Application of ECNI, a softer ionization technique than EI, can produce spectral information that is invaluable for the identification of PBDE congeners. Historically, interpretation of ECNI-generated data has been utilized rarely due to variations in instrumental conditions and configurations, thereby creating nonreproducible spectra. However, as instrumentation has evolved, parameter monitoring and control have improved greatly. This allows the analyst better control of ionization processes, reducing inconsistencies between spectra. These technological improvements permitted the exploitation of this alternative ionization technique, resulting in the generation of a ECNI spectra library for 64 PBDEs. Mass spectral interpretation permitted the confirmation of the identities of a number of BDE-209 metabolic debromination products.

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Flame-Retardants and Other Organohalogens Detected in Sewage Sludge by Electron Capture Negative Ion Mass Spectrometry

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Numerous halogenated organic compounds have been identified as pollutants of concern. Those with high persistence and hydrophobicity may concentrate in biota, sediments, and wastewater sludge. Nonetheless, the release to the environment of many remains largely unrecognized. Stabilized sewage sludge (biosolids) is increasingly being land-applied as a soil amendment. However, understanding the risks of land application has been hampered by the compositional complexity of biosolids. Compound specific analytical approaches may also underestimate environmental impact of land application by overlooking additional contaminants. However, utilizing an alternative analytical approach based on compound functional group (i.e., alkyl halides) enhanced the information content of the analysis. To illustrate, 49 organohalogens were observed by gas chromatography with electron capture negative ionization mass spectrometry in sewage sludge; 23 identified as flame-retardants: that is, PBDEs, hexabromocyclododecane, 2-ethylhexyl 2,3,4,5-tetrabromobenzoate (TBB), 2-ethylhexyl tetrabromophthalate, decabromodiphenyl ethane, 1,2-bis (2,4,6-tribromophenoxy) ethane and Dechlorane Plus. Concentrations ranged from 25 to 1 600 000 ng g^{-1} total organic carbon. An additional 16 compounds were tentatively identified as triclosan, chlorinated-methoxy triclosan, chlorinated pesticides, hexachlorobiphenyl, TBB degradation products, brominated furans and nonabromochlorodiphenyl ethers. Such an analytical approach may enhance evaluations of the risks associated with biosolids land-application and assist in prioritizing specific chemicals for future environmental fate and toxicology studies.

Introduction

A thorough knowledge of the chemical constituents present in media is critical to assessing the associated risks to the environment and human health. That being said, a targeted or selected compound approach is generally taken in regards to quantifying chemicals present. This is particularly problematic when the matrix is complex, largely uncharacterized and potentially variable with respect to the chemicals present. This is the case for sewage sludge. Application of analytical techniques that can both quantify target compounds and identify a wider range of additional chemicals based on properties of concern is indicated. Halogenated organic chemicals are some of the most bioaccumulative and persistent environmental pollutants identified to date, responsible for adverse human and environmental health effects. Such impacts were realized in the early-1970s, for example, for polychlorinated biphenyls (PCBs), leading to their ban by the end of that decade. However, nearly 40 years later PCBs remain a concern due to their recalcitrance and still are monitored diligently in a variety of environmental matrixes (e.g., water, sediment, and fish), consuming federal, state, and local resources. Recently, a new group of organohalogens, brominated flame retardants (BFRs), has received attention, particularly polybrominated diphenyl ethers (PBDEs). Manufactured since the mid-1970s, PBDEs have been used in plastics (e.g., electronic casings, computers, and television), circuit boards, polyurethane foam (furniture padding), and textiles (*1*). However, following evidence that constituents of these mixtures are being globally dispersed, bioaccumulate (*2–4*) and disrupt biological processes, for example, the endocrine system (*5, 6*), the manufacturing of two of the three formulations (penta- and octa-BDEs) was discontinued in the U.S. in December 2004. This followed their ban by the European Union earlier that year (*7*). The third formulation, deca-BDEs is currently being phase out in the U.S. with the end of all production, importation and sales by the end of 2013 (*8*).

Presumably released from their finished products, PBDEs along with other endocrine disrupting BFRs, for example, hexabromocyclododecane (HBCD), have been detected in household dust (*9, 10*). These BFRs enter waste streams and subsequently wastewater treatment plants (WWTPs) and are preferentially sequestered in sewage sludge (*11*). In the U.S., increasing amounts of stabilized sewage sludge (biosolids) are being landed-applied, 7.1 million tons in 2004 to agricultural and land restoration sites (*12*). Consequently, this may be an important route of contaminant environmental dispersal. Reports issued in 1996 and 2002 by the National Research Council (NRC) of the National Academy of Science, and findings of 2003 National Biosolids Research Summit recommended that better understanding of the chemical composition of biosolids was essential (*13, 14*). In response, the U.S. EPA initiated a new-targeted national sewage sludge survey (TNSSS) of contaminants (31 inorganics (28 metals) and 114 organic compounds, including 11 PBDE congeners) in single sewage sludge samples collected from 84 WWTPs between August 2006 and March 2007 (*15*). However, such a compound-specific analysis will only identify a limited number of chemicals present at time of collection, potentially overlooking additional contaminants that reside in sewage sludge. Application of screening approaches focusing on chemical attributes, for example, specific functional groups (e.g., alkyl halide) known to impart undesirable environmental characteristics will expand the chemical composition inventory of biosolids. This approach along with monitoring for these chemicals over a longer period of time may also provide insights as to temporal trends in usage, flux of chemicals to the environment and ultimately associated risks.

Halogenated compounds (e.g., PCBs and PBDEs) have an affinity for electrons and will form negative ions when subjected to electron capture negative ionization - mass spectrometry (ECNI-MS), some will produce a signal indicating the halogen ions themselves (e.g., chlorine produces m/z 35 ($[^{35}Cl]^-$), 37 ($[^{37}Cl]^-$), bromine m/z 79 ($[^{79}Br]^-$), 81($[^{81}Br]^-$)) (*16*). Historically, ECNI-generated full spectra have been less frequently used for structural elucidation than electron impact (EI) ionization, due to variations in instru-

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mental conditions and configurations of older instruments, resulting in nonreproducible spectra (*17*). However, as instrumentation has evolved, parameter monitoring and control have improved. Recently, ECNI-MS has been revisited for the analysis of PBDEs (*18, 19*) and methoxylated PBDEs (*19*) and found to produce valuable supplemental structural information, relative to traditional positive EI MS.

Brominated compounds can be revealed from the ECNI-generated full spectra by monitoring for a specific mass to charge ratio (m/z) (bromine m/z 79 ($[^{79}\text{Br}]^-$), 81 ($[^{81}\text{Br}]^-$). Once a particular bromine-containing scan is identified, the additional fragments in the full-scan ECNI trace can then be examined and interpreted, or compared to pre-existing or an authentic standard spectrum, and an identification achieved. Some chlorinated compounds can be evaluated similarly by monitoring for m/z 35 ($[^{35}\text{Cl}]^-$) and 37 ($[^{37}\text{Cl}]^-$). By only monitoring negative ions, interfering analytes in complex matrixes that produce positive ions are reduced, thereby simplifying the chromatogram and spectra interpretation. Utilizing the selective ECNI technique, both BFRs and other organohalogen compounds in complex samples (e.g., biosolids) may be identified, allowing for the evaluation of associated risks.

Materials and Methods

Between 2002 and 2008, processed sewage sludge from a Mid-Atlantic U.S. publicly owned WWTP was collected from the plants' drying lagoons. This facility is an activated sludge-type secondary facility. It treats 7.9 million liters per day of domestic and industrial waste, including discharges from an automotive interiors manufacturer. During each site visit (once in 2002, 2005, 2007, and 2008), several grab samples were collected along the lagoon perimeter and combined in a 1 L glass jars with Teflon lid. Each sample was presumed to represent over six-months of treated waste. The 2008 sample event occurred during sewage sludge removal, disturbing the lagoon's clay drainage bottom, which may have been inadvertently collected with this sample. Samples from each collection event were homogenized, freeze-dried and stored at <0 °C until analyzed. A 10 g aliquot of each was analyzed for total organic carbon (TOC). TOC was determined by combustion, followed by infrared detection (CE-440, Exeter Analytical, North Chelmsford, MA). (TOC results in SI Table S1 section.)

Dried 3 g samples were subjected to accelerated solvent extraction (Dionex ASE 200, Sunnyvale, CA). A surrogate standard (1 μg) of 2,3,4,4′, 5,6-hexabromodiphenyl ether (BDE-166) (Cambridge Isotope Laboratories, Inc., Andover, MA) and three chlorinated PCB retention time markers PCB-35, -65, and -204 (IUPAC nomenclature), (Accustandard, Inc. New Haven, CT) were added to each sample prior to extraction. Extracts were purified by size exclusion chromatography, (SEC, Envirosep-ABC, 350 × 21.1 mm. column; Phenomenex, Torrance, CA). Each post-SEC extract was reduced in volume, added to a solid phase 2 g silica glass extraction column (Isolute, International Sorbent Tech., Hengoed Mid Glamorgan, UK) and eluted with 3.5 mL hexane (fraction-S1), followed by 6.5 mL of 60:40 hexane/DCM (fraction-S2), and 8 mL DCM (fraction-S3). The second and third fractions, containing the compounds of interest, were reduced in volume and solvent exchanged to hexane. Decachlorodiphenyl ether (DCDE) (Ultra Scientific, North Kingstown, RI) was added as an internal standard. (See the SI for extraction method.)

Purified extracts were analyzed by GC (6890N, Agilent Tech., Palo Alto, CA) with MS detection (JMS-GC Mate II, JEOL, Peabody, MA.) using ECNI and methane reagent gas (99.99%). (See SI for ECNI-MS tuning, calibration, and settings.) Samples were introduced (1 μL) into the split/splitless injector, equipped with a glass liner (1 mm, i.d.), and separated on a 30 m DB-5HT (0.25 mm i.d., 0.1 μm, J&W Scientific, Agilent Tech.) column. The injector temperature was 300 °C and initial carrier gas (helium) head pressure was 50 psi. Four minutes after sample injection the split vent was opened and pressure reduced to 15.2 psi (flow 1.5 mL/min.). Column flow rate was kept constant (1.5 mL/min, temperature compensated) throughout the remaining portion of the analysis. Initial column oven temperature was 90 °C, held for 4 min, then increased to 150 at 30 °C/minute, then 10 °C/minute to 300 °C, and held for 20 min. It was then increased to 350 at 30 °C/minute and held at 350 °C for 5 min. Total run time was 47.7 min.

This extraction and analytical method has been previously reported for the analysis of PBDEs (*20*), which includes spiking experiments and quality control measures. To ensure data quality of the additional flame-retardants reported in this study, quality control steps were added to the analysis. These were: compound quantitation by summing the areas of two ions producing the most abundant signal (see Tables 1 and 2 for quantitation ions); a five-point calibration curve, $R^2 >$ 0.998 (Calibration standards were purchased from Wellington Laboratories, Inc., Ontario, Canada.); duplicate sample analysis relative percent difference (RPD) ranged from 4 to 37% (SI Table S2); and surrogate standard (BDE-166) recovery assessment along with an analytical batch blank analysis. Surrogate recoveries ranged from 86 to 122% (SI Table S1). Blanks did not contain any compound of interest above the detection limit.

Results and Discussion

Bromine and Chlorine Isotopic Monitoring and Compound Identification. It has long been known that GC/MS has tremendous capabilities in terms of separating and identifying constituents in environmental matrices (*21*). However, modern analytical methods have favored highly sensitive and selective methods directed at a limited list of compounds (e.g., U.S. EPA 8000 series methods) at the expense of identifying a wider range of chemicals present. With the intent of lowering the complexity of the extract, multiple purification steps are applied, simultaneously eliminating from possible scrutiny potential chemicals of concern. Alternatively, an analysis targeting a particular compound functional group (e.g., alkyl halides), requiring fewer extract purifications, may be applied. For example, a comparison of the positive EI (electron impact) -TIC (total ion chromatogram, range 10−1000 m/z) and ECNI-TIC yielded unresolved peaks in both sludge extract chromatograms, hindering compound identification (SI Figure S1a, b). However, by plotting selected information, i.e. only ions 79 and 81 m/z of the ECNI analysis (SI Figure S2a), resolution of brominated compounds were gained, enhanced further by evaluating isotopic abundances of bromine ($^{79}\text{Br} = 50.54\%$, $^{81}\text{Br} = 49.46\%$). From these exercises a total of 38 specific-brominated compounds (Table 1) were revealed in the intermediate polarity fraction (S2) and the more polar third fraction (S3) of the sewage sludge extract. Chromatographic retention time and full scan spectra (10−1000 m/z) of the observed peaks were compared to analytical standards previously analyzed by ECNI-TIC using the same GC program. This allowed the identification of 21 specific BFRs (Table 1). An additional nine brominated compounds were tentatively identified by spectral interpretation only. These were tetra- and hepta-BDE, hexa-, hepta-, and octa- brominated dibenzofurans, two partially debrominated isomers of 2-ethylhexyl 2,3,4,5-tetrabromobenzoate (TBB) (no. 1-TBB-[2Br] and no. 2-TBB-[2Br]) and two nonabromochlorodiphenyl ethers (no. 1-NBCDE and no. 2-NBCDE). By plotting 35 and 37 m/z of the ECNI-SIC (SI Figure S2b) and following the same systematic approach for bromines, but evaluating isotopic abundances for chlorine

TABLE 1. Brominated Compounds Confirmed and Tentatively Identified in Sewage Sludge by GC/ECNI-MS

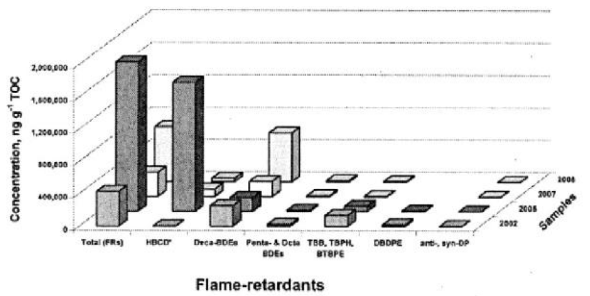
		sample						ECNI spectra major ions and intensity								
		detection				fraction										
retention time (min.)	compound (identified and tentatively identified)	2002	2005	2007	2008	S2 (%)	S3 (%)	*m/z*	int. (%)	*m/z*	int. (%)	*m/z*	int. (%)	*m/z*	int. (%)	spectrum and reference
8.12	brominated compound[a]		x			100	nd	*127*	100	*79*	33	*81*	32	*129*	25	*SI*, Figure S3a
10.98	brominated compound[a]			x		100	nd	*159*	100	*127*	65	*81*	18	*79*	17	*SI*, Figure S3b
11.02	brominated compound[a]		x			100	nd	*81*	100	*79*	98	*159*	50	*157*	30	*SI*, Figure S3c
11.32	brominated compound[a]		x	x	x	100	nd	*159*	100	*79*	60	*81*	58	*157*	55	*SI*, Figure S3d
11.57	brominated compound[a]	x	x	x		100	nd	*159*	100	*81*	68	*79*	64	*157*	50	*SI*,Figure S3e
13.48	brominated compound[a]		x			100	nd	*81*	100	*79*	94					*SI*, Figure S3f
13.82	brominated compound[a]		x			100	nd	*81*	100	*79*	99	*159*	52	*157*	48	*SI*, Figure S3g
14.08	brominated compound[a]		x			100	nd	*159*	100	*81*	75	*79*	74	*157*	53	*SI*, Figure S3h
14.75	Tetra-BDE[a]	x				100	nd	*79*	100	*81*	99	*486*	4	*326*	3	*SI*, Figure S3i
16.18	no. 1TBB-[2Br](dibrominated TBB degradation product)[a]	x	x			55	45	*79*	100	*81*	95	*279*	68	*390*	30	*SI*, Figure S3j, ref *33*
16.33	no. 2TBB-[2Br](dibrominated TBB degradation product)[a]	x	x			55	45	*79*	100	*81*	96	*279*	40	*390*	30	*SI*, Figure S3k, ref *33*
16.68	BDE-47, (2,2′, 4,4′-tetra BDE)	x	x	x	x	100	nd	*81*	100	*79*	96	*160*	29	*158*	15	refs *18*, *19*
18.23	BDE-100, (2,2′, 4,4′, 6-penta BDE)	x	x	x	x	100	nd	*79*	100	*81*	100	*403*	27	*405*	24	refs *18*, *19*
18.60	BDE-99, (2,2′, 4,4′, 5-penta BDE)	x	x	x	x	100	nd	*79*	100	*81*	99	*160*	34	*405*	10	refs *18*, *19*
18.70	TBB, (2-ethylhexyl 2,3,4,5-tetrabromobenzoate)	x	x	x	x	82	18	*81*	100	*79*	100	*356*	70	*358*	67	*SI*, Figure S3l, ref *10*
19.38	BDE-85, (2,2′, 3,4,4′-penta BDE)	x	x	x	x	100	nd	*81*	100	*79*	97	*160*	9	*403*	8	refs *18*, *19*
19.78	BDE-154, (2,2′, 4,4′, 5,6′-hexa BDE)	x	x	x	x	100	nd	*81*	100	*79*	97	*483*	18	*562*	15	refs *18*, *19*
20.33	BDE-153, (2,2′, 4,4′, 5,5′-hexa BDE)	x	x	x	x	100	nd	*79*	100	*81*	99	*563*	10	*404*	9	refs *18*, *19*
20.90	HBCD, (hexabromocyclododecane)	x	x	x	x	46	54	*81*	100	*159*	99	*79*	97	*561*	17	*SI*, Figure S3m, ref *37*
22.10	BDE-183, (2,2′, 3,4,4′, 5,6-hepta BDE)	x	x	x	x	100	nd	*81*	100	*79*	99	*562*	21	*564*	18	refs *18*, *19*
22.73	BTBPE, (1,2-bis (2,4,6-tribromophenoxy) ethane	x	x	x	x	100	nd	*79*	100	*81*	99	*251*	15	*329*	8	*SI*, Figure S3n, ref *38*
22.93	Hepta-BDE[a]	x				100	nd	*79*	100	*81*	90	*482*	14	*564*	11	*SI*, Figure S3o
23.07	Hexa-BDF[a]	x				100	nd	*642*	100	*644*	80	*640*	70	*638*	40	*SI*, Figure S3p
23.73	TBPH, (di(2-ethylhexyl)-2,3,4,5-tetrabromophthalate)	x	x	x	x	nd	100	*463*	100	*461*	73	*465*	69	*383*	59	*SI*, Figure S3q, ref *10*
24.22	BDE-202, (2,2′, 3,3′, 5,5′, 6,6′-octa BDE)	x	x	x		100	nd	*409*	100	*407*	91	*81*	90	*79*	84	ref *18*
24.88	BDE-201, (2,2′, 3,3′, 4,5,5′, 6′-octa BDE)	x	x	x		100	nd	*409*	100	*79*	77	*81*	68	*411*	68	refs *18*, *19*
25.07	BDE-197, (2,2′, 3,3′, 4,4′, 6,6′-octa BDE)	x	x	x	x	90	10	*409*	100	*81*	77	*79*	75	*407*	75	refs *18*, *19*
25.48	BDE-203, (2,2′, 3,4,4′, 5,5′, 6-octa BDE)	x	x	x	x	82	18	*81*	100	*79*	97	*720*	29	*722*	29	ref *18*
25.75	BDE-196, (2,2′, 3,3′, 4,4′, 5,6-octa BDE)	x	x	x	x	81	19	*79*	100	*81*	92	*721*	27	*642*	27	ref *18*
27.98	Hepta-BDF[a]	x	x	x		100	nd	*722*	100	*720*	95	*81*	59	*724*	59	*SI*, Figure S3r
30.15	BDE-208, (2,2′, 3,3′, 4,5,5′, 6,6′-nona BDE)	x	x	x	x	56	44	*487*	100	*489*	94	*79*	81	*81*	83	refs *18*, *19*
30.72	BDE-207, (2,2′, 3,3′, 4,4′, 5,6,6′-nona BDE)	x	x	x	x	59	41	*487*	100	*489*	98	*81*	98	*79*	90	refs *18*, *19*
32.03	BDE-206, (2,2′, 3,3′, 4,4′, 5,5′, 6-nona BDE)	x	x	x	x	61	39	*81*	100	*79*	99	*722*	83	*720*	83	refs *18*, *19*
36.52	no. 1-NBCDE, (nonabromochlorodiphenyl ether)[a]				x	100	nd	*81*	100	*79*	95	*486*	83	*442*	71	*SI*, Figure S3s
37.23	no. 2-NBCDE, (nonabromochlorodiphenyl ether)[a]				x	100	nd	*79*	100	*81*	98	*442*	68	*486*	63	*SI*, Figure S3t
37.60	Octa-BDF[a]	x				100	nd	*79*	100	*81*	87	*720*	68	*642*	48	*SI*, Figure S3u
41.73	BDE-209, (2,2′, 3,3′, 4,4′, 5,5′, 6,6′-deca BDE)	x	x	x	x	46	54	*487*	100	*489*	100	*485*	88	*491*	84	refs *18*, *19*
43.93	DBDPE, (decabromodiphenyl ethane)	x	x			100	nd	*81*	100	*79*	97	*891*	20	*893*	12	*SI*, Figure S3v, ref *27*

[a] Tentatively identified, BDE (bromodiphenyl ether), BDF (bromodibenzofuran).

TABLE 2. Chlorinated Compounds Confirmed and Tentatively Identified in Sewage Sludge by GC/ECNI-MS

retention time (min.)	compound (identified and tentatively identified)	sample: detection 2002	2005	2007	2008	fraction S2 (%)	S3 (%)	ECNI spectra major ions and intensity *m/z*	int. (%)	*m/z*	int. (%)	*m/z*	int. (%)	*m/z*	int. (%)	spectrum and reference
12.90	chlorinated compound[a]	x	x	x		nd	100	*237*	100	*35*	82	*235*	82	*239*	71	*SI*, Figure S4a
13.33	2,4,4′-trichloro-2′-hydroxydiphenyl ether[a]	x				nd	100	*252*	100	*216*	78	*254*	63	*218*	28	*SI*, Figure S4b, ref *16*
13.35	cis/trans-chlordane[a]	x	x	x	x	100	nd	*35*	100	*266*	85	*237*	77	*264*	61	*SI*, Figure S4c, ref *16*
13.58	2,3,4,4′-tetrachloro-2′-methoxydiphenyl ether[a]	x	x	x	x	67	32	*35*	100	*266*	70	*268*	46	*142*	43	*SI*, Figure S4d, ref *34*
14.00	Dieldrin[a]	x	x	x	x	nd	100	*237*	100	*35*	96	*239*	59	*235*	57	*SI*, Figure S4e, ref *16*
14.82	trans-nonachlor[a]	x	x	x		91	9	*300*	100	*302*	95	*266*	55	*264*	52	*SI*, Figure S4f, ref *16*
15.50	Hexachlorobiphenyl[a]	x		x	x	100	nd	*360*	100	*362*	84	*358*	57	*35*	31	*SI*, Figure S4g
16.08	2,3,4,4′, 5-pentachloro-2′-methoxydiphenyl ether[a]	x	x	x		100	nd	*372*	100	*374*	67	*370*	67	*336*	40	*SI*, Figure S4h, ref *34*
16.48	chlorinated compound[a]	x		x	x	nd	100	*332*	100	*315*	93	*330*	84	*317*	75	*SI*, Figure S4i
24.22	*syn*-Dechlorane Plus[b]	x	x	x	x	100	nd	*654*	100	*652*	84	*656*	63	*35*	42	,*SI* Figure S4j, ref *31*
24.88	*anti*-Dechlorane Plus[b]	x	x	x	x	100	nd	*654*	100	*652*	98	*650*	69	*656*	56	*SI*, Figure S4k, ref *31*

[a] Tentatively identified. [b] Anti- and syn-dechlorane Plus (1,2,3,4,7,8,9,10,13,13,14,14-Dodecachloro-1,4,4a,5,6,6a,7,10,10a,11,12,12a-dodecahydro-1,4:7,10-dimethanodibenzo[*a,e*]cyclooctene, CAS no. 135821-74-8 and 135821-03-3).



Total HBCD analysis by GC/ECNI-MS*

FIGURE 1. Concentrations of various flame-retardants (ng g^{-1} TOC) detected in sewage sludge.

(^{35}Cl = 75.53%, ^{37}Cl = 24.47%), 11 chlorinated compounds were observed in the second and third fractions of the extracts (Table 2). Two were identified as chlorinated flame-retardants (anti- and syn-Dechlorane Plus). Also triclosan and two chloromethoxy- derivatives (2,3,4,4′-tetrachloro-2′methoxydiphenyl ether and 2,3,4,4′, 5-pentachloro-2′methoxydiphenyl ether), several chloro-insecticides (chlordane, dieldrin and nonachlor) and a hexachlorobiphenyl were identified. (Retention time, compound names (and acronyms), fraction (S2, S3) recoveries, four-major ions and their intensities and published reference spectra are included in Table 1 (brominated compounds) and Table 2 (chlorinated compounds). Selected ECNI-TIC compound spectra of the sludge samples are presented in SI, Figures S3a−v and S4a−k.)

Of the 49 organohalogens identified during the ECNI screening process, 23 were identified as flame-retardants. Except for two PBDE congeners (BDE-201 and -202) and DBDPE, these flame-retardants were detected in each of the sludges collected between 2002 and 2008. It has previously been reported that PBDEs may accumulate in soils after repeated application of biosolids (*22*). It is likely that some of the additional flame-retardants we observed in sludges may also buildup in soils if land-applied. This warrants further quantitation and plausible source analysis of these identified flame-retardants within these sludges.

Flame-Retardant Quantitation and Source Identification. The summed concentrations of the 23 flame-retardants identified were 4-fold higher in 2005 than in 2002 (1 860 000 and 424 000 ng g^{-1} TOC, respectively) (SI Table S1). However, the concentration was lower in the 2007 sample (300 000 ng g^{-1} TOC), but was up again in 2008 (682 000 ng g^{-1} TOC) (SI Table S1). Most notable was the 1000-fold higher HBCD concentration in the 2005 sample (1 600 000 ng g^{-1} TOC). It contributed 86% of the total flame-retardant concentration of this sample, up from 0.3% observed in the 2002 sample (Figure 1). (For method comparison, results for α-, β-, γ-HBCD, and total HBCD analyzed by LC-MS are listed along with total-HBCD by GC/ECNI-MS, SI Table S1. Analytical methods GC- and LC-MS are in good agreement, RPD range of 4 to 47. Results of duplicate analysis are listed in the SI (Table S2) along with LC-MS methodology for HBCD.) These substantial quantitative variations in flame-retardants between sludge samples may be related to a variety of factors, including changes in chemical usage, manufacturing practices, and waste transfers over this 6 year period. It is noteworthy that a major automotive interior manufacturer relocated from the area in mid-2006. According to the U.S. EPA's Toxics Release Inventory (TRI, http://www.epa.gov/TRI/) this facility transferred 11 850 kg of decabromodiphenyl ether (BDE-209), one of the two BFRs listed on the TRI, to this WWTP between 2001 and 2005 (the other TRI listed BFR is 3,3,5,5-tetrabromobisphenol-A). It is presumed that this manufacturer may have used other flame-retardants, for example, HBCD. These uses may have contributed to the fluctuations seen in total flame-retardant sludge loads of these samples (Figure 1). Also, the maximum concentration reported in U.S. EPA's TNSSS for BDE-209 was 17 000 ng/g (dry weight) (*15*). However, the BDE-209 concentrations detected in our samples 2002, 2005, 2007, and 2008 (38 100, 26 400, 36 500, and 35 700 ng/g, dry weight, respectively) all exceeded the survey's maximum BDE-209 value, indicating local industries influence on sewage sludge composition. The samples collected in 2007 and 2008, after the presumed major BDE-209 contributor relocated from the area, were still twice the survey's maximum value. This may indicate additional BDE-209 sources (e.g., smaller manufacturers using <453 600 kg/year which are not required to report under the TRI requirements) also contributed substantial wastes. Also, increases in production by these smaller facilities or new manufacturers entering the area may explain the 3-fold higher BDE-209 concentration in 2008 compared to 2007 (i.e., 510 000 and 146 000 ng g^{-1} TOC, respectively) (Figure 1 and SI Table S1), again an indication of industries effect on sewage composition.

Ten lower brominated diphenyl ether congeners (BDE-28, -47, -66, -85, -99, -100, -138, -153, -154, -183) were also examined in the U.S. EPA's TNSSS. All, except for BDE-138, were detected in each of the 84 sludge samples collected. BDE-138 was found in 54 out of 84 samples. Of these 10 PBDEs, three (BDE-28, -66, and -138) were not detected in our sludge samples. (Their low percent composition (1.5% by weight of the total penta-flame-retardant formulation (*23*)) may explain their nondetect in our sludge samples.) EPA's reported totals of these seven PBDEs (BDE-47, -85, -99, -100, -153, -154, and -183) ranged from 170 to 11 200 ng/g (dry weight). Our totals for these same seven PBDEs ranged 1180−4170 ng/g, dry weight. However, the mean concentration of 2890 ng/g in our sludges was similar to that reported in the TNSSS for these same summed PBDEs, that is, 1730 ng/g (*24*). This similarity may be associated with how flame-retardants are incorporated into polymers. There are two classes of flame-retardants, those that form chemical bonds to the polymers they are added to (reactive flame-retardants) and those that do not form a chemical bond (additive flame-retardants) (*1*). The greater mobility potential of the latter chemicals, which includes PBDEs, facilitates their release from finished products. This is supported by their detection in household dust (*9, 10*). Once released, flame-retardants in such materials may enter domestic (household) waste-streams. During wastewater treatment, over 90% of PBDEs sequester into sewage sludge due to their hydrophobic nature (*11*) resulting in sludge concentrations similar to household dust (*25*). This transfer arising from domestic sources (e.g., household dust) would then appear uninterrupted and widespread, subjected to gradual society, and social changes linked to the amount and type of consumer products acquired and not subject to abrupt changes, such as disruptions in manufacturing schedules altering flame-retardant waste transfers. This may explain why there is less variation detected in our samples for the lower brominated PBDEs (penta- and octa-BDEs) compared to deca-BDEs and HBCD (Figure 1), as the latter were identified with local manufacturing usage. A domestic origin may also explain why PBDEs of the penta- and octa- formulations were detected in the TNSSS and our samples, which were collected after these formulations were discontinued (December 2004). This indicates that pre-existing, in-service products containing these flame-retardants (e.g., furniture) will remain sources to household dust, sewage sludges and soils receiving biosolids for years to come.

The two main constituents of Firemaster-550 (Chemtura Corp. CT.), TBB and 2-ethylhexyl tetrabromophthalate (TBPH), and the main component of FF-680 (Chemtura Corp.), 1,2-bis (2,4,6-tribromophenoxy) ethane (BTBPE), were also detected in each of our sludge samples (Table 1). These additive flame-retardants have been identified as replacements for the penta- and octa- PBDE mixtures, respectively (*26*). Interestingly, these flame-retardants were detected in our 2002 sludge (89 900, 33 500, and 10 200 ng g^{-1} TOC, respectively), collected over two years prior to the cessation in commercial manufacture of these PBDE products. Their total level exceeded by 6-fold the combined penta- and octa-BDE concentration and was approximately half the deca-BDE burden of the 2002 sludge (Figure 1). Total concentrations of each of the two later samples (2007 and 2008) were 91 and 85% lower, respectively, than those in the 2005 sample (SI Table S1). A higher sludge concentration ratio was also observed for the earlier 2002 and 2005 samples, 6.1 and 4.2, respectively. (Ratios were calculated by dividing the ΣTBB + TBPH + BTBPE concentration by Σ penta- + octa-BDE concentration.) When compared to the later 2007 and 2008 sample ratios (0.4 and 0.5, respectively), an apparent sludge-loading shift away from manufacturing (Figure 2) was indicated. This sludge-loading trend also coincided with the relocation of the auto interior manufacturer (mid-2006). It has also been reported that household dust contains TBB, TBPH, and BTBPE (*10*). Accordingly, domestic sources may be responsible for a significant percentage of the TBB, TBPH, and BTBPE detected in the later sludge samples.

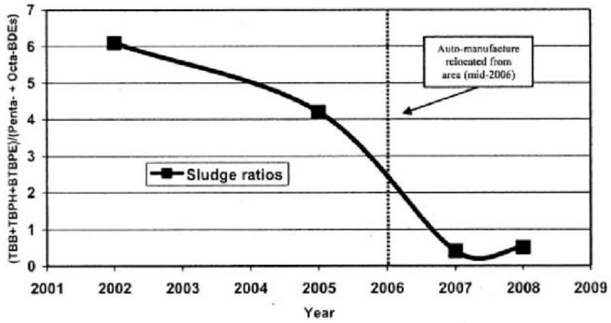


FIGURE 2. Quotient of total TBB + TBPH + BTBPE (ng g^{-1} TOC) to total penta- + octa-BDE (ng g^{-1} TOC) for the sludge sample set.

The BDE-209 alternative decabromodiphenyl ethane (DBDPE) was also detected in the 2002 and 2005 sludges (Table 1). However, DBDPE was not observed in the 2007 and 2008 samples. This may indicate a change in local commercial use of DBDPE, such as the shutdown of the above-mentioned automotive interior manufacturer, as releases from in-use products would be expected to be more consistent. DBDPE has also been detected in Swedish indoor air, sediments and sewage sludge (*27*). Ricklund et al. (2008) detected DBDPE in 40 sewage sludge samples collected from 12 different countries (*28*). The maximum reported value was 220 ng/g (dry weight). That sludge was collected from a WWTP that also received automobile industry waste. However, our DBDPE values were an order of magnitude higher (4820 and 1690 ng/g, dry weight, 2002 and 2005, respectively). The sludge in the Ricklund et al. study was collected from a WWTP that services a population of more than 100 000 in the Ruhr Region of Germany. This is approximately 10 times the population served by the WWTP providing our sludge. The cumulative wastes from the larger population served may have effectively diluted the DBDPE released from the industries, resulting in a lower sludge burden. WWTPs that serve disproportionately large industry sectors may need to monitor their sludge more closely to prevent a concentrated release of industrial chemicals by the land-application of sewage sludge (biosolids).

Two chlorinated flame-retardants were also detected in our sludge samples: anti- and syn-bis(hexachlorocyclopentadieno)cyclooctane (CAS No. 135821-74-8 and 135821-03-3, respectively). These are components of DP (Dechlorane Plus, Occidental Chemical Corp. Dallas TX). Total concentrations ranged from 112 to 175 ng g^{-1} TOC. The highest levels were for the later two samples (2007 and 2008): 175 and 156 ng g^{-1} TOC, respectively (SI Table S2). DP is a high production volume (HPV) chemical used as an additive flame retardant in electrical wires and cables, computers connectors and plastic roofing materials (*29*). The production volume of DP has been between 454 to 4540 t (MTs) since 1986, according to the U.S. EPA Inventory Update Rule 2006 (*30*). DP has been recently detected in air, sediments, and biota samples of the Great Lakes (*29, 31*). Sediment cores taken from this region indicate DP started entering the Great Lakes around 1970 and peaked 5−10 years later (*29*). Once released into the environment there appears to be shift in the dominance of the anti- to the syn-isomer, compared to the technical mixture. The anti- isomer has been reported to contribute 65% (*29*) to 80% (*31*) of the technical mixture. However, in our sludge samples the anti- isomer contributed only 39−54% (mean 46%, $n = 4$) of the DP formulation. Hoh et al., (2006) reported that isomer ratios detected in sediments and air samples collected near a DP manufacturing source resembled the technical mixture (*31*). With distance from this source the isomer ratio shifted toward the syn-isomer. The apparent greater environmental stability of the syn-isomer was hypothesized to be due to greater chlorine shielding of the syn- isomer's carbon bonds. Chemicals found in sludge are subjected to numerous aerobic and anaerobic environments during transport and treatment processes. This may facilitate degradation of the anti-isomer of DP reported in our sludge samples. Zhu et al. (2007) also detected DP in residential dust samples collected in the City of Ottawa, Canada (*32*). Of the 69 dust samples they examined, 77% had a higher syn- to anti-isomer ratio than the 1:3 of their technical mixture. This indicates that alterations may occur prior to the release of the chemicals to the outdoor environment.

From the initial GC/MS-ECNI screening, 49 organohalogen compounds were detected in our sludge samples, 39 of these were tentatively identified by ECNI- spectra interpretation. By including retention time matching of analytical standards with their ECNI-spectra, 23 of these were identified as specific brominated or chlorinated flame-retardants. These were subsequently quantified in the sludges. Of the remaining 16 tentatively identified organohalogens, four are believed to be degradates of previously described products. Two of these were identified as possible photodegradation products of the flame-retardant TBB (*33*): that is, congeners no. 1-TBB-[2Br] and no. 2-TBB-[2Br] (Table 1 and SI Figure S3j, k). It is postulated that these also may be formed during wastewater treatment. Two chloro-methoxydiphenyl ethers were also detected in the sludges (2,3,4,4′-tetrachloro-2′methoxydiphenyl ether and 2,3,4,4′, 5-pentachloro-2′methoxydiphenyl ether) (Table 2 and SI Figure S4d, h). These have been reported as transformation products of triclosan (2,4,4′-trichloro-4-hydroxydiphenyl ether, a HPV antibacterial agent) produced via microbial methoxylation and by chlorination during wastewater treatment, respectively (*34*). Two nonabromochlorodiphenyl ethers were also tentatively identified by mass spectral interpretation: no. 1-NBCDE and no. 2-NBCDE (Table 1 and SI Figure S3s, t). These may have been created by the inadvertent chlorination of a nona-PBDE (BDE-206, -207, or -208). Alternatively, they may be components of the newly introduced flame-retardant decahalodiphenyl oxide (Albemarle Corp., U.S. (*35*)), as it was only detected in the 2008 sludge sample.

Monitoring by ECNI scanning also facilitated the detection of several legacy organohalogen pesticides in the sludges. These included chlordane, dieldrin and nonachlor (SI Figure S4c, e, and f), along with hexa-, hepta-, and octa-brominated dibenzofurans (SI Figure S2o, r, and u). This suggests that land-application of sewage sludge will continue to be a source of these legacy compounds to soils. Also, the flame-retardant concentration detected in our sample set varied over the six-year sampling period. Most notably was a 1000-fold rise in HBCD concentrations between 2002 and 2005. The current risk assessment for biosolids application (*36*) is based on a select group of chemicals that does not include flame-retardants and on conclusions of a 1988 one-time survey. However, our data indicate that the contributor base of the WWTP, that is, the balance between domestic and industrial source contributions, as well as the character of the local industry (ranging from textile mills to pharmaceutical manufacturers), may control the profiles of domestic and industrial chemicals entering and ultimately leaving WWTPs via the effluents and sludge.

Our findings along with reports by the NRC (*13*) and the WERF (*14*) support the need for additional sludge chemical screening to better understand their chemical composition. Increased screenings for classes of compounds (for example, organohalogens) known to exhibit problematic environmental properties are necessary to fill the knowledge gap pertaining to sludge composition. This information is critical to properly assess attendant risks. Usage of bioassays to evaluate cumulative toxicological effects of multiple contaminants present is also indicated. Analysis to detect specific chemicals, while accurate, overlooks detection of critical contaminants. For example, the 2009 NTSSS reported that out of the 72 targeted pharmaceuticals 21% were not detected in any of the sludges tested and over half were detected less than 10% of the time (*15*). A screening and quantitation approach, as demonstrated here, can cost-effectively facilitate the identification of constituents present in complex matrices such as sludge, including degradation and metabolite products formed during wastewater treatment. This can result in better direction of the limited resources available for evaluating the risks of chemicals ultimately released to the environment through the land-application of sewage sludge.

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Supporting Information Available

Additional details and Tables S1 and S2, Figures S1a, b S2a, b, S3a−v and S4a−k. This material is available free of charge via the Internet at http://pubs.acs.org.

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Comment on "Identification of Monochloro-Nonabromodiphenyl Ethers in the Air and Soil samples from South China"

Yu et al.[1] detected three nonabromochlorodiphenyl ethers (NBCDEs) in air and soil samples from Guangzhou China and at an e-waste recycling area. Identifications were made using gas chromatographic retention times of pure reference compounds, as well as matches with full-scan mass spectra data in the electron capture negative ionization mode. Compounds included: 6′-chloro-2, 2′, 3,3′, 4,4′, 5,5′, 6-nonabromodiphenyl ether (6′Cl-BDE-206), 5′-chloro-2, 2′, 3,3′, 4,4′, 5,6,6′-nonabromodiphenyl ether (5′Cl-BDE-207) and 4′-chloro-2, 2′, 3,3′, 4,5,5′, 6,6′-nonabromodiphenyl ether (4′Cl-BDE-208). Mixed brominated/chlorinated dibenzo-*p*-dioxins and dibenzofurans have been previously detected at sites associated with anthropogenic combustion,[2] including e-waste dismantling areas.[3] Therefore, these novel brominated/chlorinated diphenyl ethers were proposed to be thermolytic transformation products of polybrominated diphenyl ethers (PBDEs) flame-retardants. However, we previously reported two NBCDEs (plus 47 organohalogens including 23 flame-retardants) in land-applied sewage sludge.[4] In that earlier study, four sludge samples were collected from a Mid-Atlantic U.S. publicly owned wastewater treatment plant (WWTP) between 2002 and 2008. Decabromodiphenyl ether (BDE-209) was the major PBDE detected in each sample, ranging from 23 600 to 38 100 ng g^{-1}, dry weight. This WWTP treats domestic and industrial wastes, including that from an autointerior manufacturer. This facility was a large-scale user of Deca-BDE (primarily BDE-209). Yu et al. also detected in thermoplastics two NBCDEs (4′Cl-BDE-208 and 5′Cl-BDE-207) at 5−8% of that of the BDE-209 contribution. They hypothesized that NBCDEs can also be formed inadvertently during the manufacture of PBDE and chlorine-containing products (e.g., PVC). They also hypothesized that the NBCDEs detected in air and soil samples collected in their study may have also originated from this manufacturing process. This would suggest that waste from our autointerior manufacturer, heavily influenced by residuals derived from flame-retardant treated thermoplastics, should also contain NBCDEs. However, NBCDEs were only detected in the 2008 sludge sample, but not in the earlier (2002−2007) samples, even though they all contained considerable amounts of BDE-209. The ratio of NBCDEs to BDE-209 (unpublished data) in our 2008 sludge sample was also similar to ratios detected in the thermoplastics by Yu et al. We do not believe that autointerior manufacturing processes have changed dramatically over the course of our sample collection period. Hence, an alternative supposition is that NBCDEs were not inadvertently formed during manufacturing, but rather that the composition of the additive flame-retardant has changed. This hypothesis was previously introduced in La Guardia et al.[4] and supported by details of an Albemarle Corporation Deca-BDE patent.[5] The patent describes the cofeeding of diphenyl oxide, or partially brominated diphenyl oxide and bromine chloride with bromine or chlorine, into a refluxing reaction mixture of bromine and a Lewis acid bromination catalyst to produce >96% BDE-209 and up to 4% by weight of two NBCDEs.[5] Similar to previous Deca-BDE formulations, this new product is used to flame-retard macromolecular materials, for example, thermoplastics, thermosets, cellulosic materials, and textile backcoating. Albemarle Corporation is a global manufacturer and supplier of flame-retardants, located worldwide including Guangzhou China, the location of Yu et al. urban air sampling. PBDEs have been manufactured since the mid-1970, with Deca-BDE formulations contributing nearly one-quarter of the global brominated flame-retardant usage. They thus have had nearly 40 years to undergo thermolytic transformation processes, but NBCDEs have only recently been detected. While NBCDEs could be formed from chlorination of PBDEs through thermolysis, as suggested by Rupp and Metzger[6] and Yu et al., we believe that a plausible alternative explanation is that they are impurities in the Albemarle Corporation product. The commercial introduction of this process also corresponds temporally with the initial detection of NBCDEs in abiotic matrixes.[1,4] Between its introduction and 2014, when Deca-BDE U.S. production is scheduled to end,[7] this "new" production process may thus produce significant volumes of NBCDEs.

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Organic Contaminants of Emerging Concern in Land-Applied Sewage Sludge (Biosolids)

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ABSTRACT: Modern wastewater treatment greatly ameliorates the release to the aquatic environment of pollutants present in industrial and residential discharges. However, the recycling of sewage sludge (also known as "biosolids") as a soil amendment presents additional challenges to the wastewater industry, as they must now also assure these complex materials do not adversely effect the environment. Concerns not only include contaminants historically tracked (e.g. heavy metals, petroleum products, PCBs and pesticides), but also those newly discovered in discharges. We recently detected four classes of emerging contaminants (polybrominated diphenyl ethers, alkylphenols and their associated ethoxylates, polycyclic musks, and triclosan) in 12 biosolid samples collected from around the U.S. These findings support the 2002 National Academy of Science conclusion that contaminants of potential toxicological concern, not previously investigated during development of the EPA Part 503-risk assessment, are present in modern biosolids and that a new National Sewage Sludge Survey and updated risk assessment need to be conducted.

INTRODUCTION

HISTORICALLY, wastewater treatment plants (WWTPs) have acted as a readily identifiable conduit for the environmental introduction of toxic chemicals from industrial and residential discharges and urban runoff. However, technological improvements in wastewater treatment processes since the enactment of the Clean Water Act of 1972 have resulted in greatly improved pollutant removal efficiencies. Partitioning of hydrophobic chemicals onto particles and their subsequent removal as sewage sludge is an integral step in the treatment process, decreasing the levels of pollutants discharged to receiving waters. Enhancements in industrial pretreatment have also diminished wastewater burdens of some chemicals before they enter the WWTP. However, despite these improvements recent studies indicate that some poorly studied contaminants of emerging concern may be released through WWTPs. Some of these emerging contaminants preferentially partition into sewage sludge and thus may also be introduced into the environment by land application of sewage sludge [1, 2]. (Stabilized sewage sludge that is land-applied as a soil amendment or fertilizer is often referred to as biosolids.) While these chemicals of concern are current-use substances, recently detected in the environment, they are currently not regulated in many countries (e.g. not designated as Priority Pollutants in the U.S.). Since the 1988 EPA National Sewage Sludge Survey (NSSS), limited efforts have been made to more fully characterize the range of environmental pollutants of concern within biosolids. However, it has been estimated that over 100,000 chemicals are currently in commerce, with up to 1000 new compounds added each year. Most of these current-use chemicals have not been adequately examined for toxicological or environmental impacts [3, 4]. Preexisting chemicals, previously thought not to present an undue risk, may merit further consideration when new uses or greater demand for them emerges. Also, the discovery of new modes of toxicity or the existence of previously unknown degradation products may merit new concerns to be registered for chemicals previously deemed "safe."

Ideally, environmentally problematic chemicals need to be identified prior to their dissemination into the environment and the potential occurrence of significant damage. But in practice the identification process is of-

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ten reactive, occurring after detrimental impacts have been realized. Some examples of chemicals that have been recognized as environmental problems in the past include; 1,1,1-trichloro-2, 2-bis (4-chlorophenyl) ethane (DDT), causing decreased reproductive success in raptors [5]; polychlorinated biphenyls (PCBs), with uptake in fishes and subsequent exposure of human consumers [6]; and recently methyl tert-butyl ether (MTBE), a gasoline additive now contaminating some drinking water supplies [7]. Recent surveys indicate additional potentially problematic chemicals are being dispersed into the environment, e.g. into surface waters [8, 9] via WWTP effluents. These include personal-care-products and pharmaceuticals, excreted parent and metabolites, as well as residues released via the deliberate disposal of unwanted and/or expired medicines into WWTP waste streams [8, 10]. Several of these biologically active chemicals have been found resistant to microbial transformation during sewage treatment (e.g., amitriptyline, codeine, and erythromycin) [11, 12]. Pharmaceutical mixtures have been shown to have adverse effects in aquatic ecosystems at dosages much lower than typically taken by humans [13]. Although, drugs receive considerable pharmacological and clinical testing, information on the environmental behavior and ecotoxicity of these biologically active substances and their metabolites are generally not available.

Chemicals that are long-lived, continuously introduced, or subject to wide dispersal are particularly problematic. Remediation of contaminants following their release into the environment is typically much more costly then preemptive routes of disposal (e.g., landfill). In cases where remediation is even possible it may be more destructive to the site than the contaminants themselves. Environmental monitoring efforts have historically focused on priority or historical pollutants [14], using analytical methods that target specific compounds at low environmental concentrations. While these approaches produce useful, accurate and defensible data needed in regulatory affairs, their specificity often obviates the ability to detect the presence of non-targeted chemicals within the environment [14].

Under the 1993 Clean Water Act, the U.S. EPA established regulations for the land application of biosolids with the intent of protecting public health and the environment. The regulation (Standards for the Use and Disposal of Sewage Sludge, Title 40 CFR, Part 503, commonly referred to as the Part 503 rule) sets chemical pollutant limits, operational standards designed to reduce pathogens and the attraction of disease vectors, and management practices. Compounds considered under this rule were selected based in part on results from the National Sewage Sludge Survey (NSSS), which was designed to detect 411 possible sewage sludge contaminants from 176 WWTPs within the U.S. [15]. Subsequently, nine metals were regulated, primarily due to their recognized toxicity and persistence in soils. Persistence is problematic as it may lead to accumulation of elevated levels over time following repeated applications of biosolids. In the so-called "Round Two" evaluation conducted in 1995 the U.S. EPA revisited the pollutants considered under Rule 503. However, a second comprehensive analytical survey of contaminants in biosolids was not conducted. Instead EPA focused largely on compounds previously considered during the original NSSS, with an emphasis on chlorinated dioxins, furans and co-planar PCBs [16]. In 2003 the U.S. EPA concluded that these compounds did not present a significant risk to human health or the environment and thus will not regulate their levels in biosolids (EPA Headquarters Press Release October 17, 2003). As public health concerns increased regarding the use of biosolids, EPA in 1999 asked the National Academy of Science (NAS) to conduct an independent evaluation reassessing the scientific basis of the Part 503 Rule. In their 2002 report, the NAS committee noted that the Part 503 Rule relied on what is now an outdated biosolid contaminant characterization [17]. Accordingly, due to changes in treatment processes and chemical uses over the last decade, there was concern over possible adverse changes in biosolid composition. The committee recommended that a new national sewage sludge survey be conducted to ensure that Part 503 Rule risk assessment assumptions are based on sound science [17]. At the 2003 Biosolids Research Summit (Proceedings of the Water Research Foundation Biosolids Research Summit 2003) performance of new national surveys of pathogen and chemical constituents of concern in biosolids were ranked as the second and third highest research priorities, following only the development of a rapid incident response study aimed at examining whether a linkage existed between biosolids land application and reports of human health impacts.

Here we report on the presence of four classes of emerging contaminants in biosolid samples obtained from several U.S. states. These include: 1) flame retardant polybrominated diphenyl ethers (PBDEs) used in thermoplastics, circuit boards, and polyurethane foam. Global PBDE demand in 1999 reached 67,125 metric tons [18]. Like PCBs, 209 different PBDE congeners are theoretically feasible and the same IUPAC PCB scheme is used for their naming. The commercial Deca-BDE (>97% decabromodiphenyl ether, also known as BDE209), constituted about 82% of the reported 1999 global tonnage consumption for all PBDEs. The Octa-BDE formulation makes up 6 % of the 1999 world PBDE market. Hepta- and octa-congeners contribute 70 to 80% of this formulation, with hexa-, nona- and the deca-congeners contributing the remainder. The Penta-BDE formulation constitutes approximately 13% of the 1999 global PBDE market. The vast majority of this, about 98%, is consumed in the U.S. to flame retard polyurethane foams used largely as padding, e.g. in furniture. Tetra-BDE and penta-BDE congeners (primarily 2,2′, 4,4′-tetrabromodiphenyl ether (BDE47) and 2,2′, 4,4′, 5-pentabromodiphenyl ether (BDE99): (Figure 1, B)) constitute about 80% of this mixture. The remainder consists of 2,2′, 4,4′, 6- pentabromodiphenyl ether (BDE100), 2,2′, 4,4′, 5′5- (BDE153) and 2,2′, 4,4′, 5,6′-hexabromodiphenyl ether (BDE154). These contribute 9.5, 4.5 and 5% of the total, respectively [19]. 2) Alkylphenol polyethoxylate (APEO) degradates, these are formed from surfactants used primarily in heavy-duty detergents. APEOs have been shown to degrade to alkylphenols such as nonyl- (NPs) and octylphenols (OPs) during aerobic and anaerobic pro-

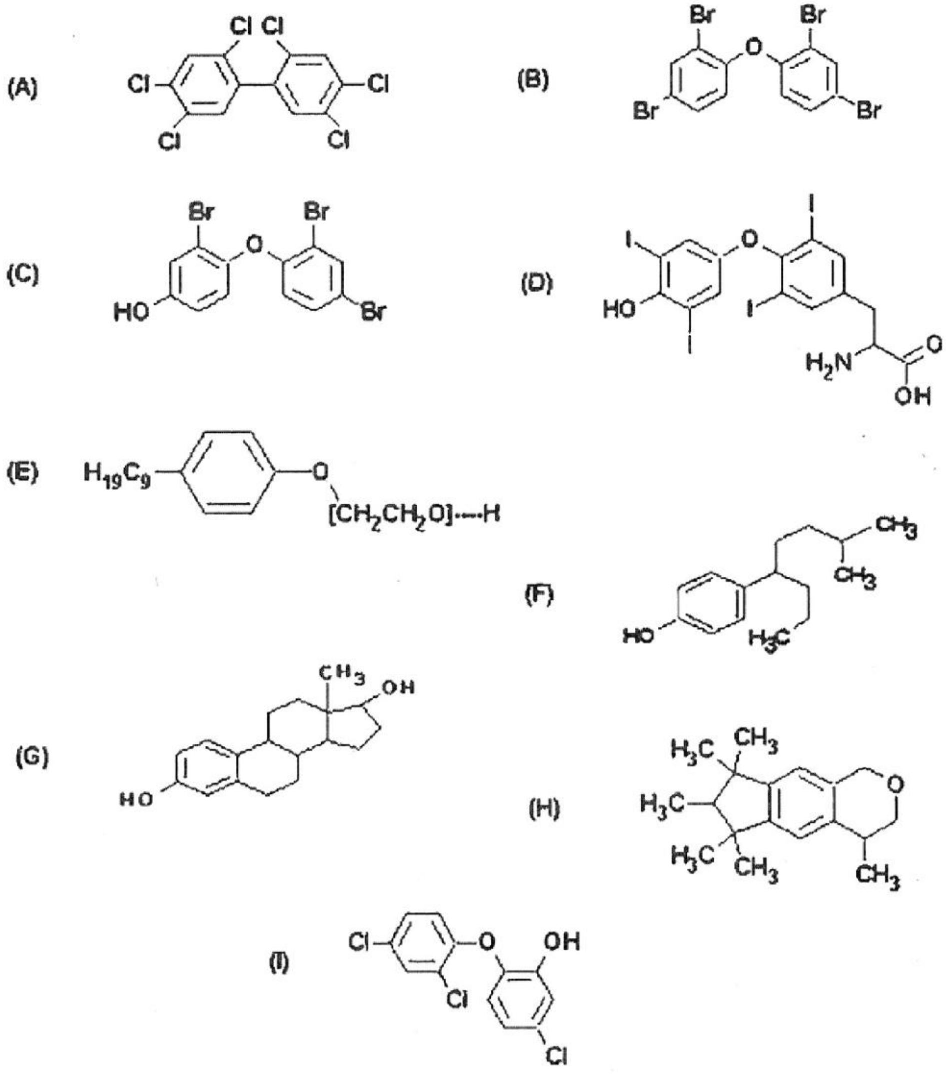

Figure 1. *Representative Chemical Structures(A) PCB (2,2′, 4,4′, 5, 5′-hexachlorobiphenyl, PCB-153); (B) PBDE (2,2′, 4,4′-tetrabromodiphenyl ether, BDE47); (C) hydroxylated BDE; and (D) thyroxin, a thyroid hormone; (E) nonylphenol polyethoxylate; (F) a representative 4-nonylphenol; (G) 17β-estradiol; (H) polycyclic musk, galaxolide (1,3,4,6,7,8-hexahydro-4, 6,6,7,8,8-hexamethyl cyclopenta[g][2]benzopyran); (I) triclosan (2,4,4′-trichloro-2′-hydroxydiphenyl ether).*

cesses, such as wastewater treatment. NPs have received recent attention due to their acute toxicity to aquatic organisms and ability to disrupt the endocrine system. The more hydrophobic OP, NP and NP mono- and diethoxylate (NP1EO and NP2EO) degradation products were analyzed due to their greater propensity to sorb to solids. 3) Polycyclic musks common as fragrances, specifically Galaxolide and Tonalide. These have been reported previously to accumulate in fish tissue and human breast milk; 4) anti-bacterial additive triclosan, which is toxic to some aquatic organisms and which may contribute to antibiotic resistance. Triclosan has also recently been reported to photolyze under natural sunlight conditions to 2,8-dichlorodibenzodioxin [20]. Structural similarities of PBDEs and triclosan to PCBs, and NPs to estradiol (Figure 1, B, I, A, and F, G), suggest potentially similar modes of action for these emerging contaminants.

Possible opportunities for lowering contaminant concentrations in biosolids and some additional emerging contaminants, reported by other researchers, will also be highlighted. Finally, we briefly discuss some European regulations, which, unlike current U.S. policies, restrict the use and/or disposal of some of these emerging contaminants of concern.

METHODS

To establish a better understanding of the organic contaminants actually present in contemporary U.S. biosolids, samples produced using different sewage sludge stabilization processes (anaerobic-digested, lime-stabilized, heat-treated and composted) and from different geographic regions (Table 1) were examined. The analytical approach employed provides the ability to detect targeted compounds, as well as screen complex matrixes for halogenated compounds (e.g., PBDEs and triclosan). A representative list of chemicals detected using this method is listed in Figure 1, (specifically Figure 1: A, B, E, F, H, I).

All samples were freeze-dried, sieved (2000 ?m) to remove large debris and stored in glass jars with Teflon® lids at <4°C until analyzed. Percent solids, Total Organic Carbon (TOC) and Total Nitrogen (TN) were determined for each sample [Table 1]. Percent solids values were determined by heating each sample at 105°C until a constant weight was established. TOC and TN were analyzed by thermal conductivity detection (Exeter CE440, Chelmsford, MA); inorganic carbon was removed by addition of hydrochloric acid. TN includes inorganic and organic nitrogen. Each sample (2–5 g) was subjected to enhanced solvent extraction (Dionex ASE 200, Sunnyvale, CA). Conditions were: two extraction cycles, pressure @ 1000 psi, temperature @ 100°C, heat 5 minutes, static 5 minutes, 60% flush, purge 180 seconds. Approximately 30 mL of dichloromethane (DCM) were used per sample. Perinaphthenone and 2,2′, 3,4,4′, 5,6,6′-octachlorobiphenyl (PCB-204) were added as surrogates prior to extraction. Extracts were reduced to 5 mL under nitrogen, and purified by size exclusion chromatography, (Envirosep-ABC®, 350 × 21.1 mm. column; Phenomenex, Torrance, CA). The column was eluted with DCM at 5 mL/min. The first 50 mL, containing high molecular weight lipids, were discarded. The next 60 mL, containing the compounds of interest, were col-

Table 1. **Biosolid characteristics.**

Sample	Location (State)	Type of Stabilization	Biosolid Classification	% Solids	% Total Organic Carbon	% Total Nitrogen
Compost-A	VA	Compost	A*	66	9.9	1.3
Compost-B	VA	Compost	A	45	18.5	2.1
Compost-C	TX	Compost	A*	64	16.1	1.6
Lime-A	VA	Lime (Alkali)	B	37	12.3	1.6
Lime-B	VA	Lime (Alkali)	B	31	24.6	2.9
Heat-A	NY/MD	Heat	A*	>95	24.9	4.0
Heat-B	NJ	Heat	A*	>95	32	2.0
AD-A	CA	Anaerobic Digestion	B	30	23.5	3.5
AD-B	CA	Anaerobic Digestion	B	39	22.2	3.8
AD-C	CA	Anaerobic Digestion	B	34	25.4	4.5
AD-D	CA	Anaerobic Digestion	B	44	20.6	3.5
AD-E	CA	Anaerobic Digestion	B	3.0	28.8	5.2

*Biosolids distributed at retail outlets for home garden usage.

lected and solvent exchanged to hexane. The partially purified extract was then introduced onto a 2 g silica column (EnviroPrep, Burdick & Jackson) and eluted with 3 mL hexane, followed by 6 mL of 60:40 hexane/DCM and 10 mL acetone. PBDEs eluted in the second fraction (60:40 hexane/DCM) and OP, NP, NP1EOs, NP2EOs, polycyclic musks, and triclosan were eluted in the third faction (acetone). Each fraction was collected separately. The retained fractions were reduced in volume and solvent exchanged to toluene. Pentachlorobenzene (0.5 µg) and p-terphenyl (10 µg) were added as internal standards, prior to gas chromatography.

Thereafter extracts were separated on a gas chromatograph (GC) and detected with a mass spectrometer (MS) (Varian Saturn 2000 GC/MS, Sugar Land, TX) operated in the electron ionization (EI) mode with a mass range of 50-650 m/z^+. The column used was a 60 m DB-5 (J&W Scientific, Folsom, CA) with a 0.25 µm film thickness and 0.32 mm inner diameter. Carrier gas was helium. The GC temperature program used was: initial column setting 75°C, hold one minute, ramp at 4°C/min, hold at 330°C for 5 min., total run time 70 min., injector 315°C. Compounds of interest were quantified with a five-point linear calibration curve using the internal standard and selected ions for each compound of interest, $R^2 > 0.991$ for each calibration curve. Curves were created from 4-tert-octylphenol, a mixture of 4-nonylphenols (Fluka Chemie AG, Switzerland), a NP1EO: NP2EO 60:40 mix (ChemService, West Chester, PA), Tonalide and Galaxolide (Cambridge Isotope Labs, Andover, MA), and triclosan (Ultra Scientific, North Kingstown, RI). Data were corrected based on surrogate recoveries, which were greater than 82 % for perinaphthenone. Selected ions were: OP 135 m/z^+, NP 135 m/z^+, NP1EO 179 m/z^+, NP2EO 223 m/z^+, Tonalide and Galaxolide 243+244 m/z^+, triclosan 288+290 m/z^+, perinaphthenone 152+180 m/z^+, and p-terphenyl 230 m/z^+.

PBDEs were initially screened and quantified using a five-point linear calibration curve and internal standard determined on a GC equipped with a halogen-selective electrolytic conductivity detector (ELCD). (PBDE standards included individual congeners, BDE-47, -100, -99, -154, -153 and -209, obtained from Cambridge Isotope Laboratories.) Analytical column type used was the same as stated previously. Carrier gas was helium. The GC temperature program used was: initial column setting 90°C, hold one minute, ramp at 4°C/min, hold at 320°C for 5 min., total run time 68.5 min., injector 320°C. It has been reported that BDE-209 may degrade if subjected to high temperatures for extended periods [21]. Therefore, the extracts were also analyzed on a 15 m DB-5 column of the same film thickness and diameter. Initial column temperature was held 2 min at 80°C, ramped at 15°C / min to 320°C and held for 3 min, then increased at 15°C / min to 350°C for 3 min. Pentachlorobenzene was used as an internal standard and all samples were corrected for PCB-204 recoveries, generally greater than 80%. PBDEs were also confirmed by GC/MS, using the same analytical columns and temperature programs.

RESULTS AND DISCUSSION

Using this screening approach we were able to identify several previously overlooked contaminants of emerging environmental concern in U.S. biosolids. Each sample contained multiple examples of these.

Polybrominated Diphenyl Ethers (PBDEs):

Sources of PBDEs released to the environment are still under investigation. However, they (primarily congeners with six or fewer bromines) have been detected in freshwater fish [22], human breast milk [23] and in biota from remote areas, e.g. deep ocean whales [24] and arctic seals [25], indicating their environmental distribution are tracking that of the PCBs. Hydroxylated-PBDEs are structurally similar to thyroxin (Figure 1, C, D) and triiodothyronine and have been shown to bind to related receptor proteins, suggesting that they may interfere with normal physiological functions [26]. The highly brominated congeners such as BDE209 have primarily been reported from sites near points of release. Their levels in biota have been reported to be low to date, presumed to be largely due to poor uptake efficiency.

PBDEs were detected in each of 11 biosolids analyzed (Table 2) for these compounds. The major PBDE congeners observed were BDE47, BDE99 and BDE209. BDE209 was detected up to 4890 µg/kg (dry wt.). For tetra- through hexa- PBDEs, few differences in concentrations were apparent as a function of WWTP sludge stabilization process used or the facility's geographical location. The commercial penta- formulation (DE-71, Great Lakes Chemical, West Lafayette, IN) in usage in the U.S. today produces a PBDE congener pattern, which closely resembles that detected in each of the biosolids. This suggests that this product, used today exclusively in polyurethane foam, may be the source of lower brominated constituents observed and that little environmental modification had occurred in the mixture's composition, indicating likely proximity to the source (Figure 2). BDE209 used in plastic casings of electronic devices and in latex back coatings on textiles, was the dominant PBDE detected, ranging 84.8 to 4890 µg/kg, dry wt. (Table 2). Although industrial contributions to the WWTP were not characterized, it is believed that major sources of Deca-BDE to the sludge may have been industrial due to: 1) the high concentrations observed; 2) the sample to sample variations in levels seen suggesting point sources; 3) the fact that its dominant use is in physically stable thermoplastics resistant to post manufacture deterioration; and 4) its extremely low water solubility and vapor pressure, limiting environmental movement. However, a recent study from Germany has reported PBDEs in dust samples from 40 households with BDE-209 ranging from 39 to 19,100 µg/kg [27], indicating that residential dischargers cannot be ruled out. It is believed that the toxicological risk of Deca-BDE is lower than the less brominated PBDEs. Its extremehydrophobicity and large molecular size likely limits its uptake by organisms. However, it may be subject to debromination, leading to production of lower brominated congeners and other products. For example, in the laboratory, photolysis by UV and sunlight [28] and metabolism exposed fish have resulted in the production of minor amounts of less brominated diphenyl ethers [29]. However, limited documentation of debromination occurring in the field has been published to date.

Deca-BDE is a high-use chemical in the U.S., and is the only PBDE listed on the EPA Toxics Release Inventory (TRI) [www.epa.gov/tri/whatis.htm]. Chemical reporting on the TRI is required if facilities produce more than 25,000 lb or use more than 10,000 lb of a listed chemical. However, there are some exceptions, e.g. a 10 lb threshold for some banned organochlorines and 100 lb for tetrabromobisphenol-A [30], another brominated flame retardant. The latter is not as bioaccumulative as the tetra- and penta- BDEs and is less likely to be released into the environment as it is chemically bonded to its matrix. Lowering the TRI reporting thresholds of all PBDE formulations to a similar level would assist WWTPs and others in identifying sources. Currently there are no U.S. regulations controlling PBDE limits in biosolids. However, due to environmental and human health concerns, the European Union (EU) and California have initiated legislation banning the Penta- PBDE and Octa-PBDE formulations. The EU directive will prohibit these formulations in the EU market by mid-2004 [31]. However, voluntary cessation by industries in many of these countries has already largely curtailed their use there. The phase-out in California is scheduled for 2008 [32]. EU rulings on Deca-BDEs are pending a risk evaluation study, to be finalized by December 2003 [31].

Alkylphenol Polyethoxylate (APEO) Degradates

Recent concerns have been raised about potential estrogenic effects of APEO degradation products. Using the yeast estrogen system (YES) it has been reported that APEOs with the hydroxyl group in the para- position (e.g. 4-NP) interact with the human estrogen receptor (hER) stimulating the synthsis of β-galactosidase

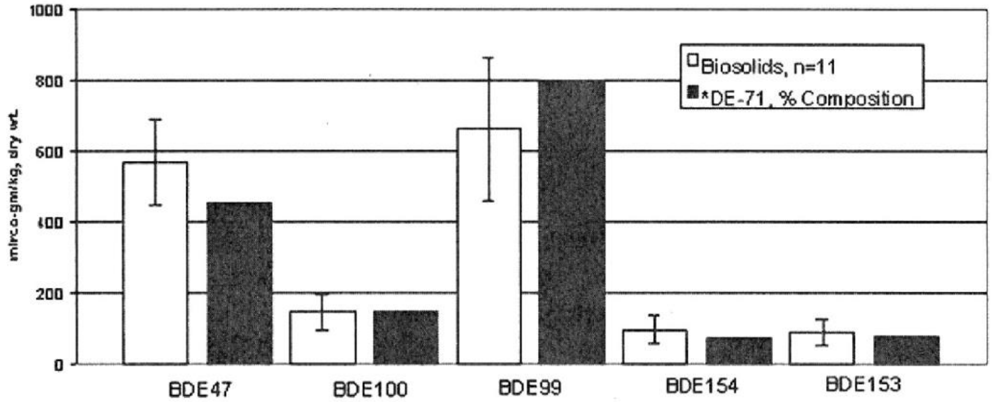

Figure 2. *"Penta-BDEs" in Biosolids Compared to DE-71 Composition. (*) Values were derived by multiplying the mean (n = 11) of the "penta-BDE" totals (BDE-47, -100, -99, -154, -153) by the % composition of each congener in the commercial formulation DE-71, as reported by Hale. R.C. et al. [22].*

Table 1. **Emerging chemicals of concern in biosolids.**

	Polybrominated diphenyl ethers (µg/kg, dry wt.)							Alkylphenol and nonylphenol polyethoxylates (mg/kg, dry wt.)					Polycyclic musks (mg/kg, dry wt.)		Antibiotic (mg/kg, dry wt.)
Sample	**-47**	**-100**	**-99**	**-154**	**-153**	**Total Penta-Like BDEs**	**-209**	**OP**	**NPs**	**NP1EOs**	**NP2EOs**	**Total APs & NPEOs**	**Galaxolide**	**Tonalide**	**Triclosan**
Compost-A	498	106	743	98.8	55.6	1501	308	<0.5	5.4	0.7	<1.5	6.1	na	na	na
Compost-B	754	167	1157	121	87.9	2287	1460	1.5	172	2.5	<1.5	176	7.0	5.6	7.4
Compost-C	536	112	516	58.2	71.8	1294	368	<0.5	14.2	<0.5	<1.5	14.2	na	na	na
Lime-A	359	88.5	513	82.6	64.3	1107	553	5.3	820	81.7	25.3	932	12.4	7.4	4.7
Lime-B	525	200	584	172	179	1660	84.8	2	119	154	254	529	na	na	na
Heat-A	518	115	\714	95.2	58.8	1501	1940	7.5	496	33.5	7.4	544	1.1	0.4	6.9
Heat-B	673	255	815	169	119	2031	4890	na	na	na	na	na	na	na	na
AD-A	605	125	572	57.2	68.9	1428	347	9.9	683	28.4	<1.5	721	17.9	9.0	5.2
AD-B	421	113	391	61	116	1102	340	12.6	720	25.7	<1.5	758	11.4	5.4	5.5
AD-C	686	129	648	61.9	67.7	1593	40	11	779	102	32.6	925	na	na	na
AD-D	674	176	613	74.5	80.6	1618	389	11.7	701	55.8	<1.5	768	na	na	na
AD-E	na	na	na	na	na	na	na	6.7	887	64.9	22.7	981	10.2	6.6	3.6

*na = not analyzed

[33] (Figure 1, F, G). OP, NPs, and NP2EOs have also been reported to induce vitellogenin production (an egg yolk precursor protein, taken up by oocytes to produce proteins) in male trout and in minnows (*Pimephales promelas*) at low µg/L concentrations [34, 35]. Others have reported effects of NPs at concentrations <1 µg/L on developing embryos and larvae of Pacific oysters, including; delays in and abnormal development (e.g. hermaphrodity and shell hinge deformities, and increased larval mortality rates [36]. Wild roach (*Rutilus rutilus*) exposed to discharges from U.K. WWTPs exhibited a high incidence of intersexuality [37]. Concentrations of NPs, NP1EOs and NP2EOs ranged up to 76 µg/L in these receiving waters [38]. Reported log octanol-water partition coefficients (K_{ow}) range from 4.12 to 4.48 for these compounds [39], indicating preferential partitioning onto sludge solids and moderate bioconcentration potential.

NPs were the major APEO degradates detected in the U.S. biosolid samples analyzed (Table 2). Concentrations up to 887 mg/kg (dry weight) were observed in anaerobically digested biosolids, but levels in composted samples were considerably lower, 5 to 14 mg/kg. In a recent study by other investigators, anaerobically digested sewage sludges from five wastewater treatment plants in central New York were reported to contain a mean NP concentration of 1500 mg/kg, with a maximum of 1840 mg/kg (dry weight) [40]. Anaerobic digestion of sludge solids has been shown to degrade the parent APEOs, favoring NPs in the final product. NP1EO and NP2EO may also accumulate in sludge solids and may be present at concentrations exceeding NPs [41]. One of our limed biosolid samples (Lime-B, which did not undergo anaerobic digestion) exhibited incomplete APEO degradation, as evidenced by the presence of high concentrations of mono- and diethoxylates, 154 and 254 mg/kg, respectively (Table 2). OP (detected up to 12.6 mg/kg, dry wt. Table 2) was generally two orders of magnitude lower than NP. However, OP has been reported to be 10 to 20 times more estrogenic than NP [41]. Natural and synthetic estrogens (e.g., estrone, 17 β-estradiol and 17 α-ethinylestradiol) have also been shown to be persistent in activated and digested sewage sludge from Germany, previously reported at up to 37, 49, and 17 µg/kg, respectively [42]. Using the 17β-estradiol equivalence (E2-Eq) factors of 0.0015 and 0.0001 for OP and NP, derived from the YES assay [43], the estrogen activity calculated for these two xenoestrogens identified in our samples set ranged from 0.5 to 98.8 µg E2-Eq/kg, indicating their potential estrogenic effects may exceed that of natural and synthetic estrogens within these samples. Also observed within the sample set was a strong positive correlation ($R^2 = 0.6919$) between NP and OP concentrations. Octylphenol polyethoxylates are used in similar applications as NPs, albeit at lower volumes, and likely follow similar degradation pathways. Their presence in sludges suggests that the simple substitution of octylphenol polyethoxylates for NPEOs would not be environmentally advantageous and could actually increase potential xenoestrogen impacts. Comparing stabilization processes (Figure 3), composting and associated aerobic microbial degradation, resulted in the lowest total AP ethoxylates/AP ratios. Composting may also be used to degrade other contaminants susceptible to aerobic degradation.

An EU initiative has set a regulation on the sum of NPs, NP1EO and NP2EO in biosolids at 50 mg/kg, dry wt. The Danish EPA has also set a 30-mg/kg limit for this same sum in biosolids. NPEOs were phased out of domestic detergents in the UK in 1976 and from all usage in Denmark, Germany, Netherlands, Sweden and the UK in the 1980's. In 1987 Switzerland banned the use of APEOs [44]. In contrast, there currently are no North American regulations controlling the usage or disposal of APEOs. However, due to their aquatic toxicity and presence in wastewater effluent APEO-degradation products have been placed on the U.S. EPA TSCA Priority Testing List [45]. Under the Canadian Environmental Protection Act, Canada's EPA has identified NPs and their ethoxylates as "toxic," indicating that further studies are needed to establish their effects on the environment [46]. U.S. EPA is also now considering water quality criteria for NPs at µg/L levels [47].

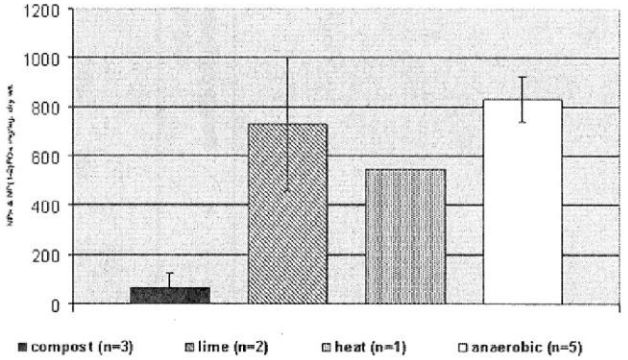

Figure 3. *NPs, NP1EOs and NP2EOs totals, grouped by stabilization process.*

Fragrance Compounds (nitro- and polycyclic musks)

Nitro and polycyclic musks (Figure 1, H) are used as fragrances in washing and cleaning products and in a variety of personal-care products, including shampoos, soaps, detergents, perfumes and skin lotions. They have been detected in finfish, shellfish and WWTP effluent [48], human milk and adipose tissue [49, 50] and river sediment samples [51]. Log K_{ow} for the nitro-musks, ketone and xylene, range from 4.1 to 5.2, whereas those for the two polycyclic musks, Galaxolide and Tonalide, have been estimated as 5.9 and 5.8, respectively. Bioaccumulation factors (wet weight basis) in trout have been reported as 4200 and 5100 for musk xylene. For bluegill sunfish (*C. dubia*), values of 597 and 1584 were published for Tonalide and Galaxolide, respectively, further underlining their potential to accumulate in aquatic organisms. Concentrations of nitro musks, their amino metabolites, and polycyclic musks have also been previously reported in sewage sludge from 12 Swiss WWTPs [52]. In that study three types of sludges were analyzed and characterized as: highly domestic, mixture (domestic, storm water runoff and low industrial) and highly industrial. The highly domestic sludge actually contained the greatest concentration of musk products, i.e., Tonalide and Galaxolide at 4161 μg/kg and 12,157 μg/kg dry wt., respectively. Nitro-musks, their amino metabolites, and other polycyclic musks were also detected ranging from less than 0.1 to 7, 49 and 843 μg/kg, (dry wt.), respectively. Nitro-musks, which are now being replaced with polycyclic musks in Europe, may degrade to amino derivatives during sewage treatment. The latter compounds appear to be more toxic than their precursors.

Tonalide and Galaxolide were detected in each of the six U.S. biosolids, which were analyzed for these compounds (Table 2.). Concentrations ranged from 0.4 to 9.0 mg/kg and 1.1 to 17.9 mg/kg dry wt. respectively, similar to the Swiss data [52]. Sample size was limited, but the data suggest that concentrations in the anaerobic-digested, limed and composted samples did not vary as a function of sewage sludge stabilization. However, levels in the single heat-treated sample (Heat-A) were 10-fold lower. The half-life for polycyclic musks in soil has been estimated to be 180 days [53], suggesting that they could persist in soil after the application of biosolids.

Triclosan

The antibacterial and antifungal agent triclosan (2,4,4′-trichloro-2′-hydroxydiphenyl ether) is structurally similar to a hydroxylated-PBDE or PCB, as well as thyroxin (Figure 1, I, C, D). This may indicate some potential to interact with the endocrine system. Triclosan is widely used in personal care products such as shampoos, soaps, deodorants, cosmetics, skin-care lotions and creams, mouth rinses and toothpastes [54, 55, 56]. Reported bioaccumulation factors for zebra fish ranged from 2532 at 3 μg/L to 4157 at 30 μg/L [54]. Fathead minnows, bluegill sunfish, *D. magna* and *C. dubia* all exhibited EC50 or LC50 concentrations in the range of 240 to 410 μg/ L. Early life stage toxicity for rainbow trout determined a no-observed-effects-value (NOEC) and a lowest-observed-effects-value (LOEC) of 34.1 and 71.3 μg/L. However, for the unicellular algae, *A. flos-aquae,* the 96-h EC50 value was only 1.6 μg/L [54]. Triclosan has been shown to be detectable for more than 30 years in anaerobic sediments [57]. It has also been observed in U.S. WWTP influents ranged from 3.8 to 16.6 μg/L, corresponding concentrations in final effluents from 0.2 to 2.7 μg/L. In aerobic and anaerobic digested sludge concentrations ranged from 0.5 to 15.6 mg/kg, dry wt. The higher levels were present in anaerobic digested sludge solids [55]. It has also been reported that activated-sludge wastewater treatment can remove more than 80% of triclosan from the waste stream and that biodegradation is the likely responsible mechanism. These observations indicate that the type of wastewater and sludge treatment process may influence its elimination [55, 56]. Triclosan was detected in all six biosolids analyzed for this compound (Table 2). Concentrations ranged from 3.6 to 7.4 mg/kg dry wt. There was little apparent association observed between triclosan concentrations and either biosolid stabilization processes or generation facility location in limited sample set examined.

CONCLUSIONS

This survey supports the hypothesis that modern U.S. biosolids contain a diverse collection of wastewater contaminants of emerging toxicological concern not considered by Part 503 Rule. This survey also demonstrates the need to develop analytical methods that can screen for multiply classes of organic contaminants in complex matrixes (e.g. biosolids). Five of the 12 samples were tested for each of the four classes of contaminants (PBDEs, APEO degradates, polycyclic musks, and triclosan)—a total of 13 individual contaminants. Two of the five samples contained all 13 contaminants, while the other three samples contained 12 of the 13 (Table 2). Previous studies have shown that sewage sludge can contain a mixture of historically-tracked contaminants (e.g. PAHs, PCBs, organochlorine pesticides). Concerns over possible additive/synergistic effects from biosolids that contain mixtures of contaminants have also been expressed. Researchers using the E-screen test, which measures the response of estrogen-sensitive MCF7 human breast cells, have shown that a combination of weak environmental estrogens can act cumulatively [58]. This suggests that the total estrogen burden or response may be a more toxicologically relevant endpoint to monitor than the concentration of single individual xenoestrogen.

Although these four classes of contaminants were detected in biosolids prior to their land application, questions still remain on their persistence, bioavailability and toxicological effects on terrestrial environments. Further studies on their fate following land application are indicated, as research is limited in this area. Also, these classes of contaminants have previously demonstrated toxicological effects on aquatic environments and there exists a paucity of studies focusing on their terrestrial impacts. Reports have shown that once land applied, decomposition of some biosolid chemical constituents can take place in aerobic environments (e.g. NPs and estradiol [59, 60]), which indicates a potential beneficial process for the elimination of some harmful wastewater residual contaminants. However, uncontrollable events, such as a rain event prior to complete contaminant decomposition, may facilitate transport by leaching contaminants or physically transport biosolid particles with associated contaminant burdens to receiving streams. For example, some researchers have reported APEOs in river sediments and have suggested that they could be derived from sewage sludge used as fertilizer in nearby fields [61]. A change in land use from agricultural to residential, a situation of increasing frequency as a result of expansion of suburbs, may acerbate human exposure. Further studies on eliminating contaminants in residuals during the wastewater treatment process should take priority; this will ultimately limit harmful exposures.

WWTP technology has primarily focused on removing contaminants from wastewater effluent, thus protecting our waterways from toxic effects to aquatic life or other impacts on downstream use. In contrast, the anthropogenic fraction of the organic constituents in biosolids, the "second effluent," has been largely considered benign following the EPA risk assessment and thus has been inadequately studied during the past decade. Interestingly, even the 1988 NSSS examined sewage sludges, not biosolids, an important distinction. Parent xenobiotic burdens in the latter are expected to be lower than in the former. However, in some cases, as illustrated by the NPEOs and NPs, breakdown products may actually be the more problematic. However, some of the contaminants detected in the present study may be reduced through additional stabilization processes, e.g. complete APEO elimination via composting. In other cases, the best approach may be removal of pollutants from the wastewater stream at the source, e.g. collection of unwanted or expired pharmaceuticals from residents prior to their disposal via WWTPs. Further studies focusing on the presence of emerging contaminants in biosolids are obviously indicated, along with efforts to more fully understand their fate during wastewater treatment and sewage sludge treatment and stabilization processes. Chemicals with structures, physical and chemical properties or persistence similar to known problematic contaminants should be of highest priority, as recapture or elimination of these may be difficult if remediation becomes necessary.

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Evidence of Debromination of Decabromodiphenyl Ether (BDE-209) in Biota from a Wastewater Receiving Stream

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Decabromodiphenyl ether (BDE-209) is a high production volume flame retardant. To date, regulation and control of its environmental release have been minimal. Once in the environment, BDE-209 may encounter conditions favoring debromination, potentially forming congeners with greater toxicity, bioaccumulation potential, and persistence. However, (photolytic and in vivo) debromination has only been demonstrated under laboratory scenarios. To examine whether debromination was likely in the field, PBDE congener profiles were tracked from a wastewater treatment plant (sludge) to receiving stream sediments and associated aquatic biota. BDE-209 and 23 additional PBDEs were detected. Sludge congener profiles resembled the commercial penta- and deca- formulations, suggesting minimal -209 debromination during wastewater treatment. Similar profiles were observed in surficial sediments at the outfall and downstream. However, sunfish (*Lepomis gibbosus*), creek chub (*Semotilus atromaculatus*), and crayfish (*Cambarus puncticambarus sp. c*) collected near the outfall contained tri- through deca-PBDEs, including congeners not detected in the commercial deca- mixture, sludges or sediments (BDE-179, -184, -188, -201, and -202). A previous in vivo laboratory study identified these as -209 debromination products. This supports the hypothesis that metabolic debromination of -209 does occur in the aquatic environment under realistic conditions. Hence assessments that assume no BDE-209 debromination may underestimate associated bioaccumulation and toxicity attributable to the less brominated congeners produced.

Introduction

Polybrominated diphenyl ethers (PBDEs) are widely used flame-retardants. They have become globally distributed, akin to other persistent organic pollutants such as PCBs. Although, BDE-47 and -99 are the most commonly reported congeners, researchers have observed nearly 40 additional congeners in various biological matrixes. Of these congeners, 13 have not been reported as components of common commercial PBDE formulations (*1*). Therefore they may result from debromination of higher brominated PBDEs. In contrast, deca-, the PBDE product of greatest historical demand and the only formulation still in production in North America and Europe, has been reported with less frequency (*2, 3*). Its primary constituent, BDE-209, has been detected at high concentrations in sediments near points of initial release (*4*). However, with the exception of birds of prey (*5*), reported concentrations in biota have been comparatively low. This has been attributed to its reportedly low bioavailability and tendency to strongly bind to sediment and soil.

BDE-209 can debrominate when dissolved in certain solvents and subjected to UV irradiation (*6*). Major homologues that have been observed include tri- through octa-PBDEs and a host of brominated dioxins and furans. Photolytic debromination of BDE-209 associated with artificial and natural sediment, soil, and sand has also been reported (*7–9*). Studies have observed an increase in the lower brominated (hexa- through nona-) PBDEs. Söderström et al. (*8*) identified BDE-47, -99, and -154 on silica gel originally amended with BDE-209. They also reported -209 dissolved in toluene and exposed to UV light generated BDE-99, -100, -153, and -154. Recently, Ahn et al. (*9*) exposed -209 adsorbed matrixes made up of clay minerals, metal oxides, and sediment to UV irradiation, lamp and natural. Tri- to nona-PBDEs were detected after 3 days of lamp-irradiation and 14 days of sunlight-irradiation in two of the six matrixes (montmorillonite (2:1 clay mineral; smectite) and kaolinite (1:1 clay mineral)). However, BDE-47 and -99 were not detected. Longer half-lifes for BDE-209 adsorbed to more complex matrixes, i.e., natural sediment or soil than artificial ones, were also observed by Söderström et al. (*8*), and Ahn et al. (*9*). This was attributed to natural soil and sediment particles being more porous and organic carbon rich than artificial ones, providing UV-radiation shielding (*8, 9*). Hua et al. (*10*) reported increasing humic acid contents deceased degradation rates for UV-irradiated BDE-209.

As much as 97% of the deca- product is formulated with plastics for electronic equipment, e.g., housings and wire coatings. This incorporation and the chemical properties of BDE-209, namely low water solubility ($<20-30\ \mu g/L$) and vapor pressure ($<10^{-6}$ mmHg at 20 °C) (*11*) retard its release and mobility. This also will limit its photodegradation and bioavailability potential. However, recent studies have shown that household dust can contain PBDEs, including BDE-209, at mg/kg levels (*12, 13*). Also, industrial discharges may release deca-. According to the U.S. EPA Toxic Release Inventory (http://www.epa.gov/tri/ (*14*)), total U.S. industrial releases on and off-site to land (e.g., air, surface water, and landfills) of BDE-209 from 1988 to 2004 averaged over 500 metric tons (MT) per year. Approximately 90 MT more per year were released to wastewater treatment plants (WWTPs). Household waste (containing PBDE-laden dust) and other unquantified industrial activities may transfer additional PBDEs to WWTPs. PBDEs may then reach the environment sorbed to particulates in the aqueous effluent (*15*) or via land applied sewage sludge (*16*).

Few studies have examined the dietary uptake and biotransformation of BDE-209. Juvenile rainbow trout (*Oncorhynchus mykiss*) were fed cod chips spiked with BDE-209. BDE-209 was not detected in the fish, but BDE-47, -99, -153, and several nonspecified hexa- to nona-PBDEs were reported (*17*). Their concentrations in liver and muscle increased with length of exposure. BDE-153, -154, and an unidentified octa-PBDE were not detected in the original deca- mixture, indicating likely transformation of BDE-209. More recently, juvenile carp (*Cyprinus carpio*) were fed BDE-209 (>98% purity) spiked food for 60 days (*18*). At the end of this study

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BDE-209 was not detected in the carp tissues, however seven other congeners were observed and accumulated over time. Two apparent metabolites were identified as BDE-154 and -155, the remainder were only identified as to their degree of bromination, i.e., as penta- through octa-PBDEs. In another experiment, dietary exposure of carp to BDE-183 or -99-spiked food, resulted in apparent metabolic debromination of both congeners (*19*), i.e. conversion of BDE-99 to -47, and -183 to -154 and an unidentified hexa-PBDE. Approximately 10% of the original doses of BDE-99 and -183 were detected as BDE-47 and -154, respectively. The extent of metabolic debromination is likely species-dependent. This would account for the low contribution of BDE-99 (<0.1%) to the total PBDEs reported in carp from the Hyco River in Virginia (U.S.) (*20*). Similar PBDE contributions were also observed in feral carp and sediments collected from a heavily industrialized portion of the Llobegat River, Spain (*21*). In a follow-up to the carp exposure study, juvenile rainbow trout were exposed in the lab to -209 via the diet for 5 months (*22*). BDE-209 concentrated in the liver. Several hepta-, octa-, and nona-PBDEs congeners also accumulated in the trout's liver. To determine whether the observed debromination was a result of metabolism by the fish, liver microsomes were prepared from both carp and rainbow trout and incubated with BDE-209. As much as 22% of the BDE-209 mass was biotransformed, primarily to octa- and nona-PBDEs, in the trout liver. About 65% of -209 was transformed to hexa-PBDEs in the carp, indicating species-dependent metabolic debromination.

From these studies, it is apparent that in the laboratory BDE-209 can undergo photolytic and biological debromination. Hence, once BDE-209 is released to the environment, it may encounter conditions conducive to debromination, contributing to the environmental burdens of the now largely discontinued, lower brominated PBDEs. However, this phenomenon has not been previously documented in an actual environmental setting. While it appears that fish took up some BDE-209 from heavily spiked food in the laboratory, questions remain as to the extent to which BDE-209 in the environment is available for uptake. Here, we evaluated congener distributions of PBDEs in WWTP sludges, and downstream receiving water sediments and aquatic biota for evidence of potential debromination.

Experimental Section

Study Site. The U.S. EPA Toxic Release Inventory (TRI) lists U.S. industry reported releases (e.g., air emissions and discharges to surface water) and transfers (e.g., landfills and WWTPs) of high production volume chemicals. Of the PBDEs, deca- is the only PBDE product tracked in this way. According to the TRI, 27 U.S. facilities released more than 4.5 MT of BDE-209 in 2001 (*14*). The total 2001 releases from these facilities were approximately 500 MT, primarily from deca-chemical manufacturing and chemical waste management facilities. The fifth largest reported amount from an individual operation, 45 MT, was from a North Carolina (NC) plactic goods manufacturer. Reported discharges were reported to be via a wastewater treatment plant (WWTP), rather than directly to surface waters. Preliminary findings (October, 2002) revealed BDE-209 at 12 μg/L in the whole WWTP effluent, with trace levels (<20 ng/L) of BDE-47 and -99 (*23*).

The WWTP is an activated sludge-type secondary facility that releases an average of 2.1-MGD of treated effluent into Marlowe Creek, downstream from the City of Roxboro, NC. During dry months, this discharge is more than 99% of the total creek flow. Marlowe Creek flows into Storys Creek, which later enters Hyco River approximately 11 km downstream from WWTP outfall. The Hyco River flows northeast from North Carolina and enters the Dan River downstream from South Boston, Virginia. The headwaters of the Hyco are dammed to form the After Bay Reservoir located approximately 35 km upstream from the Dan River. Water released from the reservoir flows down the Hyco River through rural and agricultural areas.

Sample Locations. Samples (wastewater sludge, sediments and biota) were collected in Fall 2002 (Table ST1, Supporting Information) to determine if PBDEs used in local manufacturing were transferred for further treatment to the WWTP and released via its effluent. The extent of PBDE contamination from the WWTP outfall to a site 11 km downstream was also investigated. In Fall 2005 additional samples were collected at the outfall and several locations downstream (Table ST1) to ascertain current PBDE burdens and for evaluation of detailed congener profiles as evidence of environmental debromination.

Within WWTPs, PBDEs reside primarily sorbed to solids. Hence analysis of these burdens provides an estimate of the amount introduced to the facility. Accordingly, activated sludge (4 L) was collected in 2002 and 2005. To determine release of PBDEs to the effluent-dominated receiving stream, bed sediments and aquatic biota were also collected near the outfall. The sample site chosen, approximately 15 meters downstream from the outfall, was presumed well mixed. Surficial sediments (1 L) were collected here and minnow traps were placed on both sides of the stream nearby. Traps were emptied approximately 24 h after deployment, captured biota separated by species, and transferred to holding tanks for depuration (72 hours).

Surficial sediments (1 L) were also taken upstream and several locations downstream from the outfall. Once introduced into aquatic environments, wastewater particulates with associated hydrophobic PBDEs can settle out and contribute to sediments (*24*). Sites were distributed along approximately 11 kilometers of river system and were resampled in 2005.

Methodology. Whole biota was composited and homogenized and then freeze-dried. Sediments were freeze-dried and then sieved (2000 μm) to remove large debris. Sewage sludge was centrifuged, excess water decanted, and then freeze-dried. All samples were stored in glass jars with Teflon lids at <0 °C until they were analyzed.

For PBDE determinations, each sample (0.5 g sludge, 9–10 g biota, 20 g sediment, d.w.) was extracted by enhanced solvent extraction (Dionex ASE 200, Sunnyvale, CA). A surrogate standard (1 μg) 2,2′,3,4,4′,5,6,6′-octachlorobiphenyl (PCB-204) (Ultra Scientific, North Kingstown, RI) was added prior to the extraction. Each extract was purified by size exclusion chromatography, (SEC, Envirosep-ABC, 350 × 21.1 mm. column; Phenomenex, Torrance, CA). The post-SEC fraction of interest was reduced in volume, added to a 2 g silica glass column (Isolute, International Sorbent Tech., Hengoed Mid Glamorgan, UK) and eluted with 3.5 mL hexane, followed by 6.5 mL of 60:40 hexane/DCM. The second fraction, containing the PBDEs, was reduced in volume and solvent exchanged to hexane. Pentachlorobenzene (PtClb) and decachlorodiphenyl ether (DCDE) (Ultra Scientific, North Kingstown, RI) were added for retention time markers. DCDE was also used as an internal quantitation standard. (For further information on sample extraction see the Supporting Information.) Analytical blanks ($NaSO_4$) were also extracted and analyzed with each sample batch and all results corrected based on surrogate (PCB-204) recoveries (Tables 1 and 2). Method validation including matrix ($NaSO_4$, sediment, and biota) spiking information and results, as well as replicate analyses is included in the Supporting Information (Table ST2).

Compounds of interest in the purified extracts were separated by gas chromatography (GC), (6890N, Agilent Tech., Palo Alto, CA) equipped with an on-column injector, using a 30-m DB-5HT (0.25 mm i.d., 0.1 μm, J&W Scientific, Agilent

TABLE 1. PBDEs in Wastewater Sludge and Surficial Sediments Collected in 2002 and 2005.

	collected, November 2002						collected, November 2005					
		sediments (μg/kg, %TOC), km from outfall						sediments (μg/kg, %TOC), km from outfall				
congener	sludge (μg/kg, dw)	−0.2 km	0 km	1.3 km	5.6 km	10.8 km	sludge (μg/kg, dw)	−0.2 km	0 km	1.3 km	5.6 km	10.8 km
BDE-17	nd	nd	nd	nd	25	59	14	nd	nd	nd	nd	nd
−28	nd	nd	nd	74	137	98	78	nd	nd	nd	nd	nd
−49	98	105	nd	383	330	364	95	144	nd	nd	nd	nd
−71	51	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
−47	1670	266	1180	2540	3510	2910	822	964	754	2030	3580	231
−66	125	nd	nd	nd	161	107	55	51	nd	nd	nd	nd
−100	422	115	371	645	1410	1080	305	466	372	627	1180	107
−99	1660	482	1830	3200	5540	3990	918	2210	2010	3740	5410	337
−85	255	25	87	148	249	149	108	74	nd	150	238	nd
−154	295	53	nd	nd	465	303	136	nd	nd	nd	371	35
−153	488	192	375	432	941	556	187	251	nd	525	839	123
−138	nd	nd	nd	nd	119	68	nd	nd	nd	nd	nd	nd
−183	310	nd	nd	nd	249	125	89	nd	nd	nd	nd	nd
−197	993	nd	nd	nd	156	73	171	nd	nd	nd	388	nd
−203	1190	nd	434	386	322	166	220	nd	nd	nd	553	nd
−196	1600	nd	380	771	620	210	202	nd	nd	nd	1120	nd
sum, tri- through octa-BDEs (%)	**9160 (9.4%)**	**1240 (3.3%)**	**4660 (0.3%)**	**8580 (0.3%)**	**14200 (2.1%)**	**10300 (3.2%)**	**3400 (7.9%)**	**4160 (11.1%)**	**3140 (1.6%)**	**7070 (0.3%)**	**13700 (0.6%)**	**833 (0.3%)**
−208	726	nd	3530	nd	577	nd	295	nd	nd	nd	1690	375
−207	1340	nd	5810	6660	2630	945	276	nd	nd	nd	6520	544
−206	27400	nd	67700	84000	24300	10900	1490	nd	11200	31700	35500	3120
−209	58800	36800	1630000	3150000	642000	300000	37400	33300	181000	2310000	2390000	247000
sum of nona- through deca-BDEs (%)	**88300 (90.6%)**	**36800 (96.7%)**	**1710000 (99.7%)**	**3240000 (99.7%)**	**670000 (97.9%)**	**312000 (96.8%)**	**39500 (92.1%)**	**33300 (88.9%)**	**192200 (98.4%)**	**2340000 (99.7%)**	**2440000 (99.4%)**	**251000 (99.7%)**
total PBDEs	**97400**	**38000**	**1710000**	**3250000**	**684000**	**322000**	**42900**	**37500**	**195000**	**2350000**	**2450000**	**252000**
%REC, PCB-204	73%	85%	106%	88%	81%	82%	62%	76%	69%	69%	73%	68%
%TOC	28.2	0.12	0.07	0.07	0.89	0.76	25.3	0.12	0.06	0.08	0.09	0.59

TABLE 2. PBDEs (μg/kg, % Lipid) in Biota Composites, Collected in 2002 and 2005

	collected, November 2002			collected, November 2005
congener	chub ($n = 6$)	crayfish ($n = 5$)	sunfish ($n = 13$)	sunfish ($n = 22$)
BDE-17	nd	nd	32	29
−28	285	nd	246	179
−49	618	nd	1300	855
−71	nd	nd	nd	541
−47	17200	4110	11600	7600
−66	nd	nd	1670	952
−100	3460	nd	2340	1820
−99	nd	3560	13300	5220
−85	725	nd	496	229
−154	2610	nd	2290	1880
−153	918	767	3110	2420
−188	1450	nd	884	289
−184	nd	nd	360	164
−179	1140	nd	166	137
−183	nd	nd	83	77
−202	895	87	747	243
−201	129	78	773	335
−197	nd	43	193	86
−203	117	132	74	20
−196	45	200	65	28
−208	103	143	201	67
−207	79	1920	276	73
−206	94	2650	411	133
−209	nd	21600	2880	nd
total PBDEs	**29900**	**35300**	**43500**	**23377**
%rec. PCB-204	60%	81%	90%	72%
%lipids	21.9	5.2	10.5	17.2

Tech.) column. Ion fragmentation spectra used for compound identification were produced by electron-capture negative ionization (ECNI) and electron ionization (EI) (JMS-GC Mate II, JEOL, Peabody, MA). Recently, it has been reported that 64 PBDEs (mono- through deca-PBDEs) can be reliably analyzed using a single 30-m DB-5HT (*1*). However, poor chromatography (unresolved peaks) was observed for the biota and sludge samples, perhaps due to column overloading by coextractives. Accordingly, these samples (biota, sludge, and calibration standards) were reanalyzed using a pressure pulse split/splitless injector, which greatly improved the chromatography. This injection technique has been previously reported suitable (minimum -209 thermal degradation) for mono- through deca-PBDE analysis (*25*).

The target analytes were first detected and quantified by ECNI-SIM using *m/z* 79 ($[^{79}Br]^-$), 81 ($[^{81}Br]^-$) for the PBDEs and *m/z* 35 ($[^{35}Cl]^-$), 37 ($[^{37}Cl]^-$) for the internal, surrogate, and retention time standards. Five-point quantification curves (0.998 minimum r^2 acceptance value) were generated by analyzing dilutions of a PBDE standard (Wellington Laboratories Inc., Ontario, Canada) containing 27 PBDEs ranging from mono- to deca-PBDEs. An additional seven PBDEs previously identified as potential debromination products (*22*) (Table ST3) were also analyzed. Mono-through nona-BDE concentrations ranged from 10 to 1000 pg and BDE-209 ranged from 50 to 5000 pg on-column for both injection programs. To assist in sample compound identification, relative retention indices (RRI) were calculated for both on-column and split/splitless injector programs for each of the analytical standards, as previously reported (*1*).

Along with RRI, fragmentation patterns and isotope intensity spectra produced by ECNI (scan range 10−550 *m/z*) and EI (scan range 50−1000 *m/z*, scan time 0.30 s., electron energy 70 eV) were used to identify PBDEs. (The previously stated GC conditions were used for both ECNI and EI analyses.) The predominant ions generated in ECNI spectra of PBDEs are 79 and 81 *m/z*. However, cleaving at the ether bond has also been observed for hepta-, octa-, nona-, and deca-PBDEs (*1*). These produced spectra with ion clusters centered around 328 and 330 *m/z* for $[C_6Br_3H_2O]^-$, 408 *m/z* for $[C_6Br_4HO]^-$ and 486 and 488 *m/z* for $[C_6Br_5O]^-$. For PBDE identification, bromine distributions between the two benzene rings can be determined for hepta- through deca-PBDEs by examining these fragments (*1*). In EI mode, the dominant ion clusters are centered on the molecular ion $[M]^+$ and the loss of two bromines $[M-Br_2]^+$ (*1*). Table ST3 of the Supporting Information contains the predominant ions products produced by ECNI and EI modes for compound identification used in this study.

Results and Discussion

Wastewater Sludge. Tri- through deca-PBDEs were detected in the 2002 and 2005 WWTP sludges, indicating the potential for environmental exposure through PBDE-laden effluent particulates. It has been estimated that >90% of the PBDEs entering a WWTP will ultimately reside within the sludge, with the remainder released via its effluent, primarily sorbed to suspended particulates (*15, 26*). A total of 17 PBDEs were identified in the 2002 and 18 in the 2005 sample (Table 1). The major PBDE congener in both was BDE-209 (58 800 and 37 400 μg/kg (d.w.) for the 2002 and 2005 samples, respectively), followed by BDE-99 and -47. For the tri- through octa-PBDEs, major constituents of the penta- and octa-formulations, a 55% lower sludge concentration was observed in 2005 than 2002 (Table 1). This reduction may relate to the December 2004 cessation in the U.S. manufacture of these formulations (the 2005 sample was collected in November).

The EPA TRI indicated substantial delivery of deca- to this WWTP from a major manufacturer of plastic goods. Penta- and octa- were also likely utilized to some extent but, not being high production volume chemicals, were not required to be reported in the TRI. It has also been hypothesized that releases from finished products in use, in addition to manufacturing, may contribute PBDEs to wastewaters. For example, indoor dust can contain mg/kg PBDE burdens (*27*), which can eventually enter household waste streams. Also, as products containing PBDEs age, their release may increase (*27*).

According to the TRI, 2287 kg in 2002 and 1692 kg in 2004 of BDE-209 were transferred to the WWTP examined. Prior to 2002, the TRI suggested it received over 34 000 kg of BDE-209 per year. Maximum reported transfer, 113 826 kg, occurred in 1994 (Figure SF1, Supporting Information). However, since 1999 transfers decreased 10-fold and by 26% between 2002 and 2004. This reduction may be reflected in the 41% lower -209 sludge burden in 2005 than in 2002 (Table 1). Also apparent in these sludges was a decline of the three nona- (BDE-206, -207, and -208) and three octa-BDEs (BDE-196, -203, and -197) (Table 1). It has been previously reported that -209 can debrominate under anaerobic conditions. Gerecke et al. (*28*) incubated -209 with sewage sludge collected from an anaerobic digester. They reported the appearance of several degradation products (two nona-PBDEs (BDE-207, −208) and three octa-PBDEs (tentatively identified as BDE-196, -198/203, and -197). However, no other debromination products were observed. Debromination of -209 to PBDEs with <7 bromines was also not observed in sludge collected from different stages of wastewater treatment from 11 German WWTPs (*29*).

PBDEs have been previously detected in other WWTP sludges. Hale et al. (*30*) reported a maximum BDE-209 concentration of 4890 μg/kg, mean 1010 μg/kg (d.w.), in 11 sludges (biosolids) collected from four different regions of the U.S. They also reported five additional PBDEs (BDE-47, -100, -99, -153, and -154) ranging from 1100 to 2290 μg/kg (d.w.). These congeners contributed an average 75% of the

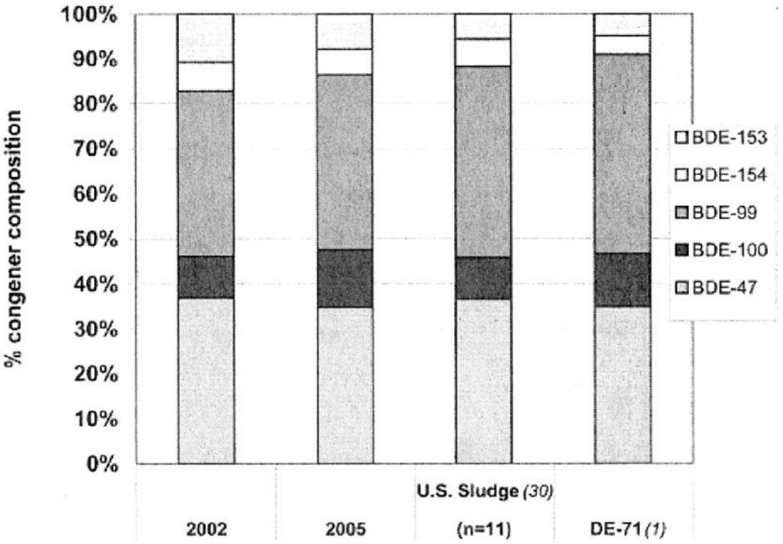

FIGURE 1. Distributions of tetra- through hexa- brominated congeners in Roxboro WWTP sludge (2002 and 2005) compared to U.S. sludges and penta-formulation (DE-71).

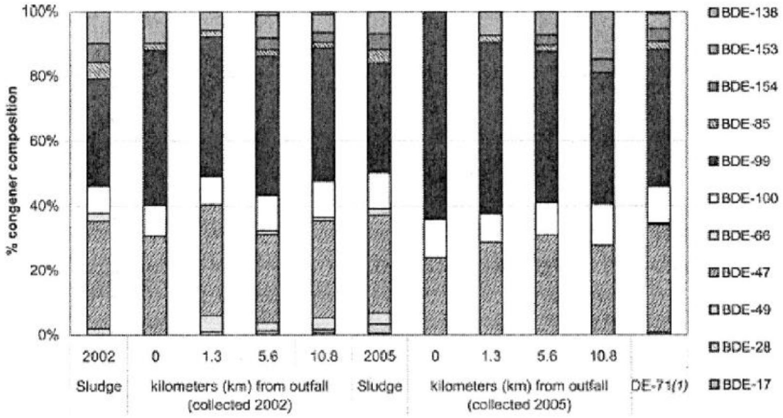

FIGURE 2. Sediment and sludge congener profiles (tri- through hexa-PBDEs), 2002 and 2005.

total PBDE sludge concentration, with the remainder predominantly from BDE-209. In one sludge, -209 contributed 70% of the total PBDEs detected. For the Roxboro WWTP sludges, BDE-209 was the major PBDE congener detected, constituting 60 and 87% of the total, respectively (Table 1). The BDE-209 concentrations here exceeded the previous survey's maximum reported -209 values by 10-fold, likely due to its usage by local plastics manufacturers. However, the sum of BDE-47, -100, -99, -153, and -154 for the 2005 Roxboro sludge (2370 μg/kg, d.w.) was similar to this survey's maximum value. The 2002 sludge burden (4540 μg/kg, d.w.) was only twice this concentration. The congener profiles in the Roxboro sludges approximated that of intact penta-technical formulations, as previously reported for other U.S. samples *(30)* (Figure 1). This suggests that BDE-209 debromination is unlikely to be a major contributor to the lower brominated PBDEs seen in Roxboro sludges.

Sediments. Surficial sediments were also collected in 2002 and 2005. Major PBDE congeners detected were BDE-209, -206, -99, and -47. A total of 20 tri- to deca-BDE congeners were observed in the 2002 samples (14 in 2005) (Table 1). BDE-209 contributed >89% of total PBDEs in each of the sediments, followed by the nona-PBDEs which constituted 3−10%. Most sediment also contained tri- through octa-PBDEs. However, their total contributions were an order of magnitude lower than that of -209 (Table 1). Interestingly, BDE-206 was the second most abundant PBDE detected, up to 84.0 mg/kg, normalized to TOC content, and its concentrations exceeded those of both BDE-47 and -99 in 8 out of 10 samples. Total PBDEs levels were actually greatest several kilometers downstream from the outfall and were still detectable at the furthest downstream collection point (10.8 km) from the outfall, where Storys Creek meets the Hyco River. Concentrations were lower upstream of the outfall (−0.2 km) in 2002 and 2005, 38.0 and 37.5 mg/kg (TOC basis), respectively (Table 1). These values indicate additional PBDE releases via urban runoff or other sources. Maximum sediment concentrations in 2002 and 2005 were detected between 1 and 6 km downstream of the outfall (3250 mg/kg and 2450 mg/kg, respectively). Further down stream (10.8 km) levels dropped (322 and 252 mg/kg, 2002 and 2005, respectively), but still exceeded by 10-fold by those upstream of the outfall (Table 1). The 2005 (5.6 km) sediment exhibited a 3-fold greater concentration than the 2002 sampling (Table 1). This may indicate variations in WWTP discharge, relocation of historic PBDE reservoirs in the river system, or simply heterogeneity within the sampling area.

BDE-209 has been shown to undergo photolytic (6, 7, 8, and 9) and microbial debromination *(31)* under selected laboratory conditions. Debromination could generate degradates with increased toxicological potential. Sediments contained similar concentrations of tri- through octa-BDEs in the 2002 and 2005 sediment sets. An exception was the 2005 sample taken 10.8 km downstream of the outfall, where

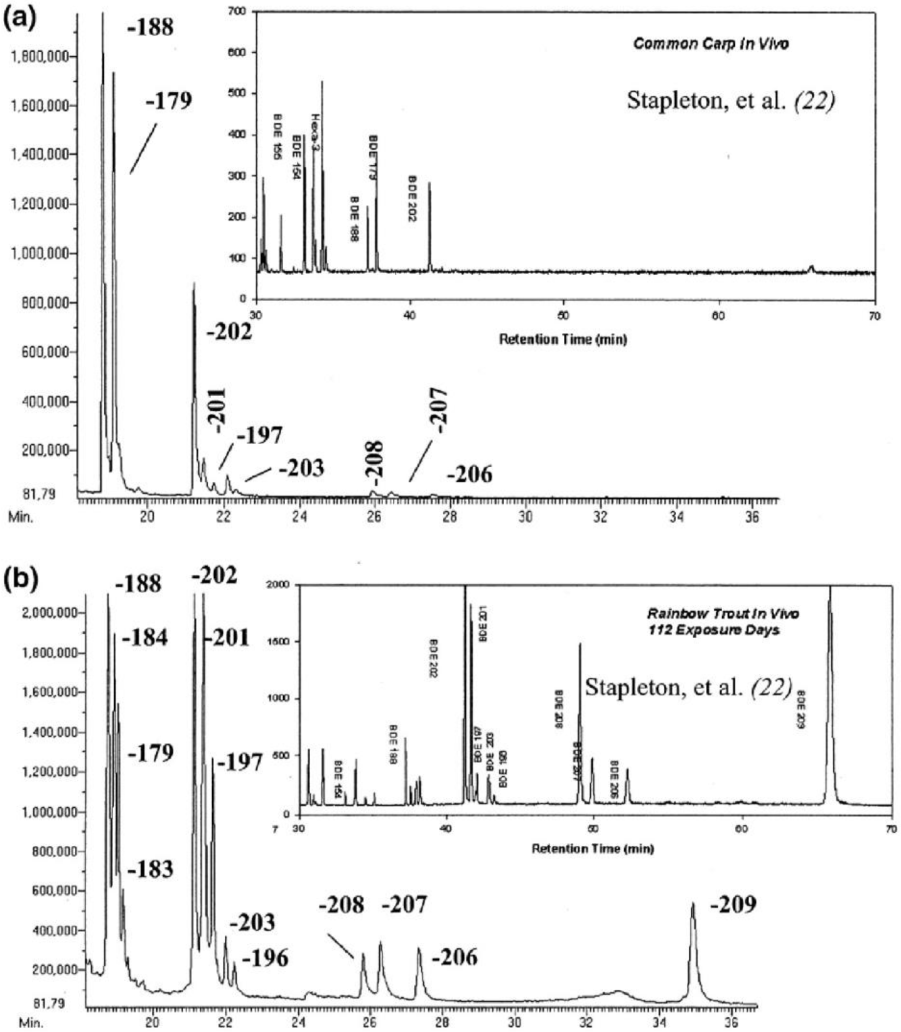

FIGURE 3. (a) ECNI-SIM (*m/z* 79, 81) chromatogram of PBDEs in Roxboro creek chub compared to common carp BDE-209 laboratory exposure study. (b) ECNI-SIM (*m/z* 79, 81) chromatogram of PBDEs in Roxboro sunfish compared to rainbow trout BDE-209 laboratory exposure study.

a 92% decrease was detected (Table 1). Concentrations of tri- through octa-BDEs also increased with distance from the outfall (Table 1). Maximal concentrations for these congeners in 2002 and 2005 in sediments 5.6 km downstream were 14.2 and 13.7 mg/kg (%TOC), respectively. Nona- and deca-BDEs levels in 2002 showed high levels at the outfall and a maximum at 1.3 km downstream (Table 1). In 2005, the 0 and 1.3 km downstream levels were lower, but the 5.6 km sample was higher. One explanation would be a decrease in releases from the WWTP in later years or downstream transport of an episodic deca release. Periodic releases of untreated sewage from this WWTP have been reported due to storm events. Further downstream (10.8 km) tri- through octa-BDEs concentrations were lower, while the BDE-209 burden was comparable to the 2002 levels. Regardless, tri- through octa-PBDEs only accounted for 3.2% or less of total PBDEs downstream from the outfall (Table 1). Others have also reported similar PBDE contributions in -209 rich sediments (>7000 μg/kg, d.w.) and attributed the less brominated congeners to commercial penta- formulations (*32, 33*). Congener profiles for the tri- through hexa-PBDEs were comparable for Marlowe/Storys sediments collected in 2002 and 2005, at the outfall and 1.3, 5.6, and 10.8 km downstream (Figure 2). BDE-47 and -99 were the major PBDEs detected and profiles generally resembled the penta-technical formulation (e.g., DE-71). Congener profiles were also similar to sludge collected from the Roxboro WWTP (the probable source of PBDEs to Marlowe/Storys Creek), indicating that minimal -209 debromination has occurred in surficial sediments along the 10.8 km creek system.

Biota. Two fish species (sunfish (*Lepomis gibbosus*, composite of 13 individuals) and creek chub (*Semolilus atromaculatus*, composite of six individuals)) and a crustacean (crayfish (*Cambarus puncticambarus sp.c*, composite of five individuals)) were collected at the outfall in 2002. In 2005, only sunfish (composite of 22 individuals) were available there. A total of 23 PBDEs were detected in the biota samples, ranging from tri- to deca- PBDEs (Table 2). BDE-47, -153, four octa-BDEs (BDE-202, -201, -203, and -196) and three nona-BDEs (BDE-208, -207, and -206) were detected in each of the biota samples. BDE-209 was observed in two of the fish samples and was only exceeded concentration-wise by the -47, -99, and -153 (Table 2). BDE-209 was also detected in the crayfish composite (21 600 μg/kg, l.w.). Interestingly, this is an order of magnitude higher than the sunfish sample (2880 μg/kg, l.w.) (Table 2). To our knowledge, this is the first report of PBDEs in crayfish. PCBs bear some structural similarities to PBDEs and have been previously reported from the river Meuse (Netherlands) in crayfish (*Orconectus limosus*). Congener profiles followed those in fish, favoring tri- through hexa-PCBs (*34*). Holmqvist et al. (*35*) compared PCBs in crayfish (*Pacifastacus leniuscukus*) from Swedish streams and lakes and found greater variability in total PCBs in stream crayfish. This was attributed to crayfish being omnivorous and hence more influenced by diverse contaminant sources within their catchment. BDE-209 accounted for 95% of the PBDEs in sediments where the Roxboro crayfish were collected. Sediment-associated dietary items likely contributed to the high BDE-209 contribution, 59% of total PBDEs. Hence benthic invertebrates could serve as a route of -209 exposure to aquatic and terrestrial predators.

BDE-47 was the second most dominant congener reported. It was lowest in crayfish (4110 ug/kg l.w.) and highest in chub (17 200 μg/kg, l.w). BDE-47 has previously been reported as the most abundant congener in Virginia fish, ranging from 45% of total PBDEs in channel catfish to 74% in carp (*20*). Congener profiles for tetra- through hexa-BDEs in the two Roxboro sunfish composites were more comparable to the penta- technical mixture, i.e., exhibited similar BDE-47 and -99 contributions.

The congener profile for the chub sample was devoid of BDE-99 and -154 concentrations were elevated compared to -153. This same congener profile was previously reported in common carp (*Cyprinus carpio*) collected from the Dan and Roanoke Rivers (*20*). Stapleton et al. (*19*) exposed common carp to BDE-99 and -183 via the diet and reported significant conversion of BDE-99 to -47 and -183 to -154. Neither congener (BDE-99 or -183) were detected in the Roxboro chub, but were observed in surrounding sediments and in other fish species at this site. The creek chub (*Semotilus atromaculatus*) and common carp (*Cyprinus carpio*) both belong to the same family (*Cyprinidae*) and may metabolize PBDEs similarly. Hence varying biotransformation capacities may contribute to the different PBDE congener profiles observed between the various fish species, as well as the crayfish.

Although BDE-209 was only detected in the 2002 sunfish, both sunfish composites did contain three nona-PBDEs. They also contained five octa-PBDEs and four hepta-PBDEs. The chub composite contained three nona-, four octa-, and two hepta- PBDEs. Of these, two octa- (BDE-201, -202) and three hepta- (BDE-188, -184, and −179) congeners were not detected in either the sludge or sediment samples. Therefore, these conceivably could be metabolic debromination products of the higher brominated PBDEs (e.g., BDE-209). In a follow-up to their initial carp exposure study, Stapleton et al. (*22*) conducted a BDE-209 dietary exposure experiment using juvenile common carp and rainbow trout (*Oncorhynchus mykiss*). After 60 days of exposure (112 days for the trout), carp whole body homogenates were extracted and analyzed for PBDEs. BDE-209 was not detected in the carp sample. However, one octa- (BDE-202), two hepta- (BDE-179 and -188) and three hexa-PBDEs (BDE-154, -155 and one unidentified hexa-BDE) were reported, indicating that carp can metabolize -209 to lower brominated diphenyl ethers. As noted previously, common carp may metabolize PBDEs similarly to creek chub. Chromatograms of the lab-exposed carp and the chub from Marlowe/Storys Creek exhibit comparable hepta- through deca- congener patterns (Figure 3a). PBDEs present were identified as one octa- (BDE-202) and two hepta- (BDE-188 and -179). These were not detected in the sediments or sludge.

In the Stapleton et al. (*22*) rainbow trout dietary BDE-209-exposure study, -209 was detected, as well as several hepta- (BDE-188, -184, 179, and -183), octa- (BDE-202, -201, -204/197, -203, and -196) and nona- PBDEs (BDE-208, -207, and -206). Uptake of -209 from food was estimated at only 3.2% (*22*). Although rainbow trout are from the Family *Salmonidae* and sunfish are *Centrachidae*, the same PBDEs (hepta- through deca-PBDEs) were detected (Figure 3b). Similar nona- through hepta-PBDEs were also detected in the 2005 sunfish sample, but -209 was not observed. Although it is likely exposure conditions varied between the in-lab -209-exposure study and at the Roxboro outfall, congener profiles for the trout and sunfish (carp and chub) were similar, suggesting analogous metabolic pathways. Stapleton et al. (*22*) confirmed that -209 debromination was performed by the fish itself by using a preparation of both trout and carp liver microsomes (*22*). These findings support the current study's conclusion that -209 is bioavailable and can undergo metabolic debromination in the field, resulting in the production of lower brominated PBDEs. Hence continued deca-BDE use may lead over time to increased PBDE burdens in organisms living in aquatic environments and terrestrial animals.

Acknowledgments

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Supporting Information Available

Additional details and Tables ST1, ST2 and ST3. This material is available free of charge via the Internet at http://pubs.acs.org.

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In Situ Accumulation of HBCD, PBDEs, and Several Alternative Flame-Retardants in the Bivalve (*Corbicula fluminea*) and Gastropod (*Elimia proxima*)

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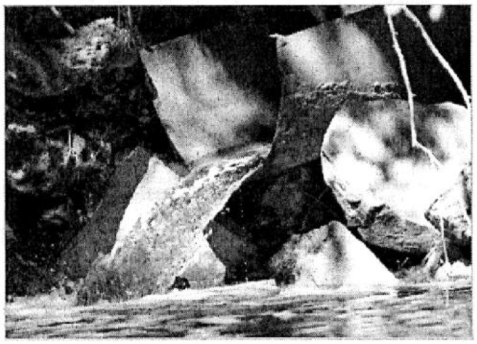
Supporting Information

ABSTRACT: Alternative brominated flame-retardants (BFRs), 2-ethylhexyl 2,3,4,5-tetrabromobenzoate (TBB), 2-ethylhexyl 2,3,4,5-tetrabromophthalate (TBPH), 1,2-*bis*(2,4,6-tribromophenoxy) ethane (BTBPE) and decabromodiphenyl ethane (DBDPE), are now being detected in the environment. However, contaminant bioavailability is influenced by the organisms' ecology (i.e., route of uptake) and in situ environmental factors. We observed that the filter-feeding bivalve (*Corbicula fluminea)* and grazing gastropod (*Elimia proxima*), collected downstream from a textile manufacturing outfall, exhibited TBB, TBPH, and BTBPE concentrations from 152 to 2230 ng g^{-1} lipid weight (lw). These species also contained additional BFRs. Maximum levels of total hexabromocyclododecane diastereomers ($\sum$HBCDs) in these species were 363 000 and 151 000 ng g^{-1} lw, and those of polybrominated diphenyl ethers ($\sum$PBDEs) were 64 900 and 47 200 ng g^{-1} lw, respectively. These concentrations are among the highest reported to date worldwide. While BDE-209 was once thought to be nonbioavailable and resistant to degradation, it was the dominant BFR present and likely debromination products were detected. Contributions of α- and β-HBCD were higher in tissues than sediments, consistent with γ-HBCD bioisomerization. Mollusk bioaccumulation factors were similar between HBCD and PBDEs with 4 to 6 bromines, but factors for TBB, TBPH, and BTBPE were lower. Despite different feeding strategies, the bivalves and gastropods exhibited similar BFR water and sediment accumulation factors.



INTRODUCTION

Brominated flame retardants (BFRs) are commonly added to constituent polymers in electronics, furniture, and textiles. With regard to the environmental properties and distribution, polybrominated diphenyl ethers (PBDEs) have been the most studied. Due to their bioaccumulation potential, persistence, and associated detrimental health effects following exposure, manufacturing of two of three PBDE formulations (i.e., Penta- and Octa-BDE) were discontinued in the U.S. in December 2004 [1] and added to the Persistent Organic Pollutants list of the Stockholm Convention in May 2009.[2] In July 2008, the third and most widely used PBDE formulation, Deca-BDE, was banned in electrical equipment in the EU [3] and will be phased out of U.S. production by the end of 2013.[4] A fourth BFR, hexabromocyclododecane (HBCD), primarily used in polystyrene insulation boards and to a lesser extent in textiles, is also considered persistent and bioaccumulative and may cause reproductive, developmental, and neurological disorders.[5,6] Under the EU's REACH program, HBCD will be phased-out of European commerce by mid-2015. In the U.S., HBCD is now under evaluation by the U.S. EPA.[7] In order to meet fire safety standards, new consumer products will need to contain alternative flame retardants. However, the environmental fate of many replacement products remains inadequately investigated.[8] Recently, several Penta- and Octa-BDE replacement products (2-ethylhexyl 2,3,4,5-tetrabromobenzoate (TBB) and 2-ethylhexyl 2,3,4,5-tetrabromophthalate (TBPH), components of Firemaster-550 (Chemtura Corp., USA) and 1,2-*bis* (2,4,6-tribromophenoxy) ethane (BTBPE), a component of Firemaster-680 (Chemtura Corp., USA)) were observed in household dust, sewage sludge, aquatic biota, and sediments.[8−10] Decabromodiphenyl ethane (DBDPE), offered as an alternative to Deca-BDE, has similar chemical structure and properties. Like Deca-BDE, DBDPE has also been detected in sewage sludge, air, sediments, fish and birds.[11,12] This compound, along with TBB, TBPH, and BTBPE, has also been reported in house and office dust.[13]

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Table 1. PBDEs, Alternative BFRs and HBCD Concentrations in Sediments and Mollusks (*Corbicula fluminea* and *Elimia proxima*)

sample matrix		sediments (ng g^{-1} TOC)				*Corbicula fluminea* (ng g^{-1} lipid)				*Elimia proxima* (ng g^{-1} lipid)			
location site [distance downstream of outfall (km)]		outfall	16.8 km	25.2 km	44.6 km	outfall	16.8 km	25.2 km	44.6 km	outfall	16.8 km	25.2 km	44.6 km
polybrominated diphenyl ethers (PBDEs)													
BDE-17	2,2′, 4-tri BDE	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
BDE-28	2,4, 4′-tri BDE	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
BDE-47	2,2′, 4,4′-tetra BDE	4470	699	309	123	5230	2980	2650	1592	8660	795	958	601
BDE-49	2,2′, 4,5′-tetra BDE	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
BDE-66	2,3′, 4,4′-tetra BDE	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
BDE-100	2,2′, 4,4′, 6-penta BDE	602	159	174	68	2030	1730	1060	804	2040	277	291	106
BDE-99	2,2′, 4,4′, 5-penta BDE	6870	1290	901	153	4080	2600	2500	1448	10 600	821	1420	518
BDE-85	2,2′, 3,4,4′-penta BDE	120	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
BDE-154	2,2′, 4,4′, 5,6′-hexa BDE	70	7	17	nd	113	56	nd	40	432	24	53	nd
BDE-153	2,2′, 4,4′, 5,5′-hexa BDE	301	112	121	nd	403	128	140	70	682	nd	nd	nd
BDE-183	2,2′, 3,4,4′, 5,6-hepta BDE	146	20	17	nd	nd	nd	nd	nd	nd	nd	nd	nd
BDE-202	2,2′, 3,3′, 5,5′, 6,6′-octa BDE	nd	nd	nd	nd	56	32	nd	nd	nd	nd	7	nd
BDE-201	2,2′, 3,3′, 4,5,5′, 6′-octa BDE	19	nd	14	nd	47	31	nd	10	nd	nd	6	nd
BDE-197	2,2′, 3,3′, 4,4′, 6,6′-octa BDE	100	37	38	nd	51	26	nd	nd	52	nd	8	nd
BDE-203	2,2′, 3,4,4′, 5,5′, 6-octa BDE	380	120	146	nd	111	47	nd	nd	79	nd	10	nd
BDE-196	2,2′, 3,3′, 4,4′, 5,6-octa BDE	554	136	153	283	99	35	nd	nd	70	nd	11	nd
BDE-208	2,2′, 3,3′, 4,5,5′, 6,6′-nona BDE	1300	559	1030	363	1220	111	78	20	341	45	73	19
BDE-207	2,2′, 3,3′, 4,4′, 5,6,6′-nona BDE	3860	1360	2140	731	2740	325	270	54	752	103	167	59
BDE-206	2,2′, 3,3′, 4,4′, 5,5′, 6-nona BDE	12 500	3160	7690	1070	4990	411	347	75	751	114	211	76
BDE-209	decabromodiphenyl ether	298 000	169 000	215 000	61 500	43 700	11 400	12 000	2388	22 700	2700	2500	2200
NBFRs: alternatives to Penta- and Octa-BDEs													
TBB	2-ethylhexyl 2,3,4,5-tetrabromobenzoate	3850	598	326	80	2220	nd	nd	nd	1740	nd	nd	nd
TBPH	di (2-ethylhexyl)-2,3,4,5-tetrabromophthalate	19 200	3120	3570	2000	1370	816	nd	37	380	nd	99	36
BTBPE	1,2-bis (2,4,6-tribromophenoxy) ethane	2000	223	229	77	153	112	nd	nd	303	nd	nd	nd
% rec. BDE-166	2,3, 4,4′, 5,6-hexa BDE	95%	129%	108%	87%	121%	120%	85%	93%	88%	71%	85%	88%
hexabromocyclododecane (HBCD)[a]													
α-HBCD	α-1, 2,5,6,9,10-HBCD	16 400	2250	2410	351	43 900	16 900	9400	***	15 200	8160	5390	4020
β-HBCD	β −1,2,5,6,9,10-HBCD	35 300	4590	3840	641	109 000	35 400	32 100	***	28 600	11 900	6500	4530
γ-HBCD	γ −1,2,5,6,9,10-HBCD	338 000	76 500	46 000	11 200	210 000	26 800	77 900	***	107 000	34 800	23 900	12 800
% rec C13-α-HBCD	α-1, 2,5,6,9,10-HB[$^{13}C_{12}$]CD	75%	61%	87%	93%	87%	100%	108%	***	74%	70%	67%	77%
%TOC or % lipid (dry weight based)		1.10%	1.60%	0.61%	0.41%	10.90%	10.45%	14.67%	11.89%	7.83%	6.00%	10.13%	9.29%

[a]*** Sample compromised and not analyzed. nd = not detected.

Toxic effects are mediated by contaminant bioavailability, which is controlled by the properties of the chemical and in situ environmental factors. Bioaccumulation in the real world is influenced by many factors. Their interplay may be too complex for simple laboratory studies to encompass. For example, lipophilic compounds (e.g., PBDEs) partition into organic-rich sediments. Benthic organisms that ingest or are in close contact with such sediments may be at greater risk of exposure. However, contaminants accumulated by such biota will eventually be redistributed to others via predation, scavenging, and other mechanisms. In freshwater environments mollusks constitute one of the largest groups, in terms of biomass, and serve as an important food source for aquatic and terrestrial predators. Over 650 species of gastropods and 300 species of bivalves inhabit freshwater systems in North America. Major molluscan feeding strategies include periphytan/detritus grazing/shredding (e.g., many gastropods) and filter-feeding (e.g., many bivalves). Bivalves have been long utilized for in situ monitoring of chemical body burdens (e.g., NOAA's Mussel Watch Program). However, 71% of native U.S. freshwater mussels are currently at risk of extinction.[14] Gastropods have also been used with increasing frequency for contaminants and effects monitoring and some are particularly sensitive to impacts such as imposex.[15] As an alternative to sacrificing native species, sampling of non-native invasive species, e.g., asian clams (*Corbicula sp.*), has been suggested for chemical exposures studies. Past studies have examined acute and chronic exposures to single and multichemical stressors including metals, surfactants, pesticides, and industrial or municipal effluents in the laboratory and the field.[14] While *Corbicula* spp. is native to Africa, Australia, and Asia, they have become established in parts of Europe and 41 U.S. states, including Hawaii.[16] This wide distribution offers the opportunity for in situ regional, continental, and intercontinental biomonitoring research using a single test organism.

On the basis of data from the U.S. EPA's Toxics Release Inventory (TRI), we previously identified a North Carolina textile-manufacturer as a likely substantial contributor of Deca-BDE to a municipal wastewater treatment plant (WWTP) and local surface waters.[17] We hypothesized that replacement BFRs may also be used at this facility and accumulate in local sediments and mollusks (e.g., *Corbicula fluminea* and *Elimia proxima*). The BFRs examined here included: HBCD, constituent congeners of the Penta-, Octa-, and Deca-BDE formulations and several PBDE alternatives (i.e., TBB, TBPH, BTBPE, and DBDPE). While laboratory-derived bioaccumulation factors are valuable for evaluating uptake potential, use of in situ exposed organisms allows consideration of bioaccumulation potential under environmentally realistic conditions. Hence, our goal was to evaluate in situ bioaccumulation of PBDE, HBCD, and alternative BFRs in two benthic invertebrates (*Corbicula fluminea* and native *Elimia proxima*) with differing feeding strategies (i.e., grazing vs filter feeding).

EXPERIMENTAL SECTION

The bivalve *Corbicula fluminea*, gastropod *Elimia proxima* and surficial sediments were collected in July 2009, from the Yadkin River, downstream of a North Carolina WWTP outfall. This treatment facility was constructed in 1968 to service a local textile mill. This WWTP was upgraded in 2003 to allow treatment of up to 16 million liters per day. According to their National Pollutant Discharge Elimination System (NPDES) permit this facility is owned and operated by a local textile manufacturer and treats ~92% industrial process wastewater and ~8% domestic sewage. Treatment includes bar and fine screening, aeration, dual clarifiers, aerobic digesters, and sludge drying beds. Mollusks and sediments were obtained at the outfall, 16.8, 25.2, and 44.6 km downstream and 0.2 km upstream (sediment only) of the outfall. Samples were collected in precleaned 1 L glass jars with Teflon lids and stored at <4 °C. At the laboratory, mollusks were rinsed with reagent grade water, shells were removed, and tissue was placed into stainless steel pans. Sediment and tissue were freeze-dried and stored at <0 °C until analyzed. Two sediment aliquots from each collection site were analyzed for total organic carbon (TOC). TOC means reported in Table 1, %RSD range 5–37%. TOC was determined by combustion and infrared detection (CE-440, Exeter Analytical, North Chelmsford, MA).

For BFR analysis dried samples (20 g sediment and ~2 g tissue) were subjected to accelerated solvent extraction (Dionex ASE 200, Sunnyvale, CA) with methylene chloride. Surrogate standards (200 ng of 2,3,4,4', 5,6-hexabromodiphenyl ether (BDE-166); Cambridge Isotope Laboratories, Inc., Andover, MA and 200 ng of ^{13}C-labeled α-HBCD; Wellington Laboratories, Ontario, Canada) were added to each sample prior to extraction. Extracts were purified by size exclusion chromatography (SEC, Envirosep-ABC, 350 × 21.1 mm. column; Phenomenex, Torrance, CA). Each post-SEC extract was reduced in volume, added to the top of a solid phase 2-g silica glass extraction column (Isolute, International Sorbent Tech.; Hengoed Mid Glamorgan, U.K.) and eluted with 3.5-mL hexane (fraction one), followed by 6.5 mL of 60:40 hexane/DCM and then 8 mL DCM (fraction two). The second fraction, containing the compounds of interest, was reduced in volume and solvent exchanged to hexane. Decachlorodiphenyl ether (DCDE; 100 ng; Ultra Scientific, North Kingstown, RI) was then added as the internal standard and the extracts were analyzed for PBDEs, TBB, TBPH, BTBPE, and DBDPE. Identification and quantitation were done by gas chromatography-selective ion monitoring mass spectrometry (GC/SIM–MS). For HBCD analysis, extracts were solvent exchanged to methanol and spiked with 100 ng of d18-labeled α-HBCD (Wellington Laboratories, Ontario, Canada), as an internal standard. These were then analyzed for α-, β-, and γ- isomers by ultraperformance liquid chromatography tandem mass spectrometry (UPLC–MS/MS). Surrogate recoveries and method blanks were evaluated along with sample analyses. Results were surrogate recovery corrected. Recoveries ranged from 71 to 129% for BDE-166 and 61 to 108% for ^{13}C-α-HBCD (Table 1). Blanks did not exhibit any targeted compounds above the quantitation limit (QL) of 1 ng g^{-1}, dw. (Details of instrumentation and operating conditions for GC/SIM-MS and UPLC–MS/MS, along with quality assurance procedures and BFR matrix-spiking experiments and results can be found in the Supporting Information, SI, section. Spiking results are listed in Table S1.) Statistical analyses were performed using SigmaStat for Windows, version 2.0. Analysis of variance (ANOVA) was used to examine differences ($P < 0.05$) between bivalve and gastropod BFR bioaccumulation factors (BAFs).

RESULTS AND DISCUSSION

Polybrominated Diphenyl Ethers. PBDEs were not detected above the 1 ng g^{-1} (dw) QL in sediments from the site 0.2 km upstream from the WWTP outfall. However, sediments at the outfall exhibited 16 of 20-targeted PBDE congeners, totaling 329 000 ng g^{-1} (normalized to TOC)

(Table 1). This concentration rivals those in sediments from the highly industrialized and populated Pearl River Delta (PRD), P.R. China, previously reported as the highest concentration worldwide.[18,19] Concentrations of $\sum$PBDEs detected in *Corbicula fluminea* and *Elimia proxima* collected at the outfall were also considerable, totaling 64 900 and 47 200 ng g^{-1} lipid weight (lw), respectively (Table 1). BDE-17, -28, -49, and -66 were not detected. PBDE sediment and mollusk profiles were dominated by congeners of the Deca-formulation and their $\sum$PBDEs decreased with distance downstream from the outfall. However, there was a notable spike in $\sum$PBDEs sediment concentration at the 23.2 km site, i.e., 228 000 ng g^{-1} TOC. This pattern was also observed in the gastropods and bivalves and may have resulted from historical changes in local industrial BFR release rates, or past river flow events (e.g., flooding or WWTP pass through). Chen et al. [20] recently documented a temporal decrease in PBDE concentrations in finfish in another North Carolina riverine system, the Hyco that exhibited extensive PBDE contamination. There, the PBDEs were believed to have entered via a municipal WWTP, following release from textiles and plastics manufacturing. PBDEs were also detected in sediments and mollusks at our most downstream station, 44.6 km from the outfall (Table 1). Concentrations at this site also exceeded those of sediment and marine mussels (*Perna viridis*) of the PRD.[19]

Relative abundance of PBDE congeners in sediments and mollusks in our study were BDE-209 $\gg -206 > -99, -207, -47 > -208$ and -100. The major constituent congener of Deca-BDE,[21] BDE-209, contributed 90 to 96% of the sediment total PBDE concentration. According to the U.S. EPA's TRI the on-site wastewater treatment facility released 6000 kg of BDE-209 to surface waters between 2000 and 2004.[17] However, TRI estimates are often not based on actual chemical measurements. Hence, their accuracy is uncertain. The log K_{ow} of BDE-209 exceeds 12 (Table S1 of the SI) indicating extreme hydrophobicity. In surface waters and wastewater streams it is largely particulate bound. In WWTPs, BDE-209 will primarily reside in sewage sludge.[22] However, treated effluents also contain entrained solids. These may be released to receiving streams and any PBDE-laden particles may eventually become incorporated in sediments. Sediments in proximity to wastewater outfalls have previously been observed to be dominated by BDE-209.[23,24] In the present study, BDE-209 was the major PBDE congener detected in both bivalves and gastropods collected at the outfall. However, the percent contribution of BDE-209 to total PBDEs was lower (37 to 67%) than in sediments. This likely relates to its strong association with particulates and attendant lower bioavailability. Moon et al. [25] reported a similar percentage contribution of BDE-209 in marine bivalves from Korean waters. In contrast, BDE-47, -99, and -100 contributed 18 to 59% of total PBDEs in these mollusks, while their contributions in sediments ranged from only 0.5 to 3.6%. This greater bioaccumulation of the lower brominated diphenyl ethers in mollusks is likely due to their intermediate log K_{ow} (<8). Chen et al. [20] reported that BDE-209 contributed less than 1% to the $\sum$PBDE in several finfish species from the Hyco River. The lower BDE-209 in finfish may be due in part to its metabolic debromination to lower brominated PBDEs (e.g., BDE-154 and -202) or decoupling of direct exposure from the sediments. This phenomenon has also been previously reported in finfish by others.[26,27]

Alternative BFRs: TBB, TBPH, BTBPE, and DBDPE. In light of market pressures and regulatory actions to reduce PBDE usage, BFR manufacturers have promoted alternatives to meet mandated fire safety standards. Of the four replacements sought, TBB, TBPH, and BTBPE were detected in sediments at the textile WWTP outfall, at 3850, 19 200, and 2000 ng g^{-1} TOC, respectively (Table 1). None of these substitutes were detected (>1 ng g^{-1}, dw) in sediments collected at our upstream site. DBDPE was not detected in any of our mollusk or sediment samples. Its absence may be due to the continued usage of Deca-BDE or the use of other alternatives, e.g., HBCD. Chen et al. [20] reported an increase in the abundance of HBCDs in finfish at another North Carolina riverine site impacted by textile-related discharges. We detected TBB, TBPH, and BTBPE in all of the sediments collected downstream from the WWTP outfall. At the 44.6 km sampling site sediment concentrations were 80, 2000, and 77 ng g^{-1} TOC, respectively. Four formulations of TBB, TBPH, and BTBPE are manufactured by Chemtura Corp. USA. The product FF-680 consists of 100% BTBPE by weight according to the manufacturer's Material Safety Data Sheet (MSDS). Firemaster-550 (FM-550), Firemaster BZ-54 (BZ-54), and DP-45 are mixtures of TBB and TBPH. Of these three formulations, only DP-45 lists compositional details on its MSDS sheet, i.e., >91% TBPH and 2−6% TBB, by weight. Components of the other two are designated as trade secrets. However, FM-550 and BZ-54 have been reported to contain TBB and TBPH.[9,28] According to Stapleton et al. [9] FM-550s TBB to TBPH ratio is 4:1. FM-550 also is reported to contain isopropylated triaryl phosphate and triphenylphosphate at approximately 50% by weight.[9] In the sediments collected at the outfall and downstream, TBPH was the primary alternative BFR we detected, comprising 77 to 93% of the total. On the basis of the available product information, this suggests that manufacturers in this area may have used primarily DP-45. However, TBPH has been reported to be less vulnerable to photodegradation and to possess a half-life twice that of TBB.[28] The change in the TBPH/TBB ratio from 5:1 at the outfall to 25:1 at the 44.6 km downstream site may result from degradative processes. However, local BFR product usage and composition may also be factors.

TBB, TBPH, and BTBPE were also detected in the bivalve and gastropod samples collected at the outfall (Table 1). In these biota, TBB was the most abundant alternative BFR detected (2220 and 1740 ng g^{-1} lw in bivalves and gastropods, respectively), followed by TBPH (1370 and 380 ng g^{-1} lw) and BTBPE (153 and 303 ng g^{-1} lw). TBB has a less extreme log K_{ow} (8.75) than BTBPE (9.15) or TBPH (11.95) (Table S2 of the SI) which suggests a greater bioaccumulation potential. Interestingly, TBB was only detected in the mollusk samples collected at the outfall, but BTBPE and TBPH were also observed in the bivalves collected at the 16.8 km downstream site (112 and 816 ng g^{-1} lw, respectively). TBPH was detected in both bivalve and gastropod samples from the 44.6 km site, 37 and 36 ng g^{-1} lw, respectively. The sediment concentration of TBPH was 25-fold higher at this site than TBB or BTBPE.

Hexabromocyclododecane. Data on HBCD levels in the North American environment, and the U.S. in particular, are limited. While its use here is generally assumed to be lower than in the EU, this presumption is based on outdated 2001 market demand statistics. These indicated that in North America HBCD consumption is less than 10% of PBDEs and constitute less than 17% of the global HBCD market demand.[29] However, 2006 U.S. production/import volumes were between 4535 and 22 679 tonnes.[30] This represents a 2- to 10-fold increase in HBCD demand from 2001 to 2006. It is possible that current North American HBCD consumption may now rival that of the EU (9840 tonnes for 2010).[31] In our outfall sediment ΣHBCD (α-, β-, and γ-HBCD) was the most abundant BFR at 390 000 ng g^{-1} TOC, total (Table 1). ΣHBCD was also detected at every collection site downstream from the outfall, ranging from 88 300 to 12 200 ng g^{-1} TOC. However, HBCD was not detected (>1 ng g^{-1}, dry weight) at the upstream site. The technical HBCD formulation consists of three diastereomers: γ-HBCD isomer (75−89%), followed by β-HBCD (10−13%) and α-HBCD (1−12%).[5] However, HBCD-treated finished products (e.g., insulation boards and textiles) may contain higher proportion of the α-diastereomers than that of commercial HBCD mixtures.[32,33] It has been reported that γ-HBCD can isomerize at temperatures above 160 °C, forming α-HBCD, e.g. during polystyrene production; and the drying and curing of treated textiles.[33] Isomerization of β- and γ- to α-HBCD has also been observed in aquatic organisms.[11] HBCD in most marine mammals has been reported to be >80% α-HBCD.[32,34] The isomer composition reported in house dust [presumably released from finished products, e.g., building materials (EPS and XPS) and textiles] is 33% α-, 11% β- and 56% γ-HBCD.[35] Similar HBCD isomer profiles were observed in sewage sludge (42% α-, 6% β-, and 52% γ-HBCD) from a municipal WWTP that received wastewater from an automotive interiors manufacturer.[10] Differing degradation rates between isomers have also been reported for sewage sludge and sediments.[36,37] For example, Davis et al. [37] observed β- and γ-HBCD degradation rates in sludge 28% to 47% lower than those in freshwater sediments. However, the γ-isomer is typically the most abundant HBCD stereoisomer reported in sediments.[32,34] In our sediment sample, γ-HBCD represented >87% of $\sum$HBCD set, (Table 1). The release of the technical HBCD product (75−89% γ-HBCD) to waste streams by industries that utilize HBCD in their products and from HBCD production and processing facilities may explain this pattern.[20] However, γ-HBCD's 2-fold greater degradation rate contrasts with the γ-HBCD-dominated sediment isomer profiles observed tens of km downstream from the presumed sources.

ΣHBCD was also the most abundant BFR detected in the biota samples. At the outfall ΣHBCD concentrations in the gastropods and bivalves were 151 000 and 363 000 ng g^{-1} lw, respectively. At the 44.6 km site, the ΣHBCD concentration in gastropods was 21 400 ng g^{-1} lw. (Unfortunately, the bivalve sample from the 44.6 km site was compromised during HBCD sample preparation and was not analyzed.) The dominant diastereomer, γ-HBCD, constituted 34 to 71% of ΣHBCD in every biota sample (except for the bivalve sample collected at the 16.8 km site). Although their log K_{ow}s [range 5.07−5.47 (Table S2 of the SI)] would indicate similar bioavailability, the percent contribution of α- and β-HBCD compared to γ-HBCD was higher in the mollusks (29−66%) than in the sediments (8.1−13%) at these collection sites. This may reflect biologically mediated isomerization of γ-HBCD in aquatic invertebrates.[32] Alternatively, it may relate to lower bioavailability of γ-HBCD, which exhibits the lowest water solubility of the three diastereomers (i.e., 48, 15, and 2 μg L^{-1} for α-, β- and γ-HBCD, respectively [38]).

Biota-Sediment Accumulation Factors (BSAFs). The BSAF is a useful tool for assessing the bioaccumulation of lipophilic compounds in aquatic environments. It is defined as follows: BSAF = C_t /C_s; where C_t = a substance's lipid-normalized steady-state concentration in tissue of an aquatic organism and C_s = its sediment organic carbon-normalized concentration. A BSAF exceeding 1.0 implies that bioaccumulation has occurred.[39] Laboratory-derived BSAF measurements are often used to predict tissue concentrations in wildlife. However, since ecosystems are complex and rarely at steady state, BSAFs in the environment often vary more than laboratory-determined estimates.[40,41] Feeding strategies and biotransformation capabilities may further impact BSAFs. However, strong correlations from site- and species-specific determinations have been observed between log transformed BSAF and log n-octanol/water partition coefficient (log K_{ow}) values for nonionic halogenated organic compounds (e.g., PBDEs). These often exhibit a parabolic behavior, with maximum BSAF values for compounds with log K_{ow} values between 6 and 8 (e.g., BDE-47, -99, -100).[42] (See SI Table S2 for log K_{ow} values.)

Log BSAFs determined for the BFRs we detected in *Corbicula fluminea* and *Elimia proxima* ranged from −1.9 to 1.1. A parabolic relationship was observed between log BSAF values and corresponding log K_{ow} values for both mollusks (Figure 1a,b), consistent with previously published observations. However, BASFs for α- and β-HBCD isomers exceeded expectations, suggesting preferential accumulation of α- and β-HBCD in bivalves and gastropods. In contrast, the BASF values for γ- HBCD were <0. Our log BSAF values for the PBDEs

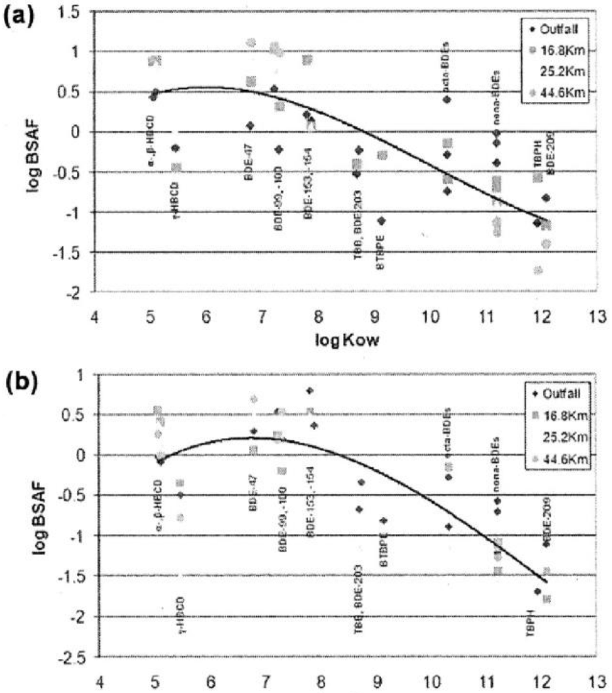

Figure 1. (a) Relationship between log biota-sediment accumulation factors (log BSAF) in *Corbicula fluminea* and log K_{ow} for several flame-retardants. A log BSAF > 0 indicates greater accumulation in tissues than sediments. Solid line indicates best fit polynomial third order, r^2 = 0.677. (b) Relationship between log biota-sediment accumulation factors (log BSAF) in *Elimia proxima* and log K_{ow} for several flame-retardants. A log BSAF > 0 indicates greater accumulation in tissues than sediments. Solid line indicates best fit polynomial third order, r^2 = 0.677.

were approximately half those previously reported [42] for the field-exposed marine mussel (*Modiolus modiolus*); but were closer to reported log BSAF values of PCBs. This may relate to discrepancies in the species assessed or ecological or environmental differences in the two studies. In both studies, however, a parabolic pattern was apparent for the PBDEs, with maximum BSAF values for congeners with log K_{ow} between 6 and 8 (i.e., BDE-47, -99, -100, -153, and -154). At the collection site adjacent to the Yadkin River textile outfall, BSAFs for BFRs with log $K_{ow} < 10$ were lower than those at the other stations; potentially indicating greater bioavailability with distance downstream (Figure 1a). This was also observed by Debruyn et al. [42] for PCBs in mussels collected downstream from a municipal wastewater outfall. In our study, BFRs with log K_{ow} values >8 (PBDEs with greater than seven bromines, TBB, TBPH, and BTBPE) exhibited negative log BSAFs, suggesting low bioavailability for both bivalves and gastropods. Despite differences in feeding strategies, a strong correlation was observed for log BSAF values between bivalves and gastropods (r^2 of 0.772, slope 0.875) (Figure S1 of the SI), indicating similar BFR accumulation in these mollusks (ANOVA, $P > 0.05$).

Bioaccumulation Factors (BAFs). The BAF_w is defined as the ratio of a substance's lipid-normalized concentration in an aquatic organism (C_t) to its organic carbon-normalized concentration in sediment pore water (C_w), i.e., $BAF_w = C_t / C_w$.[39] Sediment pore water concentrations (C_w) may be estimated from $C_w = C_s/f_{oc}K_{oc}$, where f_{oc} is the organic carbon fraction of the sediment. (Organic carbon–water partition coefficients (K_{oc}) are listed in Table S2 of the SI.) Hydrophobic compounds (compounds with log $K_{oc} > 4$) in the aquatic environment partition preferentially into sediment organic matter. If the log BAF_w of a chemical is equal to its log K_{oc}, then partitioning is considered to be controlled by passive diffusion. This is indicated by the dotted-line in Figure 2. However, the composition of the organic matter (e.g., black carbon), as well as environmental conditions (e.g., salinity), can also influence the bioavailability of chemicals.[43,44] Lower than predicted accumulation was observed in both bivalves and gastropods in our study, i.e., compounds with a log $K_{oc} > 6.9$ (PBDEs with eight or more bromines and TBPH) fell below the indicated dotted line (Figure 2). An exception was BDE-201 in bivalves collected at the outfall. A possible explanation may be in vivo formation of BDE-201 via debromination of higher brominated diphenyl ethers such as BDE-209. Roberts et al. [45] exposed BDE-209 to carp hepatic microsomes which formed BDE-201 and other PBDEs and Ikonomou et al. [46] suggested that mechanisms other than biomagnification may be the cause for the unexpected PBDE congeners they observed in aquatic biota. We also observed a greater than predicted accumulation potential for tetra-, penta-, and hexa-BDEs indicated by their log BAF_w exceeding their log K_{oc} (Figure 2.). Different patterns were also observed for the HBCD isomers (Figure 2). The log BAF_w of γ-HBCD was less than predicted. In contrast, the log BAF_w for α- and β-HBCD suggested higher bioavailability. However, the log K_{oc} of the latter two isomers are slightly lower (<0.4) than that of γ-HBCD. As previously mentioned, α-HBCD can be formed by the bioisomerization of γ-HBCD and this may be a factor in the latter's depletion. The log BAF_w for TBB and BTBPE were also below the predicted accumulation (dotted-line). TBB and BTBPE have log K_{oc} values less than those of tetra-, penta-, and hexa-PBDEs; indicating lower sediment organic carbon associations. While this may also indicate metabolic alteration of these compounds, brominated metabolites of TBB, TBPH, and BTBPE were not observed. However, hydroxylated and debrominated degradates have been observed in BTBPE-dosed laboratory rats.[47] TBB and TBPH have also been reported to undergo photodegradation [28] and possible TBB debromination products have been detected in sewage sludge.[10] As was the case for the BSAFs, the slope of the line comparing BAF_ws between the two mollusk species approximated 1.0 (slope 0.980, r^2 of 0.858) (Figure S2 of the SI), indicating similar BFR accumulations (ANOVA, $P > 0.05$), despite different feeding strategies.

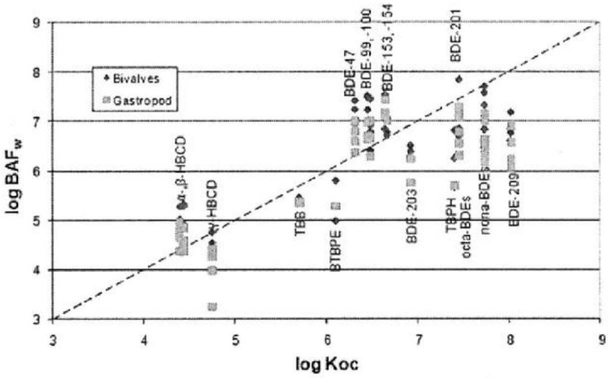

Figure 2. Relationship between log bioaccumulation factors (log BAF_w) and log Koc for various BFRs in *Corbicula fluminea* (bivalve) and *Elimia proxima* (gastropod). Dotted line indicates accumulation according to the equilibrium partitioning theory.

While PBDE congeners with four to six bromines exhibited the highest BSAFs and BAF_ws, PBDEs with 8–10 bromines (e.g., BDE-209) still appeared bioavailable. Due to the substantial local discharge of the highly brominated congeners, they contributed 40 to 82%, by weight, of the $\sum$PBDE body burden of the mollusks. The log BSAFs of α- and β-HBCD were comparable to PBDEs with 4 to 6 bromines (e.g., BDE-47, -99, and -154), although their accumulation may have been augmented by isomerization of γ-HBCD. BSAFs and BAFs for BTBPE, TBB, and TBPH were generally an order of magnitude lower than those of BDE-47, -99, -100, -153, and -154 (constituents of the commercial Penta-formulation) indicating lower potential for accumulation. However, several octabrominated BDE congeners appeared to bioaccumulate. Several of the alternative BFRs appeared to be environmentally persistent, as indicated by their detection (e.g., TBPH 2000 ng g^{-1} TOC) in sediments 44.6 km downstream from their probable point of release. In conclusion, as the usage of HBCD, TBB, TBPH, and BTBPE may be increasing due to recent and pending restrictions on PBDEs, further research is needed on their sources, release mechanisms, environmental fate and distribution, as well as potential biological effects.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Additional details on method development, quality assurance, matrix-spiking experiments, and results along with Tables S1 and S2 and Figures S1 and S2. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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Brominated Flame-Retardants in Sub-Saharan Africa: Burdens in Inland and Coastal Sediments in the eThekwini Metropolitan Municipality, South Africa

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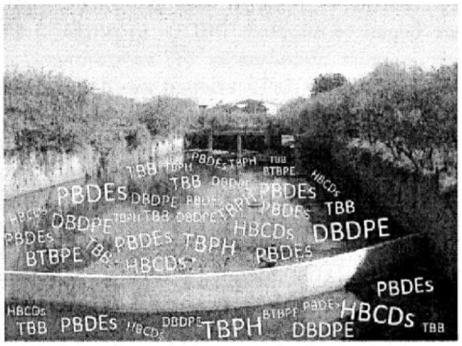
Supporting Information



ABSTRACT: Brominated flame-retardant (BFR) additives are present in many polymeric consumer products at percent levels. High environmental concentrations have been observed near cities and polymer, textile, and electronics manufacturing centers. Most studies have focused on European, North American, and Asian locales. Releases are likely rising most dramatically in countries with weak environmental and human health regulation and enforcement, demand for electrical and electronic equipment (EEE) is escalating, and importation of waste EEE occurs. Several African countries meet these criteria, but little data are available on burdens or sources. To better understand the extent of BFR environmental dissemination in a southern African urban community, inland and coastal sediments were collected in the eThekwini metropolitan municipality, South Africa, and analyzed for polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCD), 2-ethylhexyl 2,3,4,5-tetrabromobenzoate (TBB), 2-ethylhexyl 2,3,4,5-tretabromophalate (TBPH), 1,2-bis (2,4,6-tribromophenoxy) ethane (BTBPE), and decabromodiphenyl ethane (DBDPE). BFRs were detected in all samples ($n = 45$). Concentration data are presented on total organic carbon (TOC) normalized basis. ΣBFR ranged from 114 to 47 100 ng g^{-1}. Decabromodiphenyl ether was detected in 93% of samples (mean concentration 3208 ng g^{-1}) followed by TBB at 91% (mean conc. 545 ng g^{-1}). Durban Bay is strongly influenced by urban runoff and tidal hydrology, and sediments therein exhibited ΣPBDE concentrations ranging from 1850 to 25 400 ng g^{-1} (median conc. 3240 ng g^{-1}). These levels rival those in the heavily impacted Pearl River Delta, China. BFRs likely enter the South African environment during manufacture of BFR-containing products, during and following product use (i.e., after disposal and as a result of materials recycling activities), and from nonpoint sources such as atmospheric fallout and urban runoff. These results underline the need to investigate further the environmental burdens and risks associated with BFRs in developing countries.

INTRODUCTION

South Africa is the world's 28th richest country and Africa's leading economy. In 2011 the gross domestic product (GDP) was $408.2 billion (USD), up 344%.[1] South Africa's industrial base includes substantial automotive, chemical, and telecommunications sectors (representing 6%, 5%, and 7% of GDP, respectively).[2] Along with its expanding economy, South Africa is also in the midst of a consumer product and technological revolution. For example, the number of in-use mobile phones (64 million) now exceeds its population, up from 17% of its adults in 2000 to 76% in 2010.[3] The use of mobile computing (e.g., laptops) is also rising, with an expected annual growth of 20% (>650 000 units sold in 2007).[4] However, as the demand for electrical and electronic equipment (EEE) grows, so does waste EEE (WEEE). It has been predicted that by 2018 developing nations will dispose of more end-of-service computers than developed countries.[5] In addition, nearly 80% of all WEEE generated in developed countries is currently exported to developing nations.[6] WEEE recycling is growing in Africa, facilitated by low labor costs and high demand for raw materials. It is estimated that 1.3 million metric tonnes (MT) of WEEE is disposed annually in the sub-Saharan countries of Benin, Cote d' Ivoire, Ghana, Liberia, and Nigeria.[7] WEEE contains both valuable and hazardous materials. The environmental release of toxicants (e.g., chlorinated dioxins and heavy metals) from unregulated WEEE salvaging has been observed.[8] This is of concern for countries with weak environmental and human health practices. Africa's sub-Saharan region ranked last according to Yale University's 2010 Environmental Performance Index (EPI) of 163 countries on 25 performance indicators covering environ-

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mental public health and ecosystem vitality (http://www.epi2010.yale.edu). Of the 41 sub-Saharan countries, South Africa's EPI ranked 15th and when compared to 16 other countries with similar GDP/capita, ranked last.

Plastics account for 21% by weight of WEEE[9] and may be recycled and reformed into new products.[10] Alternatively, plastics are often burned to facilitate access to the valuable metals or simply for debris disposal. WEEE-associated plastics typically contain persistent, bioaccumulative chemicals such as polybrominated diphenyl ethers (PBDEs).[11] PBDEs are brominated flame retardants (BFRs) added to textiles and polymers used in electronics and furniture. Two PBDE formulations, i.e., Penta-BDE and Octa-BDE, were added to the Persistent Organic Pollutants (POPs) list of the Stockholm Convention in May 2009, restricting their production.[12] In July 2008 the third and most widely used PBDE formulation, deca-BDE, was banned in EEE in the EU [13] and is scheduled to be phased out of U.S. production by the end of 2013.[14] Replacement brominated and nonbrominated FR additives have begun to supplant PBDEs in products and hence may become more abundant in the environment. Also, outdated EEE discarded by the developed world is exported and sold in the developing world, [7] these near-end-of-lifecycle EEE can contain harmful chemicals (i.e., PBDEs) now prohibited from newly manufactured EEE. Some WEEE exporters have exploited a loophole in the UNEP Basel Convention which prohibits the trans-boundary export of WEEE, but allows for the export of functioning or reparable electronics. Some deceptive exporters have padded shipments with up to 75% irreparable electronics (i.e., WEEE).[15] The United States, the world's largest WEEE producer, is expected to produce over 3 million MT annually by 2020 and has not yet ratified the Basel Convention.[16] With the exception of cathode ray tubes (e.g., computer monitors), this has allowed U.S. shippers to legally export WEEE wherever desired, as long as the export goal is declared as "recycling".[15]

Elevated BFR levels have been associated with densely populated urban centers, in turn correlated with the amount of BFR containing products therein.[17−19] However, while Europe was the world's third largest consumer of PBDEs,[20] air concentrations of these chemicals detected off the sub-Saharan coast of West Africa (0.95−3.10 pg m^{-3}) were reported to be comparable to those off Europe's coast (1.13−3.30 pg m^{-3}).[21] This was attributed to EEE and WEEE's salvaging. Seawater samples taken from the southern hemisphere Agulhas Current retroflection region (~400 miles southwest of Cape Town, South Africa) exhibited PBDE levels >2.5 times those detected in the English Channel (2.19 and 0.81 pg L^{-1}, respectively).[21] PBDEs have also been detected in South African sediments,[22] sewage sludge and wastewater effluent,[23] landfill leachate,[24] house dust,[25] and human breast milk.[26] According to Frost and Sullivan (2010), the South African plastics manufacturing sector is the largest consumer of BFRs, valued at approximately 2.5 million (USD).[27] However, this only accounts for <0.04% of the global 2005 BFR consumption. In contrast the U.S. represented 38% (564 000 MT), Europe 33% (489 000 MT), Japan 10% (160 000 MT), and other Asian countries 18% (269 000 MT) of worldwide demand.[28] Therefore, emissions of the chemical product by South African manufacturers may be a minor source, but emissions from components produced elsewhere and assembled in South Africa, those from in-use or discarded goods or from recycled materials (e.g., vehicles, WEEE), may be a more significant source.

Recently another BFR (i.e., hexabromocyclododacane (HBCD)), primarily used in polystyrene insulation boards and to a lesser extent textiles, has been detected in South African bird eggs [29] and human breast milk from Ghana.[30] HBCD is also considered a persistent, bioaccumulative, and toxic (PBT) chemical. Exposure to HBCD may cause reproductive, developmental, and neurological disorders.[31,32] Like PBDEs, HBCD will be phased-out of European commerce by 2015 under EU's REACH program. In the U.S., HBCD and 20 additional flame retardants are now under evaluation by the EPA.[33] According to the EPA, "These chemicals were selected because they are likely to persist in the environment, bioaccumulate in people and/or have high exposure potential...". These flame-retardants likely are also entering the African environment. Therefore, to gain a better understanding of BFR environmental dissemination in developing countries of the Southern Hemisphere, sediments from urbanized inland to coastal waterways in the eThekwini metropolitan municipality, South Africa were evaluated for BFR burdens and patterns.

EXPERMENTAL SECTION

Inland and coastal surficial sediments ($n = 45$) were collected in August 2011 from Durban Bay and 13 rivers in the eThekwini metropolitan municipality, South Africa. A map of the municipality and Durban Bay along with sample IDs, locations (latitude and longitude), river/bay names, and site associated population densities are listed in the Supporting Information (Figure S1 and Table S1). The municipality is located in the KwaZulu-Natal Province on the northeast coast of South Africa, which includes Durban, the third largest city in South Africa and home to the Port of Durban (Durban Bay), the largest container terminal in the Southern Hemisphere. The combined municipality (land area 2292 km^2) is the largest city (population 3.5 million) on Africa's east coast, with a population density of 1,513 inhabitants/km^2. Sediments were collected using a van Veen grab and stored in precleaned glass jars at <0 °C. Samples were freeze-dried, homogenized, and stored in glass jars at <0 °C until analyzed.

Details of the methods for the analysis of polybrominated diphenyl ethers (PBDEs) and the alternative-BFRs (alt-BFRs) 2-ethylhexyl 2,3,4,5-tetrabromobenzoate (TBB), 2-ethylhexyl 2,3,4,5-tretabromophalate (TBPH), 1,2-bis (2,4,6-tribromophenoxy) ethane (BTBPE), and decabromodiphenyl ethane (DBDPE) by gas chromatography (GC) and three α-, β-, and γ-hexabromocyclododecane (HBCD) isomers by liquid chromatography (LC) mass spectrometry are described by La Guardia et al..[34] The method was modified to permit determination of these BFRs in a single extract by ultra-performance liquid chromatography (UPLC)/atmospheric pressure photoionization (APPI) tandem mass spectrometry (MS/MS). The 18 targeted BFRs, their acronyms, and chemical formulations are listed in Table S2. All BFR analytical standards were supplied by AccuStandard, Inc. (New Haven, Ct., U.S.A.). A good agreement between analytical results generated by GC high-resolution mass spectrometry and LC APPI-MS/MS has been previously demonstrated for these BFRs and an additional 18 halogenated flame-retardants in fish tissue extracts.[35] For BFRs in sediments, the method was validated by the analysis of a BFR-fortified matrix (sodium sulfate ($NaSO_4$)) and also the standard reference material (SRM) 1944 (New York/New Jersey Waterway Sediment, National Institute of Standards and Technology (NIST)). BFR-fortified matrix recoveries ranged from 86 and 121% (Table S3) and the relative percent difference (RPD) for ΣPBDEs and ΣHBCDs in SRM 1944 were compared to NIST certified values. Their RPD was 35% and 58%, respectively (Table S4). This is the first reported values for alt-BFRs (i.e., TBB, TBPH and BTBPE) in SRM 1944 (Table S4). DBDPE was not detected above the detection limit, consistent with previously reported literature data.[36] Briefly, ~20 g (dry weight) of the sample was subjected to accelerated solvent extraction (ASE 200, Dionex, Sunnyvale, CA, U.S.A.) with dichloromethane (DCM). A surrogate standard (200 ng of 2,3,4,4′,5,6-hexabromodiphenyl ether (BDE-166); Cambridge Isotope Laboratories, Inc., Andover, MA) was added to each sample prior to extraction. Extracts were purified by size exclusion chromatography (SEC, Envirosep-ABC, 350 × 21.1 mm^2 column; Phenomenex, Torrance, CA, U.S.A.). Each post-SEC extract was solvent exchanged to hexane, reduced in volume and added to the top of a solid phase 2-g silica glass extraction column (Isolute, International Sorbent Tech.; Hengoed Mid Glamorgan, U.K.). Each column was eluted with 3.5 mL of hexane (fraction one), followed by 6.5 mL of 60:40 hexane/DCM and 8 mL of DCM (fraction two). The second fraction, containing BFRs, was reduced in volume and solvent exchanged to methanol. Decachlorodiphenyl ether (DCDE; 400 ng; Ultra Scientific, North Kingstown, RI, U.S.A.) was then added as the internal standard. Analytes in these purified extracts were chromatographically separated by UPLC (Acquity UPLC, Waters Corporation, Milford, MA, U.S.A.) operated in the gradient mode (100% methanol (A1) and 100% water (B1)), equipped with a C18 UPLC analytical column (Acquity UPLC BEH C18, 1.7 μm, 2.1 × 150 mm^2, Waters Corp.). Analytes were ionized by APPI, the dopant (acetone) was introduced at 150 μL/min by a liquid chromatography pump (LC-20AD, Shimadzu Corporation, Kyoto, Japan), and product ions were detected by a triple quadrupole mass spectrometer (3200 QTrap, AB Sciex, Framingham, MA, U.S.A.) operated in the multiple reaction monitoring (MRM) mode. For BFRs transition ions m/z 79 ($[^{79}\mathrm{Br}]^-$) and 81($[^{81}\mathrm{Br}]^-$), and m/z 35 ($[^{35}\mathrm{Cl}]^-$), 37($[^{37}\mathrm{Cl}]^-$) for DCDE were used for quantitation. Further details of the sample preparation and UPLC APPI-MS/MS operating conditions are listed in Table S5. Along with BFR-fortified matrix and SRM analysis, method performance was validated by processing blanks and duplicates with each sample set and monitoring for surrogate recovery of each sample. BFRs were not observed in the blanks above the detection limit (>0.6 ng g^{-1} dry weight (dw). Surrogate recoveries ranged from 73 to 132% and are listed along with sample results in Table S6. Duplicate analysis results are listed in Table S3. Total organic carbon (TOC) was determined by combustion followed by infrared detection (CE-440, Exeter Analytical, North Chelmsford, MA) and results are listed in Table S6. Statistical analyses were performed using Minitab 16.1.0 for Windows. Regression analysis was used to determine the relationship ($P < 0.05$) between ΣBFR concentration and population density (inhabitants/km^2).

RESULTS AND DISCUSSION

Multiple BFRs were observed in surficial sediments from rivers, estuaries and Durban Bay. At least one of the 18 targeted BFRs was detected in each of the 45 samples (Table S6); indicating that BFRs are ubiquitous within the region. Frequency of detection was highest for alt-BFRs (98%), followed by ΣPBDEs (93%) and ΣHBCD (69%) (Table 1). The major constituent of the Deca-formulation, BDE-209,[37] was the most frequently detected individual BFR (93%) followed by TBB (91%). The frequency of detection for ΣPenta-BDE was 67% (Table 1). This higher rate for the alt-BFR TBB may be a consequence of the 2004 discontinuation of Penta-BDE and its replacement with TBB.[38] Although the alt-BFRs had a higher frequency of detection, PBDEs were typically the dominant concentration contributor, as indicated by a 58.8% mean percent composition (Figure 1a). PBDE concentrations were followed by ΣHBCD (28.2%) and Σalt-BFR (12.9%). BDE-209 contributed 85.5% to ΣPBDEs, whereas ΣPenta-BDEs (BDE-47, −100, −99 and −153) contributed 11.3% (Figure 1b, mean concentration 423 ng g^{-1}, Table 1). PBDE congener profiles within these sediments generally resembled the profiles of technical products with BDE-209 being the dominant Deca-BDE congener at 97% (mean) and 3% for BDE-206. The Penta-profiles within these sediments were dominated by BDE-47 and -99, at 41% and 49%, respectively. There is a lack of BFR production and usage available for countries in the Southern Hemisphere. However, in Europe, 7500−10 000 MT of Deca- was sold in 2011 (**VECAP 2012**).[39] Prior to the Penta- and Octa-BDE 2004 production restrictions, the global 2001 demand for Deca-BDE was 56,100 MT and was followed by Penta- and Octa- at 7500 and 3790 MT, respectively.[20] It has been observed that the biota-sediment accumulation factors (BSAFs) for PBDEs decrease for congeners with greater than six bromines.[34] This indicates a lower uptake rate for the BDE-209 in these sediments. However, BDE-209 has been observed to undergo debromination in the aquatic environment and hence less brominated degradates may contribute to the cumulative PBDE bioaccumulation and toxicity.[40]

Table 1. Sediment BFR (Congener and Totals) % Detection Rate (%DR) and Concentration (ng g^{-1}, TOC) Range, Median and Mean[a]

analytes	%DR	range	median	mean
BDE-28	0%	nd	nd	nd
BDE47	49%	nd to 3790	nd	174
BDE-66	0%	nd	nd	nd
BDE-85	0%	nd	nd	nd
BDE-100	7%	nd to 246	nd	7
BDE-99	67%	nd to 3050	47	209
BDE-154	0%	nd	nd	nd
BDE-153	18%	nd to 588	nd	33
Σpenta-BDEs	**67%**	**nd to 7430**	**64**	**423**
BDE-183	33%	nd to 707	nd	35
BDE-206	47%	nd to 1360	nd	84
BDE-209	93%	nd to 44 500	784	3208
Σocta- and deca-BDEs	**93%**	**nd to 45 900**	**837**	**3320**
ΣPBDEs	**93%**	**nd to 46 300**	**1390**	**3750**
TBB	91%	nd to 13 900	150	545
TBPH	60%	nd to 899	54	96
BTBPE	13%	nd to 616	nd	34
DBDPE	62%	nd to 1840	70	171
Σalt-BFRs	**98%**	**nd to 13 900**	**383**	**825**
α-HBCD	62%	nd to 10 400	133	600
β-HBCD	42%	nd to 4970	nd	173
γ-HBCD	67%	nd to 18 700	146	1030
ΣHBCDs	**69%**	**nd to 27 500**	**349**	**1800**
ΣBFRs	**100%**	**114−47 100**	**2,280**	**6380**

[a] nd < 0.6 ng g^{-1} dry weight.

For HBCD, sediment profiles (Figure 1c) were dominated by γ-HBCD (57.1%) followed by α-HBCD (33.3%) and β-

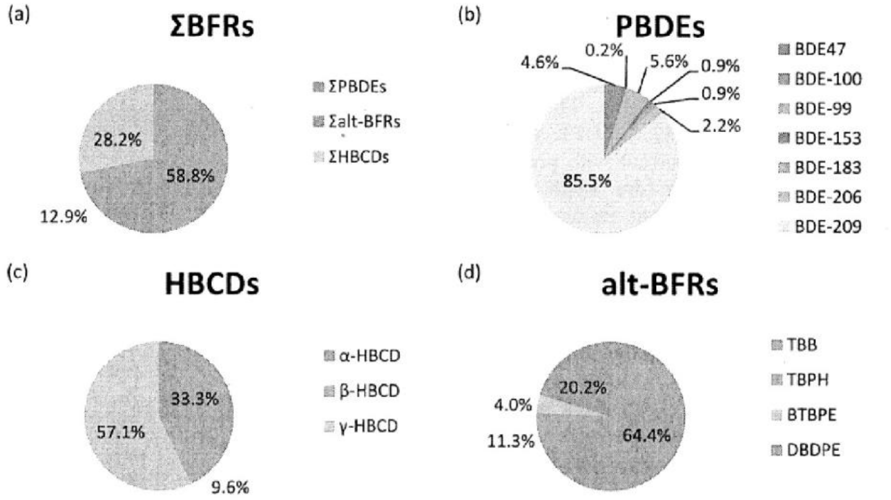

Figure 1. Mean percent contribution of (a) ΣBFRs, (b) PBDEs, (c) HBCDs, and (d) alt-BFRs sediment concentration.

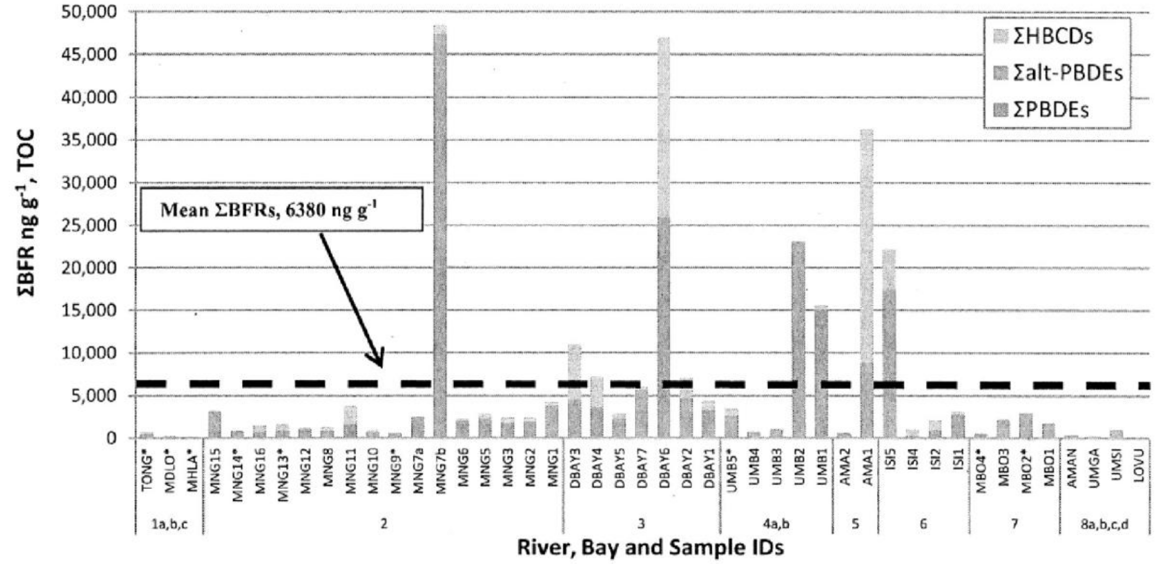

Figure 2. ΣBFR (ΣHBCD, Σalt-BFRs, and ΣPBDEs) concentrations (ng g^{-1}, TOC) in sediment samples from the eThekwini metropolitan municipality in order of river location (north to south) and river sample location (inland to coast). (*Denotes sample sites located downstream from a wastewater treatment works outfall.) River and Bay IDs: 1a-Tongaat R., 1b-Mdlota R., 1c-Mhlanga R., 2-Mngeni R., 3-Durban Bay, 4a-Umbilo R., 4b-Umhlatuzana R., 5-Amanzimnyama R., 6-Isipingo R., 7-Mbokodweni R., 8a-Amanzimtoti R., 8b-Umgababa R., 8c-Umsimbazi R., 8d-Lovu R.

HBCD (9.6%). Annual European usage of HBCD was 10 000–12 500 MT in 2011, up from 7500–10 000 MT in 2009 and 2010.[39] The frequency of detection (Table 1) for the ΣHBCD (69%) was lower than the other two BFR classes: ΣPBDEs 93% and Σalt-BFR 98%. However, the mean ΣHBCD concentration, 1800 ng g^{-1} (maximum 27 500 ng g^{-1}) was >4-times the mean concentration of ΣPenta-BDE, 423 ng g^{-1} (max conc. 7430 ng g^{-1}). (Concentration data are presented on total organic carbon (TOC) normalized basis.) This could be of concern considering the BSAF for HBCD (log BSAF $\approx$ 1) in mollusks is similar to BSAFs of BDE-47 and −99, dominant constituent congeners of the restricted Penta-BDE formulation. Each of the alt-BFRs was detected in these sediments. Their profiles (Figure 1d) were dominated by TBB (64%), DBDPE (20%), TBPH (11%), and BTBPE (4.0%).

The sediment ΣBFR concentrations in the eThekwini metropolitan municipality ranged from 114 to 47,100 ng g^{-1} (Table 1). Traversing the municipality north to south it is evident that BFR concentrations increased near the urban center, with six sites exceeding the mean (ΣBFR 6380 ng g^{-1}, indicated by the dash line in Figure 2) by several fold. Excluding these sites (i.e., MNG7b, DBAY6, UMB2, UMB1, AMA1, and ISI5) from the data set, regression analysis of the remaining sites (n = 39) indicated a weak relationship between population density and ΣBFRs (r^2 = 0.14). However, this relationship was statistically significant (P = 0.038). BFR containing products used domestically are one potential source.[17−19] The lowest BFR sediment concentrations were detected in rural areas. These estuarine sites are located in the municipality's northern end (Tongaat (1a), Mdloti (1b) and Mhlanga (1c)) and southern end (Amanzimtoti (8a), Umgababa (8b), Umsimbazi (8c), and Lovu (8d), ΣBFR < 741 and <1010 ng g^{-1}, respectively (Figure 2). The municipality is a mix of residential and industrial areas that range from densely populated informal settlements to lavish tourist resorts, intertwined with small business and large international manufacturing and chemical producers. To facilitate drainage and prevent inland flooding most of the natural river systems within the urbanized area have been canalized to receive runoff during storm events. These systems have also become catchments for trash and illegal dumping. A number of wastewater treatment plants also discharge into the rivers. All only perform primary treatment of wastewater. ΣBFR concentrations exceeding the mean were not detected downstream of the outfalls (sample sites #TONG, MDLO, MHLA, MNG14, MNG13, MNG9, UMB5, MB04 and MB02), although this can to some extent be explained by the fact that the rivers are relatively fast flowing and the sediment has a low organic fraction (Figure 2). The highest ΣBFR level, 47 100 ng g^{-1}, was detected downstream from the Umgeni Business Park (#MNG7b). The Business Park is divided by the Mngeni River, which flows through an area containing a mix of commercial and manufacturing industries. BDE-209 was dominant (44 500 ng g^{-1}), contributing 95% to the ΣBFRs. This was the furthest upstream collection site for this river. Hence, the source was not delineated. However, the five sites located downstream (sites #MNG6, 5, 3, 2 and 1) all had BDE-209 levels greater than the municipality's median BDE-209 value of 784 ng g^{-1}. Site #ISI5, located in another business district on the canalized lower reaches the Isipingo River, exhibited the fifth highest ΣBFR concentration at 22 300 ng g^{-1}. BDE-209 was the dominant flame retardant (11 500 ng g^{-1}), followed by γ-HBCD and Σpenta-BDE (3860 and 2760 ng g^{-1}, respectively). This site drains a large industrial area where automotive manufacturing and salvaging takes place. A large pile of scrap automotive interiors (e.g., polyurethane foam) was observed at this site (Google Earth, imagery date June 16, 2012). This site is also located adjacent to a large commercial WEEE recycler.

Another potential BFR source (sites #UMB1 and #UMB2) is located on the Umbilo and Umhlatuzana rivers, which flow through the central part of the eThekwini municipality. These rivers drain mostly urban (70%) areas. The remainder of the drainage is primarily bushland, grassland, and forest. The rivers converge just before the entering Durban Bay, where the sixth highest ΣBFR sediment concentration was detected (#UMB1, 15 600 ng g^{-1}). This site is located in Bayhead Marshalling Yard, a large rail yard servicing the Bay. ΣBFRs were dominated by BDE-209, at 14 400 ng g^{-1}. However, site #UMB2 located on the Umbilo River upstream from site #UMB1 had the fourth highest ΣBFR concentration (24 000 ng g^{-1}), dominated by TBB (13 900 ng g^{-1}), followed by ΣPenta-BDEs (7430 ng g^{-1}). TBB is a component of the commercial mixture Firemaster-550 (Chemtura Corp. (USA)) which also contains TBPH at a TBB/TBPH ratio of 4:1. TBPH was detected in this sample at 899 ng g^{-1}, producing a TBB/TBPH ratio of 16:1. This suggests a different chemical composition or manufacturer or different relative fate or transport for these flame-retardants. This was hypothesized by Stapleton et al., who reported TBB/TBPH ratios ranging from 1:20 to 50:1 in indoor dust samples.[41] The third highest ΣBFR site (#AMA1, 36 300 ng g^{-1}) was on the Amanzimnyama River where it passes through the Bayhead Marshalling Yard, before flowing into the southern side (extreme upper reach) of Durban Bay. The ΣHBCD level here (27 500 ng g^{-1}) was the highest reported in these sediments and contributed 76% of the ΣBFRs. The second highest ΣBFR concentration (47 000 ng g^{-1}) was located in Durban Bay (#DBAY6) at the mouth of a culvert draining surface runoff from area consisting of a mix of industrial and mainly residential concerns. At this site, BDE-209 was again the dominant BFR (24 500 ng g^{-1}). However, it also exhibited the second highest HBCD concentration in our study (21 100 ng g^{-1}). The HBCD technical formula is dominated by the γ-isomer and exhibits a α:γ ratio of 0.09. Similar ratios have been observed in abiotic media such as sediments, soils and landfill leachate.[31] Enrichment of α-HBCD may occur following exposure of HBCD-treated textiles and thermoplastics to elevated temperatures (e.g., ≥160 °C)[42] or through biotransformation. [31] In finished textiles the α:γ ratio has been reported to be 0.52 to 1.06, which indicates enrichment of the α-isomer by preferential absorption by the product of the α-isomer or γ-isomer thermal rearrangement.[43] At this site (#DBAY6) the total HBCD profile in sediments was dominated by γ-HBCD at 88.6% followed by α-HBCD at 9.0% and 2.1% for β-HBCD, producing a α:γ ratio of 0.10; indicating the HBCD technical product as the likely source. The α:γ ratio at the other sites within the municipality ranged from 0.21 to 4.28. Both HBCD and BDE-209 are commonly used to treat textiles. Hence, a facility making or using textiles might have been a contributor here.

Within and near to Durban Bay (sites #AMA1, UMB1, and DBN1 through 7), the mean ΣBFR sediment concentration (15,300 ng g^{-1} TOC) was 2-times higher than other sediments from eThekwini municipality sites. The Bay has an area of 8.29 km^2 and receives inputs from the Umbilo, Umhlatuzana and Amanzimnyama rivers and urban runoff from culverts draining the city of Durban. The total catchment area of all three rivers is 195 km^2 and falls entirely within the eThekwini municipal boundary. All three catchments are canalized at their lower reaches and highly transformed. Over 70% of the catchment is considered urban (23% natural) and 20% of this area consists of impervious surfaces. These factors have all altered the Bay's hydrology causing present day runoff into the Bay to be 74% greater than it was prior to its human disturbance.[44] PBDEs, HBCDs, and alt-BFRs were all detected in sediment at each Bay sample ($n = 7$). The ΣBFR ranged from 2900 to 47 000 ng g^{-1}. BDE-209 was the dominant BFR and concentrations ranged from 1740 to 24 500 ng g^{-1}. With the exception of sites #DBAY6 and #DBN3, BFR concentrations in the Bay were fairly consistent between locales, from 2900 to 7240 ng g^{-1}. This suggests a likely common source. However, a wider range in concentrations was observed for HBCD (349 to 3640 ng g^{-1}), possibly indicating a more localized source (e.g., textile manufacturing). The HBCD α:γ-isomer ratio varied from 0.64 to 3.21, which may indicate releases from finished products (e.g., textiles or building insulation material) washing into the Bay from the urban environment. Except for sample #DBN1; TBB, TBPH, and DBDPE were detected in each of the Bay's samples, ranging from 363 to 1460 ng g^{-1}. (TBB was not detected in sample #DBN1 above the detection limit of 0.6 ng g^{-1}, dry weight.)

Compared to other aquatic systems around the world, Durban Bay's PBDE sediment concentrations were relatively high, especially considering the reported lack of flame-retardant demand in Africa. Demand figures for 2001 suggested that North America accounted for 95% of the global Penta-BDE consumption and 44% of that for Deca-BDE.[20] Studies have revealed that PBDE concentrations in marine biota from California's coast are among the highest in the world, doubling as rapidly as every 2–4 years in some species, presumably

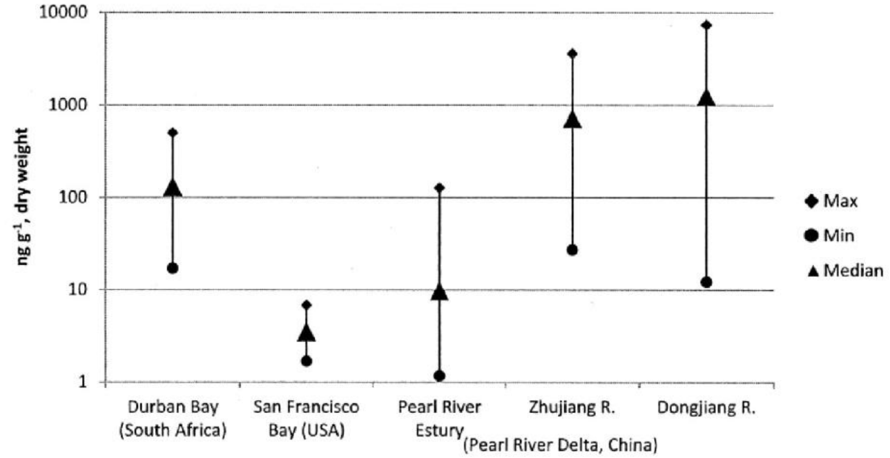

Figure 3. ΣPBDE (sum of BDE-28, −47, −99, −153, and −209) sediment concentrations (ng g^{-1}, dry weight) for Durban Bay (South Africa), San Francisco Bay (U.S.A.), and Pearl River Delta (PRD), China.

exacerbated by California's stringent flammability standard for polyurethane foam (Technical Bulletin 117 (TB117)).[45] However, the ΣPBDEs (i.e., ΣBDE-28, −47, −99, −153, and −209) detected in Durban Bay sediments (17 to 497 ng g^{-1}, dw (median concentration 131 ng g^{-1}, dw)) were an order of magnitude higher than those of San Francisco Bay (1.68 to 6.76 ng g^{-1}, dry weight (dw) (median 3.51 ng g^{-1}, dw)) (Figure 3).[46] PBDE concentrations in Durban Bay also rivaled those reported in the Pearl River Estuary, China (1.17 to 127 ng g^{-1}, dw (median 9.7 ng g^{-1}, dw)) (Figure 3). The two major rivers of the Pearl River Delta (PRD), the Zhujiang and Dongjiang, flow through the world's most densely urbanized region (population 120 million) and a major electronics manufacturing center. The median PBDE sediment concentrations in these rivers were reported as 718 and 1250 ng g^{-1} dw, respectively (Figure 3). The city of Dongguan is considered to be the largest manufacturing base of electronic products in the PRD region and is estimated to produce one of every three computers worldwide.[47] If the levels of BFRs detected within Durban Bay were associated with manufacturing, global markets would indicate a large manufacturing presence in South Africa. Therefore, particulates carried by the three rivers (i.e., Umbilo, Umhlatuzana, and Amanzimnyama) that enter Durban Bay from the south, after washing through large residential and industrialized areas, are probable proximate sources. Particulate material is deposited, because of weak currents and long water residence time in the upper part of the Bay due to a unique hydromorphology. The Bay's opening is in opposition to the south-flowing Mozambique Current of the Indian Ocean. This sets up littoral drifts, moving sand northward along the East African cost, forming sandbars that have historically closed the Bay. These factors (i.e., urban runoff and the Bay's hydromorphology) may account for flame-retardant levels exceeding those found in other urban bays (e.g., San Francisco Bay). Our findings suggest that, as local demand for polymer products and electronics escalates and if the area remains a center for WEEE, BFR concentrations may increase in the sub-Saharan environment. This underlines the need to further investigate the environmental burdens and risks associated with BFR use in developing countries of the Southern Hemisphere.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Additional details on instrument methodology, quality control/quality assurance, duplicate analysis, spiking experiments, and their results along with Figure S1 and Tables S1−S6. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Notes

The authors declare no competing financial interest.

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Halogenated flame-retardant concentrations in settled dust, respirable and inhalable particulates and polyurethane foam at gymnastic training facilities and residences

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Gymnast

ABSTRACT

Halogenated flame-retardants (FRs) are used in a wide array of polymer-containing products. Animal studies and structure-activity modeling exercises suggest that FR exposure may result in detrimental toxicological effects. Workers with extended contact with such polymers (e.g., electronic dismantlers, carpet installers and aircraft personnel) have previously been observed to exhibit elevated body burdens of FRs, e.g., polybrominated diphenyl ethers (PBDEs). Recently, elevated PBDE blood levels were also reported in a non-occupational exposure group, gymnasts. These levels were hypothesized to be related to the large volumes of FR-treated polyurethane foam in gymnastics facilities. To further our understanding of workers' potential exposure, we analyzed FR concentrations in indoor dust and size-fractionated air particulates (respirable (<4 μm) and inhalable (>4 μm)) from gymnastic studios. Values were compared to samples from the homes of coaches employed at these facilities. Polyurethane foam blocks (i.e., pit foam) were also analyzed to characterize potential FR sources. FRs examined included those used to flame-retard polyurethane foam: 8 PBDE congeners, two brominated components of Firemaster 550 (2-ethylhexyl 2, 3, 4, 5-tetrabromobenzoate (TBB) and bis(2-ethylhexyl) 3, 4, 5, 6-tetrabromophthalate (TBPH)) and three chlorinated organophosphates (tris(2-chloroethyl) phosphate (TCEP), tris(1-chloro-2-propyl) phosphate (TCPP) and tris(1,3-dichloro-2-propyl) phosphate (TDCPP)). Several additional FRs not used in polyurethane were also evaluated. These have also been detected in indoor dust and air and may also lead to adverse health effects. These include: BDE-183 and its replacement product (1, 2-bis(2, 4, 6-tribromophenoxy) ethane (BTBPE), two congeners of the deca-BDE formulation (BDE-206, -209) and their replacement decabromodiphenyl ethane (DBDPE) and hexabromocyclododecane (α-, β-, γ-HBCD), and tetrabromobisphenol-A (TBBPA)). Pit foam contained multiple FRs at cumulative concentrations of 12,100 to 25,800 μg g^{-1}, or 1.2% to 2.6% by weight. TBB and TBPH were the most abundant FRs detected, followed by TDCPP and several PBDEs. The mean total FR burden detected at the gyms was 8.6-fold higher (574 μg g^{-1}) than that observed in the house dust samples (66.8 μg g^{-1}). However, the polyurethane additives TBB and TDCPP were the only FRs that exhibited significantly greater levels ($P < 0.05$) in gym than house dust. Mean levels of five FRs (BDE-99, -100, -153, -209 and TDCPP) were also higher in respirable particulates from the gyms than the homes and four FRs (BDE-47, TBB, TBPH and DBDPE) were higher at the homes than the gyms; these differences were not significant ($P > 0.05$). Several additional FRs were detected in inhalable particulates; mean levels of BDE-66, -206 and TCPP were higher in the homes and BDE-47, -85, -99, -100, -153, -209, TBB, TBPH, and TDCPP were higher at the gyms. But, only the polyurethane additives i.e., BDE-100, TBB and TDCPP were significantly greater ($P < 0.05$) in inhalable particulates from the gyms than at the homes. In conclusion, polyurethane foam collected from gymnastic studios exhibited a variety of FR compositional signatures; likely reflective of changes in FR usage over time and by different manufacturers. FR concentrations and compositional signatures also differed between settled dust, respirable and inhalable particulates between the gyms and homes. Concentrations of FRs used in polyurethane foam were higher in gym air and dust compared to homes, particularly TBB and TDCPP — which were also the primary FRs detected in the pit foam samples. Although these results should be interpreted with caution, as the sample size was small, these findings do suggest that FR concentrations observed in dust and air particulates from the gymnastic studios are further evidence that individuals frequenting these environments are at greater risk for exposure to these polymer additives.

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1. Introduction

To slow ignition and the spread of fire, halogenated flame-retardants (FRs) have been widely added to many polymeric consumer products, often at percent levels. As a result of the dissemination of these products and resulting degradates, FRs are now detected globally (Sjödin et al., 2003). Some FRs (e.g., polybrominated diphenyl ethers (PBDEs)) are environmentally persistent, can bioaccumulate and disrupt biological processes, e.g., the endocrine system (Legler and Brouwer, 2003 and Kim et al., 2014). The manufacture of PBDEs is now restricted in the U.S. (USEPA, 2006) and in the European Union (EU) (Kemmlein et al., 2009). These FRs were also added to the Persistent Organic Pollutants (POPs) list of the Stockholm Convention in May 2009 (Stockholm Convention, 2009), restricting their use in signatory countries. But products purchased prior to restrictions still remain in use and new products containing alternative-flame-retardants may present additional heath concerns (e.g., U.S. EPA, "EPA Announces Chemicals for Risk Assessment in 2013, Focus on Widely Used Flame Retardants." (USEPA, 2013) and European Food Safety Authority (EFSA), "Scientific opinion on emerging and novel brominated flame retardants (BFRs) in food." (European Food Safety Authority, 2012)).

For the general public, the primary route of FR exposure is through incidental ingestion of contaminated indoor dust (Wu et al., 2007; Stapleton et al., 2009; Carignan et al., 2013). In addition, inhalation may be a contributing exposure pathway. Allen et al. (2007) examined potential inhalation using personal samplers worn by study participants. They observed that inhalation may be more important (up to 22% for BDE209 of total exposure) than previously hypothesized due to the presence of a cloud of suspended particles generated by participant activities. Dust particle contamination may arise through FR migration from or physical deterioration of additive-treated products (Besis and Samara, 2012). Higher PBDE body burdens have been detected in certain occupationally-exposed subpopulations, i.e., those employed in polymer, textile and electronics manufacturing and dismantling of electronic waste (Besis and Samara, 2012); as well as workers frequenting environments laden with FRs, e.g., aircraft personnel (Strid et al., 2014). Recently, elevated PBDE serum levels were also detected in gymnasts (Carignan et al., 2013). This was hypothesized to be due to exposure within training facilities, which typically contain large volumes of PBDE-treated polyurethane foam (e.g., landing mats and loose foam pits) which, over time may break down to small dust-sized fragments.

Over 5 million Americans participate in gymnastics annually, over 50 million amateur gymnasts worldwide (International Gymnastics Federation, 2014), and over 900,000 individuals engage in gymnastics 100 or more days per year (USA Gymnastics, 2014)). Most gymnasts are female (>70%), 80% are under the age of 18 years old (USA Gymnastics, 2014), which may place them at a greater risk of adverse health consequences to PBDE exposure. Also, consideration of the full complement of xenobiotics one is exposed to is important as these may interact toxicologically (Silva et al., 2002). Besides PBDEs, PBDE replacement-FRs have also been detected in dust from gymnastic studios including: 2-ethylhexyl 2,3,4, 5-tetrabromobenzoate (TBB), 2-ethylhexyl 2,3,4, 5-tetrabromophalate (TBPH), tris(1-chloro-2-propyl) phosphate (TCPP) and tris(1, 3-dichloro-2-propyl) phosphate (TDCPP) (Carignan et al., 2013). Both TBB and TBPH have demonstrated endocrine disruption potentials (Saunders et al., 2013) and TDCPP an analog of TCPP, was used in children's sleepwear until manufacturers voluntarily withdrew it in 1977 due to mutagenicity concerns. TDCPP is now listed as a carcinogen under California Proposition 65 (California EPA, 2011). Under the EU's toy safety directive both TCPP and TDCPP are considered carcinogens, restricting their use in children's toys, starting December 21, 2015 (EU, 2014).

Elevated FR levels in gymnastics training facilities would also affect coaches and other gym staff, in addition to the gymnasts themselves, potentially experiencing greater exposure than the general population. While gymnasts may spend less time in the facility than coaches, who may spend 40 or more hours per week in the facility, gymnasts may take-in higher levels of airborne dust due to increased rate and depth of breathing during exercise. It has been observed at room temperature PBDEs have a high affinity for air particulates and exhibit low concentrations in the vapor state (Allen et al., 2007). These dust particulates may then penetrate deep inside the lung when exercising. Coaches and gymnasts both may also accumulate FR-contaminated dust on skin and clothing. Therefore, to further our understanding of occupational and residential FR exposure of coaches and gymnasts, we analyzed dust and air particulates collected at training facilities and in the homes of coaches. Samples were analyzed for PBDEs, their replacements and two additional FRs shown to cause adverse health effect e.g., disrupt thyroid hormone function; hexabromocyclododacane (HBCD), used in polystyrene foams in building materials and textiles (Ema et al., 2008; Ibhazehiebo et al., 2011) and tetrabromobisphenol-A (TBBPA) used in epoxy resins of printed circuit boards (Kitamura et al., 2002; Huang et al., 2013), all recently detected in indoor dust (Dodson et al., 2012) and air samples (Karlsson et al., 2007). Compositions of polyurethane foam products collected from these training facilities were also compared to indoor dust and particulates in air samples to investigate possible sources.

2. Materials and methods

2.1. Sample collection

2.1.1. Polyurethane block sampling method

Four polyurethane foam blocks were collected from the foam pit at the Seattle Gymnastics Academy (SGA), Seattle Washington, USA, Gym#4 (samples #4a–4d). (The SGA has four locations (Gym#1–#4) within Seattle's metropolitan area.) The loose foam pit contained over 100 blocks varying in age, as individual blocks are replaced as needed. Blocks were then selected ("old to new") based on their color (fading) and degree of pitting which may be an indication of age (*see* Appendix A, *Supplementary data, Figure SI-1 Image of polyurethane foam blocks*). Also, one new 2013 foam block (#4e) from Gym#4's storage was sampled, along with a block (#2a) from Gym#2's foam pit. The coach from Gym#2 indicated that this particular type of block appeared to be more resistant to pitting. Its acquisition date was estimated as prior to 2007. Each block was placed in doubled sealed plastic bags at the gym and remained in these bags until analyzed. Prior to analysis a portion of the outer section of blocks #4a–4e was removed at the laboratory, divided into three sections and each section was analyzed for FRs. A single composite of block #2a was also analyzed for FRs.

2.1.2. Dust sampling method

Settled dust samples (~1 g) were collected from SGA gymnasium floors at each of the four training facilities and residences of the four coaches (one from each of their four training facilities) using a Eureka® Mighty-Mite® (model 3670G) vacuum, fitted with a cellulose filter (Whatman 2800-199) held in the crevice tool at the inlet with a stainless steel ring as previously demonstrated by Wu et al. (2007) and Schreder and La Guardia (2014). The gymnasium floors consisted primarily of wall-to-wall carpet-bonded foam. To collect a sample that represents exposure throughout the training facility dust sampling was not conducted within ~2 m of uncovered polyurethane foam (e.g., foam pit). At the homes, settled dust was collected from main living areas; i.e., kitchen, living room, bedroom, office, and dining room. Dust was collected by moving the crevice tool slowly across the floor, including bare floor and carpet. After collection, all dust samples were placed in double sealed plastic bags and stored at <4 °C until analyzed.

2.1.3. Air sampling methods

To compare indoor air exposure levels at the training facilities to house levels, the four coaches were instructed to collect 8-hour air samples, once during a normal work day (e.g., coaching gymnasts) and once at their residences doing normal indoor activities (e.g., light house cleaning, viewing television). Air particulates were collected using an AirChek 2000 pump (flow rate 2 L min^{-1} or 0.002 $\text{m}^3\ \text{min}^{-1}$) with an Institute of Occupational Medicine (IOM) Sampler (SKC, Eighty Four, PA, USA) equipped with a stainless steel cassette assembly. The IOM sampler meets air particulate sampling criteria established by the American Conference of Governmental Industrial Hygienist (ACGIH) and Occupational Safety and Health Administration (OSHA) (SKC, 2014). Two size classes of air particulates were examined: 1) inhalable particulates (>4 μm, nominal), that can either enter the lung airways (trachea, bronchi and their branches) or are trapped on the mucosa of the nose, mouth and lungs and then are expelled or swallowed. These were collected with a MultiDust® foam disc of a specific porosity (D_{50}) of 4 μm (D_{50}: a particle aerodynamic diameter for which 50% of the particles penetrate). 2) Smaller respirable air particulates (<4 μm, nominal) which are able to penetrate deep inside the lung's gas-exchange regions were collected on a 25-mm, 1.0 μm glass fiber filter placed behind the foam disc. Both the foam disc and filter were placed inside the stainless steel cassette assembly, positioned at the IOM inlet. This design, i.e., positioning the cassette at the IOM inlet, reduces the possibility of contaminating the sample by first drawing the air sample through the foam disc and filter without it coming in contact with the collection equipment (e.g., pump). All collection equipment and cassettes were cleaned and assembled at the laboratory and stored in double sealed plastic bags prior to shipment to the field. After collection, all cassettes were placed in pre-cleaned SKC's transportation clip with cover and stored <4° C in double sealed plastic bags until analyzed.

2.2. Analytical protocols

2.2.1. Target compounds

All samples (dust, discs, filters and foam) were analyzed for brominated flame-retardants (BFRs): PBDEs (BDE-28, -47, -66, -85, -99, -100, -153, -154, -183, -206, -209), α-, β-, γ-HBCD, and TBBPA. Alternative-BFRs (alt-BFRs): BTBPE, DBDPE, TBB and TBPH; and three chlorinated organophosphate flame-retardants (ClOPFRs): TCEP, TCPP, and TDCPP. For a list of FRs nomenclature and CAS# refer to Appendix A, Supplementary data *Table SI-1, Nomenclature of targeted brominated and chlorinated flame-retardants.*

2.2.2. Extraction, purification and analysis

Briefly, polyurethane foam (~100 mg, dry weight (dw)), dust (~100 mg dw, sieved to 300 μm), air particulate samples (disc and filter) and laboratory blanks ($NaSO_4$, baked at 400 °C >4 h) were spiked with surrogate standards (BDE-166, AccuStandards, Inc. New Haven, Ct. USA) and deuterated tris(1,3-dichloro-2-propyl)phosphate (*d*TDCPP, Max Planck Institute for Biophysical Chemistry, Germany) and extracted with methylene chloride (DCM) in a Dionex ASE 200 accelerated solvent extractor (Sunnyvale, CA, USA) at 100 °C and 68 atm. All solvents used were residue grade (Burdick & Jackson, Muskegon, MI, USA). Each extract was then purified on a 2 g silica solid phase extraction column (International Sorbent Tech.; Hengoed Mid Glamorgan, UK) eluted with 3.5-mL hexane (Fraction 1), followed by 6.5 mL of 60:40 hexane/DCM and then 8 mL DCM (Fraction 2) and 5 mL 50:50 acetone/DCM (Fraction 3). Fraction 2 contained the PBDEs, alt-BFRs and HBCDs. Fraction 3 contained the ClOPFRs and TBBPA. Decachlorodiphenyl ether (DCDE, AccuStandards, Inc.) was then added to each fraction as the internal standard. Analytes in Fraction 2 and 3 were further separated by ultra-performance liquid chromatography (UPLC, Waters Corp. Milford, MA, USA) and analyzed by atmospheric pressure photoionization tandem mass spectrometry (APPI/MS/MS, Q-Trap3200 MS, AB Sciex, Framingham, MA. USA). The five point calibration curves used for quantitation of each of the 22 targeted FRs were constructed from analytical standards provided by Accustandards, Inc. Further details of the UPLC-APPI/MS analysis can be found in La Guardia et al. (2013) and Schreder and La Guardia (2014).

2.3. Statistical analysis

To determine if individual FR levels (e.g., BDE-47) in the dust and air samples were greater within the gyms than the homes; their respective mean concentrations were compared by 2-sample T-tests (Minitab 16, version 16.1.0; Minitab Inc.). However, because of the small sample size (n = 4) data normality could not be reliably checked therefore the test had low statistical power. Low statistical power will bias the test to fail to find a statistical difference that may have been detected with a larger sample size. Therefore caution should be exercised when interpreting results. Also, for comparison each data set (home or gym) needed to contain at least three values out of four above detection limit; non-detected analytes were substituted with values of one-half their respective detection limit.

3. Results and discussion

3.1. FRs in polyurethane foam blocks

3.1.1. Quality control (QC) overview for foam blocks

The four foam blocks collected from Gym#4's foam pit (samples #4a to 4d) and a new foam block from storage (#4e) were analyzed in triplicate. A single composite of block #2a from Gym#2 was also analyzed (Table 1). The percent relative standard deviation (%RSD) between the triplicate analyses for a majority of the individual FRs was < 30%; none exceeded 71%. The %RSD range for the $\sum$ FRs was 5.1–11% (Table 1). Standard deviations >30% RSDs were observed when the individual FR result neared the limit of detection, 0.1 μg g^{-1} (Table 1). Except for two samples (#2a and 4c) surrogate recoveries were good; BDE-166: recovery range 97–128%, RSD 3–10% and *d*TDCPP: recovery range 86–109%, RSD 5–12%. Interferences with the surrogate (BDE-166) in sample #2a and *d*TDCPP in sample #4c were caused by high concentrations of pre-existing FRs co-eluting with the surrogates. Most of the foam blocks contained FRs at percent by weight concentrations. Therefore, quantitation required final extracts to be diluted 10- to 100-fold and re-analyzed. To check for the possibility of laboratory contamination, a laboratory blank was analyzed with each sample set. The blanks did not contain any of the analytes above the detection limit (0.1 μg g^{-1}). All results were corrected for surrogate recoveries, except where interferences were encountered (i.e., for the analytes that eluted in the third fraction of sample #2a and the second fraction of sample #4c).

3.1.2. Foam block analysis

Several FRs (i.e., BTBPE, DBDPE, HBCD (α-, β-, γ-), and TBBP-A) not commonly used to flame-retard polyurethane foam were not detected in the foam blocks above detection limits (Table 1). The FR composition between each of the six blocks varied substantially (e.g., TDCPP, 0.12% to 93.4%). However, the $\sum$ FRs within each block were similar; i.e., range from 12,100 to 25,800 μg g^{-1} (dw) (Fig. 1). Also, the low %RSD (5.1–11%) between the $\sum$ FRs triplicate analysis may indicate that FRs are evenly distributed throughout the block (Table 1 and Fig. 1, %RSD indicated by the solid bars). Each block contained multiple FR formulations, except the newest foam block (#4e) which contained primarily (>99%) TBB and TBPH, constituents of Firemaster 550 (Chemtura Corp., Middlebury, CT, USA). Carignan et al. (2013) reported polyurethane foam blocks purchased prior to the U.S. 2004 penta-BDE discontinuation of production were also treated with the FRs: TBB, TBPH, TDCPP and triphenyl phosphate (TPP), at 2 to 6% by weight. Newer blocks obtained by Carignan et al. (2013) contained either TDCPP or a formulation similar to Firemaster 550 (i.e., TBB, TBPH and TPP). TBB and TBPH were also the major FRs that we detected in four of the blocks (i.e., #2a, 4a, 4b and 4d), contributing > 61.5% to the total FR content, followed by TDCPP contributing 2.8% to 4.5% (Fig. 1). Block #4c contained primarily TDCPP, which contributed 93.4% of total FR content, followed by ΣTBB_TBPH at 6.3%. The newest foam block (#4e)

Table 1
Polyurethane foam block (pit foam) flame-retardant mean concentrations (μg g^{-1}, dry weight) and relative standard deviations (%RSD). Shaded columns indicate FRs commonly used in the treatment of polyurethane foam.

Analytes	2a (n=1)	4a (n=3)	%RSD	4b (n=3)	%RSD	4c (n=3)	%RSD	4d (n=3)	%RSD	4e (n=3)	%RSD
BDE-28	28.9	nd	–	nd	–	nd	–	nd	–	nd	–
BDE-47	1720	64.7	21%	166	13%	18.2	6%	11.4	33%	0.3	68%
BDE-66	113	0.4	36%	1.2	19%	0.2	59%	0.1	24%	nd	–
BDE-85	518	4.2	24%	11.4	30%	0.9	12%	0.6	44%	0.0	71%
BDE-100	834	13.7	16%	44.7	42%	3.9	14%	2.5	41%	0.1	42%
BDE-99	1490	84.1	16%	232	10%	18.0	17%	12.5	36%	0.4	56%
BDE-154	444	5.1	25%	17.6	37%	1.4	17%	1.1	27%	nd	–
BDE-153	475	6.5	27%	19.3	32%	1.4	21%	1.0	42%	0.1	58%
Σpenta-BDEs	5620	179	18%	492	15%	43.9	12%	29.3	35%	0.9	59%
BDE-183	36.6	0.3	12%	0.8	14%	0.1	70%	0.2	48%	0.4	24%
BDE-206	nd	0.1	58%	nd	–	nd	–	nd	–	nd	–
BDE-209	0.4	0.4	9%	0.4	15%	0.4	21%	0.4	29%	0.1	15%
TBB	8300	8050	7%	13700	12%	1400	11%	13300	19%	12500	6%
TBPH	2030	2850	9%	5220	10%	225	22%	5710	17%	5430	4%
ΣTBB+TBPH	10300	10900	7%	19000	12%	1630	13%	19000	18%	17900	5%
BTBPE	nd	nd	–	nd	–	nd	–	nd	–	nd	–
DBDPE	nd	nd	–	nd	–	nd	–	nd	–	nd	–
α-HBCD	nd	nd	–	nd	–	nd	–	nd	–	nd	–
β-HBCD	nd	nd	–	nd	–	nd	–	nd	–	nd	–
γ-HBCD	nd	nd	–	nd	–	nd	–	nd	–	nd	–
% recovery (BDE-166)	–[a]	128%	8%	123%	5%	101%	3%	97%	10%	108%	6%
TCEP	1.9	nd	–	nd	–	1.6	28%	nd	–	nd	–
TCPP	8.0	10.9	20%	9.1	11%	41.4	60%	6.9	6%	nd	–
TDCPP	746	990	12%	549	21%	24100	11%	784	22%	22.1	42%
ΣClOPFRs	756	1000	12%	558	21%	24100	11%	791	22%	22.1	42%
TBBPA	nd	nd	–	nd	–	nd	–	nd	–	nd	–
% recovery (*d*TDCPP)	107%	86%	12%	88%	5%	–[a]	–	93%	8%	109%	6%
Σ*FRs*	16700	12100	5.9%	20100	11%	25800	11%	19800	5.9%	18000	5.1%

[a] Surrogate interference not quantified, nd < 0.1 μg g^{-1}.

contained only trace amounts (<0.2%) of TDCPP. The block from Gym#2 (#2a) exhibited the greatest level of Σpenta-BDEs (5620 μg g^{-1}) (Table 1), representing 33.6% of its total FR content (Fig. 1). Sample #4a and 4b blocks, judged oldest by visual inspection, also contained Σpenta-BDEs: 179 μg g^{-1} and 492 μg g^{-1}, respectively, representing 1.5% and 2.4% of their total FR contents (Fig. 1). TBB and TBPH were the dominant FRs detected in the block from Gym#2, contributing 61.5% of the total FR content. In the two "older" (#4a, #4b) blocks, TBB and TBPH comprised 90% and 95% of the total FR content (10,900 μg g^{-1} and 19,000 μg g^{-1}), respectively (Fig. 1). The TBB: TBPH ratio in Firemaster 550 has been reported to be 4:1 (Stapleton et al., 2008), within our blocks ranged from 2:1 to 6:1. This indicates either degradation of the parent compound or use of alternative formulations.

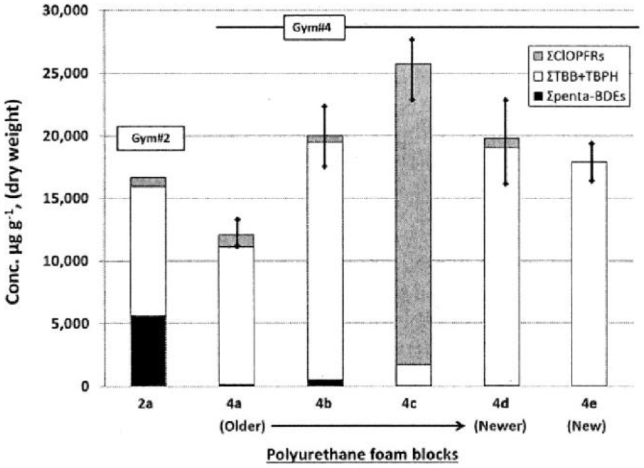

Fig. 1. Polyurethane foam block FR mean (n = 3) totals, contributions and %RSD (solid bar).

3.2. FRs in dust from gyms and homes

3.2.1. Quality control (QC) overview for settled dust

Surrogate recoveries for *d*TDCPP were good (mean 115%, SD 30%) for each of the dust samples. Recoveries of surrogate BDE-166 for 7 of the 8 samples were also good, mean 96%, SD 18%, recovery for Gym#2 dust was not quantifiable (Table 2). As discussed previously, this anomaly was related to high innate PBDE levels in the dust, resulting in coelution of congener BDE-154 with the surrogate (BDE-166). Interference with BDE-166 could be reduced by reducing the sample amount extracted (e.g., to less than 100 mg), but this might compromise the representative nature of the sub-sample. Therefore, as in the case for the foam samples, this dust was not reanalyzed. All analytes (except those that elute in the second fraction of sample Gym#2) were corrected based on surrogate recoveries (Table 2). A laboratory blank was analyzed with each set of gym and residential dust samples extracted. TBB and TBPH were the only analytes detected above the detection limit (0.1 μg g^{-1}) in the blank associated with the gym dust samples. These values (0.2 and 0.1 μg g^{-1}, respectively) were <2% of concentrations detected in the gym dust samples, and were subtracted from results Gym#1–#4.

Table 2
House and gym dust flame-retardant concentrations (µg g^{-1}, dry weight) and statistical analysis (2-sample T-test, $P < 0.05$). Shaded columns indicate FRs commonly used in the treatment of polyurethane foam.

Analytes	House #1	House #2	House #3	House #4	Mean (house)[a]	Gym #1	Gym #2	Gym #3	Gym #4	Mean (gym)[a]	Mean (>) conc.[b]	2-sample T[c]
BDE-28	nd	nd	nd	nd	0.05	nd	nd	nd	nd	0.05	*	–
BDE-47	2.6	0.7	2.8	1.4	1.88	0.8	138	6.2	30.3	43.8	G	0.141
BDE-66	nd	nd	nd	nd	0.05	nd	1.9	nd	0.2	0.55	G	–
BDE-85	0.2	0.1	0.3	0.1	0.18	0.1	18.3	1.1	1.4	5.23	G	0.165
BDE-100	0.9	0.4	1.0	0.4	0.68	0.3	51.4	3.0	8.1	15.7	G	0.15
BDE-99	3.9	1.5	5.6	2.3	3.33	1.2	141	17.0	40.4	49.9	G	0.118
BDE-154	0.5	nd	0.7	0.3	0.39	0.1	26.3	2.3	1.7	7.60	G	0.165
BDE-153	0.3	0.2	0.6	0.2	0.33	0.2	29.4	1.6	2.0	8.30	G	0.171
∑penta-BDE					6.89					131		
BDE-183	nd	nd	nd	nd	0.05	nd	nd	nd	0.2	0.09	G	–
BDE-206	nd	nd	0.1	nd	0.06	0.5	0.8	0.1	0.6	0.50	G	–
BDE-209	1.8	2.4	4.3	0.6	2.28	15.7	nd	3.2	20.6	9.89	G	0.07
TBB	1.7	7.1	1.0	0.5	2.58	73.5	13.9	12.3	63.5	40.8	G	0.050[e]
TBPH	1.3	3.1	0.7	2.3	1.85	41.3	6.3	4.8	44.9	24.3	G	0.065
∑TBB+TBPH					4.43					65.1		
BTBPE	nd	nd	0.4	nd	0.14	nd	nd	0.1	nd	0.06	H	–
DBDPE	0.4	0.6	2.9	0.2	1.03	1.1	nd	0.5	0.1	0.44	H	0.224
α-HBCD	nd	nd	nd	nd	0.05	nd	nd	nd	nd	0.05	*	–
β-HBCD	nd	nd	nd	nd	0.05	nd	nd	nd	nd	0.05	*	–
γ-HBCD	nd	nd	nd	nd	0.05	nd	nd	nd	nd	0.05	*	–
% recovery (BDE-166)	88%	75%	94%	76%	–	89%	–[d]	106%	134%	–	–	–
TCEP	3.6	0.3	5.1	1.0	2.50	0.7	1.6	1.8	0.6	1.18	H	0.168
TCPP	20.2	6.6	86.8	27.8	35.4	2.3	10.9	2.6	1.5	4.33	H	0.09
TDCPP	17.9	7.5	27.1	2.7	13.8	458	578	334	69.5	360	G	0.025[e]
∑ClOPFRs					51.7					366		
TBBPA	nd	nd	nd	nd	0.05	0.9	0.2	0.9	0.7	0.68	G	–
% recovery (dTDCPP)	98%	90%	160%	139%	–	121%	99%	127%	155%	–	–	–
∑FR (mean)	–	–	–	–	66.8	–	–	–	–	574	G	–

nd < 0.1 µg g^{-1}, [a] nd were substituted with values of one-half the detection limit (0.05 µg g^{-1}), [b] Greater (>) analyte mean concentration for the Gym (G), House (H) or no difference (*) [c] Statistical analysis (2-sample T-test) was completed for each analyte sample set (i.e., gym and house) containing three or more results > nd, [d] Surrogate interference, not quantified [e] Significantly greater $P < 0.05$.

3.2.2. Settled dust analysis

Seven of the 22 targeted FRs (i.e., BDE-28, -66, -183, TBBPA and α-, β-, γ-HBCD) were not detected in the house dust samples above detection limits. Four (i.e., BDE-28 and α-, β-, γ-HBCD) were not detected in the gym dust samples (Table 2). BDE-28 and -66 are minor constituents of the penta-BDE technical formulation, DE-71 (Chemtura Corp., formally Great Lakes Chemical Corp., West Lafayette, IN), ~0.25%; La Guardia et al. (2006). BDE-183 is a major component of the octa-BDE formulation used in wire casings. TBBPA is primarily used as a reactive FR in electronics and HBCD is most commonly used in polystyrene insulation boards. Thus, our failure to detect these seven FRs in the dust was not unexpected.

A total of 18 FRs were detected in the gym dust, 14 at concentrations higher than measured in dust from the coaches' homes (Table 2). The total mean gym dust concentration (∑FR 574 µg g^{-1}) was nearly an order of magnitude higher, than from the homes (66.8 µg g^{-1}). A noticeable difference was also observed between the gym and house dust FR profiles (Fig. 2). This may reflect the types, amounts and age of FR treated products within these environments. For example, the dominant FRs detected in gym dust by Carignan et al. (2013) were constituents of the penta-BDE formulation (median range 370 to 950 µg g^{-1}), which may have been contributed by the penta-BDE treated polyurethane blocks present in the gyms. We observed ClOPFRs, also used to treat polyurethane foam, were dominant FRs in both the house and gym dust. TDCPP contributed 63% to the gym mean total, while TCPP contributed 53% to the house mean total (Fig. 2). Similar house dust concentrations (ΣFRs range 3.1–121 µg g^{-1}, mean 25.9 µg g^{-1}) and compositional profiles (e.g., TCPP contributed 65% to the total mean) were also observed by Schreder and La Guardia (2014) in dust collected from homes located in the state of Washington, USA. This

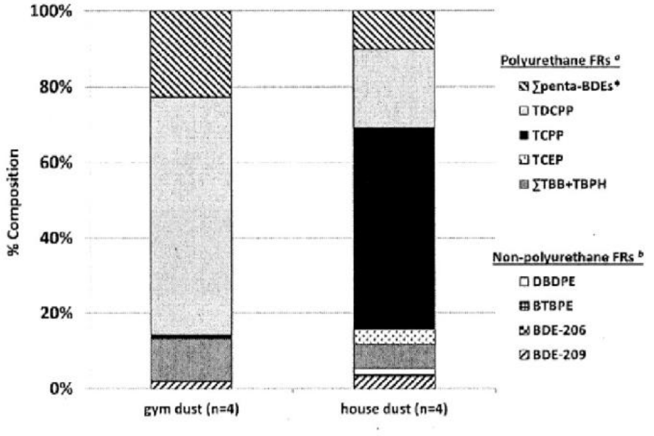

Fig. 2. Gym and house dust FR percent composition (∑penta-BDEs, BDE-47, -85, -99, -100, -153 and -154. [a] FRs used to treat polyurethane. [b] FRs not used in the treatment of polyurethane.).

may be related to the replacement of TDCPP with TCPP in new consumer products. TDCPP is a suspected carcinogen and scheduled to be discontinued by the end of 2015 by its major U.S. producer ICL Industrial Products (ICL-IP) (ICL, 2012). According to U.S. EPA's 2014 Chemical Data Reporting (CDR) nearly 20,000 tonnes of TCPP is produced by ICL-IP annually, an increase of over 5000 tonnes from the previous 2010 CDR reporting and nearly three times that of TDCPP (USEPA, 2014). Our higher FR gym dust levels were similar to dust levels observed by Allen et al. (2013), in commercial aircraft (median 502 μg g^{-1}) in which components must meet stringent aviation flammability standards. However, compositionally those aircraft-associated dusts were dominated by BDE-209 (>98%), with TDCPP contributing <1%.

Mean concentrations of TBB and TDCPP in gym dust were significantly higher than dust concentrations from the homes of the coaches (2-sample T-test, $P < 0.05$) (Table 2). The mean TBPH dust concentrations from gyms and homes were 24.3 and 1.85 μg g^{-1}, respectively. These means were not significant due to the high variability (range 4.8 to 44.9 μg g^{-1}) in the gym samples (Table 2). TBBPA was only detected in the gym dust samples (range 0.2–0.9 μg g^{-1}). Schreder and La Guardia (2014) detected TBBPA in 95% of their Washington state house dust samples (range <0.1 to 6.6 μg g^{-1}), which is statistically similar to the gym dust levels. The mean of the major congeners of the penta-BDE formulation (i.e., the sum of BDE-47, -85, -100, -99, -153) contributed 23% or 123 μg g^{-1} to the gym dust's, compared to 10% or 6.4 μg g^{-1} to the residential dust. Interestingly, our Σpenta-BDE gym dust levels (131 μg g^{-1} mean, range 2.80 to 406 μg g^{-1}) were lower than those observed by Carignan et al (2013), but higher than those reported by Allen et al. (2013) in aircraft dust (median 2.5 μg g^{-1}, range 0.8 to 64.2 μg g^{-1}), and Christiansson et al. (2008) (mean 75.3 μg g^{-1}, range 0.33 to 586 μg g^{-1} (*24*)). Christiansson et al. (2008) also reported elevated blood levels of penta-BDEs (i.e., BDE-28, -99, -100, -153 and -154) in nine passengers following an intercontinental flight compared to pre-flight blood levels. However, mean concentrations of the individual penta-BDEs detected in our gym dust were not significantly greater ($P > 0.05$) than those detected in dust from the coaches' homes (Table 2). Also, mean BDE-183 dust concentrations (<0.1 μg g^{-1} home, 0.1 μg g^{-1} gym) and its replacement BTBPE (0.1 μg g^{-1} home, 0.1 μg g^{-1} gym) and BDE-209 (2.3 μg g^{-1} home, 9.9 μg g^{-1} gym) and its replacement DBDPE (1.0 μg g^{-1} home, 0.4 μg g^{-1} gym), were not statistically greater at the gym (Table 2). Note that these four FRs are used primarily in thermoplastics and not in the polyurethane foam, common in gymnast equipment (Section 3.1.2. *Foam block analysis*).

3.3. Air (gym and home)

3.3.1. Quality control (QC) overview

Prior to sample collection five MultiDust® foam disc and glass fiber filters from each batch supplied from the manufacture were analyzed for FRs. None of the 22 targeted FRs were detected above the detection limit (0.1 ng m^{-3}). However, during the analysis of the air samples (respirable and inhalable) several PBDE congeners (i.e., BDE-47, -99,

Table 3
House and gym air (respirable, <4 μm) flame-retardant concentrations (ng m^{-3}) and statistical analysis (2-sample T-test, $P < 0.05$). Shaded columns indicate FRs commonly used in the treatment of polyurethane foam.

Analyte	Blank	House#1	House#2	House#3	House#4	Mean (house) [a]	Gym#1	Gym#2	Gym#3	Gym#4	Mean (gym) [a]	Mean (>) conc. [b]	2-sample T [c]
BDE-28	nd	nd	nd	nd	nd	0.05	nd	nd	nd	nd	0.05	*	–
BDE-47	3.0	2.1	nd	2.7	8.5	3.34	nd	5.7	4.2	2.3	3.06	H	0.452
BDE-66	nd	nd	nd	nd	nd	0.05	nd	nd	nd	nd	0.05	*	–
BDE-85	nd	nd	nd	nd	nd	0.05	nd	nd	nd	nd	0.05	*	–
BDE-100	6.8	1.0	nd	1.6	1.5	1.04	1.8	0.6	5.5	1.6	2.38	G	0.161
BDE-99	4.9	1.2	nd	nd	11.1	3.10	nd	7.7	9.6	4.8	5.54	G	–
BDE-154	nd	nd	nd	nd	nd	0.05	nd	nd	nd	nd	0.05	*	–
BDE-153	0.7	1.5	nd	nd	3.0	1.15	nd	6.7	nd	1.0	1.95	G	–
Σpenta-BDE						8.83					13.1		
BDE-183	nd	nd	nd	nd	nd	0.05	nd	nd	nd	nd	0.05	*	–
BDE-206	nd	nd	nd	nd	nd	0.05	nd	nd	nd	nd	0.05	*	–
BDE-209	6.9	9.1	nd	6.0	nd	3.80	nd	13.4	8.3	3.9	6.41	G	–
TBB	6.4	14.2	nd	16.2	55.2	21.4	nd	20.2	7.2	18.2	11.4	H	0.245
TBPH	2.9	nd	nd	9.0	18.6	6.93	nd	5.6	3.2	12.8	5.41	H	–
ΣTBB+TBPH						28.3					16.8		
BTBPE	nd	nd	nd	nd	nd	0.05	nd	nd	nd	nd	0.05	*	–
DBDPE	nd	nd	nd	12.3	8.2	5.15	nd	nd	nd	nd	0.05	H	–
α-HBCD	nd	nd	nd	nd	nd	0.05	nd	nd	nd	nd	0.05	*	–
β-HBCD	nd	nd	nd	nd	nd	0.05	nd	nd	nd	nd	0.05	*	–
γ-HBCD	nd	nd	nd	nd	nd	0.05	nd	nd	nd	nd	0.05	*	–
% recovery (BDE-166)	92%	97%	89%	93%	69%	–	97%	91%	83%	78%	–	–	–
TCEP	nd	nd	nd	nd	nd	0.05	nd	nd	nd	nd	0.05	*	–
TCPP	nd	nd	nd	nd	nd	0.05	nd	nd	nd	nd	0.05	*	–
TDCPP	nd	nd	nd	nd	26.4	6.64	32.0	36.5	34.2	34.5	34.3	G	–
ΣClOPFRs						6.74					34.4		
TBBPA	d	nd	nd	nd	nd	0.05	nd	nd	nd	nd	0.05	*	–
% recovery (*d*TDCPP)	75%	121%	96%	114%	87%	–	98%	94%	93%	84%	–	–	–
ΣFR (mean)	–	–	–	–	–	53.2	–	–	–	–	71.2	G	–

nd < 0.1 ng m^{-3}, [a] nd were substituted with values of one-half the detection limit (0.05 ng m^{-3}), [b] Greater (>) analyte mean concentration for the Gym (G), House (H) or no difference (*)
[c] Statistical analysis (2-sample T-test) was completed for each analyte sample set (i.e., gym and house) containing three or more results > nd.

-100, -153 and -209), TBB and TBPH were detected in the laboratory blank (Table 3). These values were subtracted from the results in the respirable and inhalable (house and gym) air samples. Surrogate recoveries were good for both silica gel fractions of the respirable and inhalable air samples (BDE-166 mean 91%, SD 11% and *d*TDCPP mean 105%, SD 15%). All results were corrected for surrogate recoveries.

3.3.2. Respirable air particulate (<4 μm) analysis

Several PBDEs (i.e., BDE-28, -66, -85, -154, -183 and -206), BTBPE, HBCD (α-, β-, γ-), TCEP, TCPP and TBBP-A were not detected above the detection limit (0.1 ng m^{-3}) in either gym or house respirable air samples (Table 3). The mean total FR concentration in respirable gym air was 34% higher than respirable home air, 71.2 and 53.2 ng m^{-3}, respectively. TDCPP was detected in one house respirable air sample, at 26.4 ng m^{-3} and detected in all of the gym samples, ranging from 32.0 to 36.5 ng m^{-3} (Table 3), contributing 49% of the respirable air FR content. TBB and TBPH were the major FRs detected in the home respirable air samples, contributing 60% to the total mean (Fig. 3). ΣPBDEs (BDE-47, -85, -99, -100, -153 and -209) were also detected in respirable air contributing 27% (gym) and 26% (home) to the total FR means (Table 3). DBDPE was not detected in the respirable particle fraction of gym air. DBDPE was only detected in two house respirable air samples: 12.3 and 8.2 ng m^{-3} (Table 3).

3.3.3. Inhalable air particulate (>4 μm) analysis

Total mean FRs detected in the inhalable particulate fraction of air from the gyms were 10-fold higher, 779 ng m^{-3}, than the respirable air fraction of the gyms. Higher FR levels were also observed in larger air particulates collected at an electronic recycling facility (Julander et al., 2005). Wei et al. (2009) found that >80% of total PBDEs were associated with the <150 μm fraction of indoor home and car dust (pre-sieved to <420 μm). This has ramifications as to the relative route of exposure, as larger particulates do not penetrate into the gas-exchange region of the lung, but may be absorbed through the mucus membranes of the respiratory and digestive tracts. Along with the nine FRs detected in the respirable air fraction several additional FRs were detected in the inhalable particulate air samples (i.e., BDE-66, -85,-206 and TCPP). However, BDE-28, -154, -183, BTBPE, HBCD (α-, β-, γ-), TCEP, TBBP-A and DBDPE were not detected above detection limit (0.1 ng m^{-3}) in either gym or home samples (Table 4). Both house and gym inhalable air samples were dominated by TCPP and TDCPP, contributing 65% and 94% to the total FR means, respectively (Fig. 3). Like the respirable air, the mean total FR inhalable air levels were slightly higher in the gym (779 ng m^{-3}) compared to the home, mean 624 ng m^{-3}. However, TCPP was the dominant FR in one house sample (house#1, 1360 ng m^{-3}), contributing 91% to the total (Table 4). TCPP also was the major FR detected in the settled house dust (Section 3.2.2. *Settled dust analysis*) but, concentrations did not correlate with house total (respirable and inhalable) air particulate analysis, $R^2 = 0.059$. Similar poor correlations between air and settle house dust were also observed for the other FRs. However, within the gymnasiums the concentrations of the major constituents of the penta-BDE formulation (i.e., BDE-47, -99, -100 and -153) were strongly correlated ($R^2 > 0.603$) between dust and total air particulates. BDE-209, TBB, TBPH, TCPP and TDCPP levels in gymnasium dust did not correlate well with gymnasium air totals ($R^2 < 0.350$). These observations may be related to partitioning of penta-BDEs released from older gym products approaching steady-state between dust and air particulates. In contrast, newer polymer products that contained alternative-FRs (e.g., TBB and TBPH components of Firemaster 550) may not have yet neared this condition. However, caution should be exercised when interpreting results due to small sample size (n = 4) and variations (e.g., amount and age of equipment) between locations.

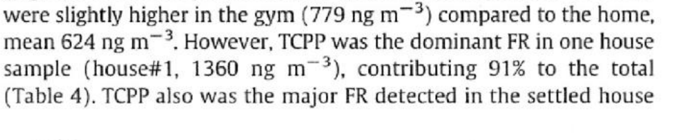

Fig. 3. Gym and house air (respirable and inhalable) FR percent composition (∑ penta-BDEs, BDE-47, -85, -99, -100, -153 and -154. [a] FRs used to treat polyurethane. [b] FRs not used in the treatment of polyurethane.).

TDCPP concentrations in inhalable air samples from the gyms ranged from 125 to 397 ng m^{-3} (Table 4). The mean TDCPP level in the gym inhalable air samples was significantly greater ($P = 0.022$) than in the home samples (Table 4). The mean penta-BDE level (sum of BDE-47, -85, -100, -99 and -153) in the gym inhalable air samples (mean 82.5 ng m^{-3}, range 35.8–166 ng m^{-3}) was slightly higher than those detected by Carignan et al. (2013) (mean 69.1 ng m^{-3}, range 19.7–119 ng m^{-3}) and over 15-times higher than in the home samples (mean 4.85 ng m^{-3}, range 0.85–4.85 ng m^{-3}); contributing 11% and 0.7% to the mean total FR contents, respectively (Fig. 3). Although, BDE-66 and -206 were only detected in one inhalable air sample from the homes and not in the gyms, the concentrations of BDE-47, -85, -100, -99, -153 and -209 were greater in the gym-derived samples (Table 4). But only, BDE-100 was significantly greater ($P = 0.039$) (Table 4). Strid et al. (2014) detected several PBDEs, including those of the deca-BDE formulation, in cockpit air samples collected during flight. Although those levels were lower than our gym air samples, and closer to air levels detected in homes of the coaches (*This could be related to the type of sampler and lower flow rate used by Strid* et al.), the pilots and cabin crew had BDE-153 and -154 blood levels significantly higher than the blood levels of their control group which included 31 individuals working in schools, local environmental and health administration offices without any known occupational exposure to FRs. Carignan et al. (2013) also detected several congeners of the penta-BDE formulation in blood samples of collegiate female gymnasts (n = 11), their BDE-153 mean concentration was 4–6.5 times higher than the general U.S. population.

The mean inhalable air concentration of TBB was also significantly greater ($P = 0.026$) from the gyms than the homes (Table 4). TBB inhalable air concentrations at the gym ranged from 46.4 to 220 ng m^{-3}, with a mean of 143 ng m^{-3}. In the homes levels ranged from 2.6–55.4 ng m^{-3}, with a mean of 22.0 ng m^{-3} (Table 4). TBPH concentrations were not significantly greater ($P = 0.120$). TBPH concentrations in the inhalable air from the gyms ranged from 4.9 to 71.9 ng m^{-3}, with a mean of 34.3 ng m^{-3}, while home levels ranged from <0.1 to 18.3 ng m^{-3}, with a mean of 8.61 ng m^{-3} (Table 4).

3.4. Physical activity exposure rate

For some occupations the work environment may exacerbate exposure, e.g., personnel working within FR-laden aircraft (Allen et al., 2013; Christiansson et al., 2008). Elevated PBDE blood levels have also been reported in collegiate gymnasts, attributed to the high amount of FR-containing materials within training facilities (Carignan et al., 2013). Here, we also reported generally higher FR dust and air levels in gymnastic studios than the homes of coaches employed by these gyms (Section 3.2.2. Dust analysis and 3.3.3. Inhalable air particulate (>4 μm) analysis). Human activities within these environments may also contribute to exposure. For example, children (<6 years old) ingest 2 to 3-times more dust than adults (>21 years old) (USEPA, 2011). This

Table 4

House and gym air (inhalable, >4 μm) flame-retardant concentrations (ng m^{-3}) and statistical analysis (2-sample T-test, $P < 0.05$). Shaded columns indicate FRs commonly used in the treatment of polyurethane foam.

Analyte	House#1	House#2	House#3	House#4	Mean (house) [a]	Gym#1	Gym#2	Gym#3	Gym#4	Mean (gym) [a]	Mean (>) conc. [b]	2-sample T [b]
BDE-28	nd	nd	nd	nd	0.05	nd	nd	nd	nd	0.05	*	–
BDE-47	1.9	2.3	1.4	0.2	1.45	20.2	82.7	10.1	19.0	33.0	G	0.078
BDE-66	nd	nd	nd	1.5	0.41	nd	nd	nd	nd	0.05	H	–
BDE-85	nd	nd	nd	2.0	0.54	3.0	2.6	1.6	1.5	2.18	G	–
BDE-100	1.9	0.5	1.3	1.7	1.35	6.9	15.4	5.4	4.4	8.03	G	0.039 [d]
BDE-99	nd	4.3	0.5	nd	1.23	44.6	58.5	18.5	20.1	35.4	G	–
BDE-154	nd	nd	nd	nd	0.05	nd	nd	nd	nd	0.05	*	–
BDE-153	nd	nd	0.3	0.7	0.28	6.1	5.1	1.3	3.1	3.90	G	–
Σpenta-BDE					5.36					82.7		
BDE-183	nd	nd	nd	nd	0.05	nd	nd	nd	nd	0.05	*	–
BDE-206	nd	nd	nd	1.6	0.44	nd	nd	nd	nd	0.05	H	–
BDE-209	nd	3.8	nd	0.1	1.00	7.6	8.2	7.7	9.4	8.23	G	–
TBB	55.4	16.9	13.0	2.6	22.0	178	220	46.4	129	143	G	0.026 [d]
TBPH	18.3	6.6	9.5	nd	8.61	5.5	71.9	4.9	54.8	34.3	G	0.12
ΣTBB+TBPH					30.6					177		
BTBPE	nd	nd	nd	nd	0.05	nd	nd	nd	nd	0.05	*	–
DBDPE	nd	nd	nd	nd	0.05	nd	nd	nd	nd	0.05	*	–
α-HBCD	nd	nd	nd	nd	0.05	nd	nd	nd	nd	0.05	*	–
β-HBCD	nd	nd	nd	nd	0.05	nd	nd	nd	nd	0.05	*	–
γ-HBCD	nd	nd	nd	nd	0.05	nd	nd	nd	nd	0.05	*	–
% recovery (BDE-166)	106%	88%	99%	93%	–	114%	93%	76%	91%	–	–	–
TCEP	nd	nd	nd	nd	0.05	nd	nd	nd	nd	0.05	*	–
TCPP	1360	209	294	279	536	184	219	525	136	266	H	0.21
TDCPP	61.3	32.0	69.2	37.7	50.1	397	213	125	240	244	G	0.022 [d]
ΣClOPFRs					586					510		
TBBPA	nd	nd	nd	nd	0.05	nd	nd	nd	nd	0.05	*	–
% recovery (*d*TDCPP)	116%	102%	128%	108%	–	127%	105%	119%	114%	–	–	–
ΣFR (mean)	–	–	–	–	624	–	–	–	–	779	G	–

nd < 0.1 ng m^{-3}, [a] nd were substituted with values of one-half the detection limit (0.05 ng m^{-3}), [b] Greater (>) analyte mean concentration for the Gym (G), House (H) or no difference (*) [c] Statistical analysis (2-sample T-test) was completed for each analyte sample set (i.e., gym and house) containing three or more results > nd, [d] Significantly greater $P < 0.05$. Blank results are listed on Table 3.

results in greater PBDE exposure in children than their parents, likely in part through greater hand-to mouth transfer behavior of children (Lunder et al., 2010; Stapleton et al., 2012). In addition, specific activities (e.g., coaching) and greater physical exertion with attendant elevated breathing rates likely contributes to heightened FR exposure. According to U.S. EPA's 2011 Exposure Factors Handbook (USEPA, 2011), the sedentary ventilation rate (i.e., when sitting) for females 21–31 years of age (the age range of coaches included in this study) is 0.004 m^3 min^{-1} (50th percentile). During moderate intensity activities (e.g., walking) this rate increases over 5-fold (0.0219 m^3 min^{-1}), and up to 10-fold (0.0431 m^3 min^{-1}) during high intensity activities (e.g., jogging/running). Therefore, along with environmental monitoring, activities within these spaces should also be considered when assessing FR exposure.

4. Conclusion

The polyurethane foam blocks in gymnastic pits contained multiple FRs at total concentrations of 12,100 to 25,800 μg g^{-1}, or 1.2% to 2.6% by weight. TBB and TBPH were the major flame-retardants in four of the five pit blocks from Gym#4, the fifth block contained primarily TDCPP. TBB and TBPH were also detected in the block from Gym#2, which also contained congeners of the penta-BDE formulation contributing 33.6% (Σpenta-BDEs) of the total FR load, respectively. Penta-BDEs were detected in two of the "older" blocks from Gym#4 as well, contributing < 2.4% of their totals. Although, polyurethane foam blocks appear to be a likely source of FRs within the gym, other foam-containing products (e.g., landing mats and equipment padding) which were not tested, were also present in each gym and may be additional sources.

To determine if occupational exposure to FRs are potentially greater at gymnastic facilities versus non-occupational exposure, gymnasium floor dust was analyzed and FR levels compared to those in house dust from four coaches' homes. The mean of the total FR levels detected at the gyms were 8.6-fold higher (574 μg g^{-1}) than the total mean detected at the homes (66.8 μg g^{-1}). Of the 22 targeted FRs analyzed, 18 were detected in gym and 15 in house dusts. Two FRs (i.e., TBB and TDCPP) used primarily to treat polyurethane foam were significantly greater ($P < 0.05$) in gym than house dust. TCPP and TDCPP also used to treat polyurethane foam were also the primary FRs detected in gym and house dust, contributing 78% and 64% to the mean total FR burdens, respectively. *Please note that dust from the foam pit was not collected or considered in exposure models and that exposure may be higher when activities involve the loose foam pit.*

Although, FR concentrations in settled dust and air particulates were only correlated for a few penta-BDE constituent congeners in the gymnast studios, analysis of airborne particulate samples indicated higher mean levels (both respirable and inhalable) at the gyms than the homes for BDE-99, -100, -153, -209 and TDCPP. The Deca-BDE replacement DBDPE was the only FR detected at higher concentrations at the homes in both respirable and inhalable air fractions. The polyurethane additives BDE-100, TBB and TDCPP were likewise significantly greater ($P < 0.05$) in the inhalable air fraction at the gyms than in the homes.

These were also among the most abundant FRs detected in the gym floor dust. However, compared to the polyurethane foam blocks, PBDEs in settled dust were at considerably lower proportions than TDCPP and TBB, suggesting alternative dust sources, e.g., residuals of previously-used PBDE-containing products within the gym or differential partitioning of the compounds. These findings indicate that there are multiple sources and types of FRs within gymnastic studios and that exposure rate of occupants are likely higher than in the home. In addition, activities of gymnasts and training personnel within these facilities may further accentuate exposure.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.envint.2015.02.014.

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Article

Human Indoor Exposure to Airborne Halogenated Flame Retardants: Influence of Airborne Particle Size

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Abstract: Inhalation of halogenated flame-retardants (HFRs) released from consumer products is an important route of exposure. However, not all airborne HFRs are respirable, and thus interact with vascular membranes within the gas exchange (alveolar) region of the lung. HFRs associated with large (>4 μm), inhalable airborne particulates are trapped on the mucosal lining of the respiratory tract and then are expelled or swallowed. The latter may contribute to internal exposure via desorption from particles in the digestive tract. Exposures may also be underestimated if personal activities that re-suspend particles into the breathing zone are not taken into account. Here, samples were collected using personal air samplers, clipped to the participants' shirt collars (n = 18). We observed that the larger, inhalable air particulates carried the bulk (>92%) of HFRs. HFRs detected included those removed from commerce (i.e., polybrominated diphenyl ethers (Penta-BDEs: BDE-47, -85, -100, -99, and -153)), their replacements; e.g., 2-ethylhexyl 2,3,4,5-tetrabromobenzoate (TBB or EH-TBB); bis(2-ethylhexyl) 3,4,5,6-tetrabromophthalate (TBPH or BEH-TEBP) and long-produced chlorinated organophosphate-FRs (ClOPFRs): tris(2-chloroethyl)phosphate (TCEP), tris(1-chloro-2-propyl)phosphate (TCPP or TCIPP), and tris(1,3-dichloro-2-propyl)phosphate (TDCPP or TDCIPP). Our findings suggest estimates relying on a single exposure route, i.e., alveolar gas exchange, may not accurately estimate HFR internal dosage, as they ignore contributions from larger inhalable particulates that enter the digestive tract. Consideration of the fate and bioavailability of these larger particulates resulted in higher dosage estimates for HFRs with log $K_{oa} < 12$ (i.e., Penta-BDEs and ClOPFRs) and lower estimates for those with log $K_{oa} > 12$ (i.e., TBB and TBPH) compared to the alveolar route exposure alone. Of those HFRs examined, the most significant effect was the lower estimate by 41% for TBPH. The bulk of TBPH uptake from inhaled particles was estimated to be through the digestive tract, with lower bioavailability. We compared inhalation exposure estimates to chronic oral reference doses (R*f*Ds) established by several regulatory agencies. The U.S. Environmental Protection Agency (EPA) R*f*D levels for several HFRs are considered outdated; however, BDE-99 levels exceeded those suggested by the Dutch National Institute for Public Health and the Environment (RIVM) by up to 26 times. These findings indicate that contributions and bioavailability of respirable and inhalable airborne particulates should both be considered in future risk assessments.

Keywords: flame retardants; particle size; indoor air quality; human exposure; polyurethane; bioavailability

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1. Introduction

Halogenated flame-retardants (HFRs), such as polybrominated diphenyl ethers (PBDEs), are used in a wide array of polymer-containing consumer products, and have been widely detected in indoor environments (e.g., air, dust, and surface films) [1–3]. To meet Californian (USA) 1975 home furniture flammability standards (Technical Bulletin 117 or TB 117 [4]), the Penta-BDE technical formulation, formally marketed as DE-71 (Great Lakes Chemical Corp. now Chemtura Corp., Philadelphia, PA, USA) and Bromkal 70-5DE (Chemische Fabrik Kalk, Köln, Germany), among others, were added to furniture padding (polyurethane foam) to slow ignition and the spread of household fire. These Penta-BDE formulations contained PBDEs with primarily 4–6 bromines [5]. Due to human and environmental health concerns, PBDEs of the Penta- and Octa-BDE technical formulations were added in May 2009 to the Persistent Organic Pollutants (POPs) list of the Stockholm Convention, restricting their usage globally in signatory countries [6]. However, PBDEs will continue to be released into the environment from products with long lifetimes such as furniture and automobiles. Recycled materials may also reintroduce PBDEs. For example, carpet underlayment (known as rebond) may contain up to 1000 ppm (0.1% by weight) of Penta-BDEs [7]. Usage restrictions have led to the introduction of alternative halogen-containing flame retardants, including 2-ethylhexyl 2,3,4,5-tetrabromobenzoate (TBB or EH-TBB) and bis(2-ethylhexyl) 3,4,5,6-tetrabromophthalate (TBPH or BEH-TEBP). Usage of chlorinated organophosphate flame retardants (ClOPFRs) also increased, including tris(1-chloro-2-propyl)phosphate (TCPP or TCIPP) and its analog tris(1,3-dichloro-2-propyl)phosphate (TDCPP or TDCIPP). TDCPP is a suspected mutagen, formerly used in children's sleepwear until its voluntarily withdrawal in 1977 [8]. (The nomenclature for HFRs is listed within the Supplementary Materials Table S1). These and other HFRs, some lacking comprehensive toxicity testing, have now been detected in homes and other indoor environments [9].

Partitioning of HFRs between the gas and particle-phases has been described by the gas/particle partition coefficient, K_p ($m^3 \cdot \mu g^{-1}$), ($K_p = (C_p/C_{TSP})/C_g$), where C_p and C_g are the organic compound concentrations ($pg \cdot m^{-3}$) in the particle and gas phases, respectively, and C_{TSP} is the concentration of total suspended particles in the air ($\mu g \cdot m^{-3}$). Cetin et al. (2008) [10] observed a strong correlation (R^2 = 0.70–0.98) between log K_p and log octanol-air partitioning coefficient (K_{oa}) for several PBDEs on airborne organic matter. Using the K_{oa}-based equilibrium model, Cetin et al. (2008) observed at indoor temperatures (25 °C) that 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) (log K_{oa} 10.5), with 4 bromines, reaches equilibrium within 100 days with >80% residing in the particle phase at equilibrium. In contrast, PBDEs with >5 bromines (including the fully brominated diphenyl ether, BDE-209, log K_{oa} 18.4) require 100–1000 days, with >95% residing in the particle phase. Several additional adsorption models (i.e., Mackey and Junge-Pankow) have also described atmospheric gas-particle partitioning and suggest HFRs with a log $K_{oa} > 11$ have a greater tendency to reside on organic particulates (HFRs log K_{oa} and their partitioning coefficients are listed on Table S1). However, An et al. (2011) [11] observed that the Junge-Pankow model underestimated the sorption of most PBDE congeners in indoor air samples collected at electronic waste dismantling and incineration plants. They reported that PBDE air concentrations during working hours were 25 times higher than during non-working hours. Allen et al. (2007) [12] observed clouds of suspended particles generated by office workers' activities (e.g., walking), resulting in greater air levels of PBDEs. Using exposure monitors affixed to individuals, Ferro et al. (1999) [13] observed that normal human activities increased levels of airborne particle matter (PM) >10 μm by 6–11 times and coarse PM (2.5–10 μm) by 2–11 times, compared to stationary indoor monitors during active periods. They observed little difference in levels of fine PM (<2.5 μm) [13,14].

Larger particles may also carry higher HFR burdens, associated with polymer fragments. Polymers often contain percent by weight levels of HFR additives. Using a stainless-steel test chamber, Rauert and Harrad (2015) [15] investigated PBDE transfers from source materials (i.e., plastic television casings containing penta-BDEs, 2,2′,3,4,4′,5′,6-heptabromodiphenyl ether (BDE-183) and BDE-209). They observed no detectable transfers to dust by volatilization at 22 °C and 60 °C, but did detect transfer from fragments abraded following motion that mimicked repeated wiping/moving/bumping of the source product. Large dust particles, approximately 30 μm in diameter, examined by electron microscopy revealed that BDE-209, having a log K_{oa} of 18.4, was highly associated (1000 ppm or 0.1% by weight) with polymer/organic matrix, suggesting fragments of HFR-treated products are transferred to dust [16]. Hale et al. (2002) [17] reported that Penta-BDE-containing polyurethane became brittle and disintegrated within four weeks of exposure to direct sunlight. These observations, along with partitioning models, suggest that polymer additives with log $K_{oa} > 10$ are less likely to volatilize at room temperature. Once incorporated into carbon-rich organic polymers of consumer products, such HFRs are more likely to remain within this matrix (e.g., polyurethane foam). However, as these polymer products fragment, HFR-containing particulates are released to the indoor environment.

Dietary dust intake via incidental ingestion is widely accepted as the major exposure route for HFRs for most individuals [18–20]. However, air inhalation and dermal absorption are also contributing paths [21,22]. Schreder et al. (2016) [23] estimated inhalation and dust intake exposure of several HFRs for adults and children and observed "exposure may be much higher—by one or more orders of magnitude—than previously believed based on dust ingestion as a primary exposure route" (p. 503). However, total inhalation exposure can be underestimated if the route of contaminant uptake, i.e., alveolar uptake of volatile compounds or uptake via the digestive tract of larger airborne particulates inhaled and swallowed, and the bioavailability of the contaminant dictated by the route of uptake are not taken into account. La Guardia and Hale (2015) [24] and Schreder et al. (2016) [23] detected Penta-BDEs, TBB, TBPH, and ClOPFRs, used to flame-retard polyurethane foam, in indoor dust and air samples. Indoor environments examined included gymnasiums at gymnastics facilities, with their high use of polyurethane foam products to protect against impact injuries, residences of the gymnastics coaches working within these gymnasiums, and a combination of residences, offices, and other locations. Both studies were conducted in the greater Seattle, WA, USA area. All samples were collected using personal exposure monitors, clipped to the participants' shirt collars. This allowed air collection within the participants' breathing zone while they were engaged in daily activities. Two air particulate fractions were obtained with each sample: (1) inhalable particulates (>4 μm, nominal) which deposit primarily in the upper respiratory tract and; (2) respirable air particulates (<4 μm, nominal) which can penetrate deep inside the lung's gas-exchange regions. Both studies included the same HFRs. These display a wide range of log K_{oa} values (5.31 to 16.9, Table S1) which may dictate the particle size tendency and therefore zone of deposition within the respiratory tract.

To expand our understanding of human exposure to airborne HFRs, both data sets (La Guardia and Hale, 2015 [24] and Schreder et al., 2016 [23]) were further analyzed and HFR air particulate levels within each environment were compared. Air particulate fractions (respirable and inhalable) for each HFR were evaluated and total inhalation uptake through the alveolar region of the lung and digestive tract was estimated. These estimates were then compared to total alveolar uptake. Total daily inhalation HFR exposure rates were evaluated and compared to established chronic reference dose (R*f*D) values. Finally, we discuss the human exposure implications of the air fraction exposure model for airborne HFRs in indoor environments.

2. Materials and Methods

2.1. Sampling Demographics

A total of 18 indoor environments were sampled. These included 14 common indoor spaces (i.e., residence/office (n = 10) [23], four coach residences, and four gymnasiums [24]). The gymnasiums contained substantial amounts of flame retardant-treated polyurethane foam, previously associated with increased gymnast PBDE blood levels [20]. Adults (n = 10), primarily office workers, were recruited to collect residence/office/"other" air samples during a 24-h day while engaged in normal activities (e.g., at home, work, traveling to and from home and work, shopping, socializing, and during sleep). Personal samplers (see Section 2.2. Air Sampling Methods) were worn at breathing zone level. Duration of collection ranged from 12.9 to 24.6 h, and air volumes ranged from 1.55 to 2.95 m^3.

Four coaches at four separate gymnasiums were recruited to collect 8-h air samples during a normal workday (e.g., coaching gymnasts) and 8-h air samples at their residences while doing normal daytime indoor activities (e.g., light house cleaning, viewing television, etc.). Time of collection ranged from 6.07 to 8.41 h, and air volumes ranged from 0.728 to 1.01 m^3. All participants resided in greater Seattle, WA, USA.

2.2. Air Sampling Methods

Air particulates were collected using an AirChek 2000 pump (flow rate 2 $L{\cdot}min^{-1}$) with an Institute of Occupational Medicine (IOM) Sampler (SKC, Eighty Four, PA, USA) equipped with a stainless steel cassette assembly. The IOM sampler meets air particulate sampling criteria established by the American Conference of Governmental Industrial Hygienists (ACGIH) and the Occupational Safety and Health Administration (OSHA) [25]. The IOM is capable of collecting two size classes of particulates: inhalable particulates and respirable particulates. Inhalable particulates (>4 μm, nominal), which deposit primarily in the upper respiratory tract, were collected with a MultiDust® foam disc (SKC, Eighty Four, PA, USA) of a specific porosity (D_{50}) of 4 μm (D_{50}: a particle aerodynamic diameter for which 50% of the particles penetrate) (Figure S1, MultiDust® foam disc Certificate of Conformity, performance criteria 85%, ±10%). This was placed inside the stainless steel cassette assembly, positioned at the IOM inlet. Respirable air particulates (<4 μm, nominal), which are able to penetrate deep inside the lung's gas-exchange regions, were collected on a 25-mm, 1.0 μm glass fiber filter placed behind the foam disc within the cassette. (Direction of air flow: The air sample was drawn though the IOM sampler by the AirChek 2000 pump, first passing through the MultiDust® foam disc followed by the glass fiber filter. This configuration keeps the sample from contacting the sampling equipment, preventing contamination by the IOM sample housing and pump.). Sampling equipment and cassettes were cleaned and assembled at the laboratory and stored in double sealed plastic bags prior to shipment to the field. After collection, all sample cassettes were placed in SKC's transportation clip with cover and stored <4 °C in double-sealed plastic bags until analyzed.

The AirChek 2000 pumps were also calibrated at the laboratory prior to shipping. Upon their return, calibrations were verified. All pumps were within manufacturer's specifications (±5% of set-point). Therefore, no adjustments to recorded field values (e.g., collection times or volumes) were made.

2.3. Target Compounds, Extraction, Purification, and Analysis

Air particulate samples (disc and filter) were analyzed for HFRs: Penta-BDEs (BDE-47, -85, -99, -100, and -153); 2-ethylhexyl 2,3,4,5-tetrabromobenzoate (TBB); bis(2-ethylhexyl) 3,4,5,6-tetrabromophthalate (TBPH); and chlorinated organophosphate FRs (ClOPFRs: tris(1,3-dichloro-2-propyl)phosphate (TDCPP or TDCIPP), tris(2-chloroethyl)phosphate (TCEP), and tris(1-chloro-2-propyl)phosphate (TCPP or TCIPP) (Table S1), as described in La Guardia and Hale (2015) [24]. Briefly, disc and glass fiber filters were subjected to accelerated solvent extraction (ASE 200, Dionex, Sunnyvale, CA, USA) with dichloromethane (DCM). Surrogate standards (500 ng of 2,3,4,4′,5,6-hexabromodiphenyl ether (BDE-166); Cambridge Isotope Laboratories, Inc., Andover, MA, USA and 6000 ng of deuterated tris(1,3-dichloro-2-propyl)phosphate (*d*TDCPP); MPI for Biophysical Chemistry, Goettingen, Germany), were added to each sample prior to extraction. Each post-ASE extract was solvent exchanged to hexane, reduced in volume, and added to the top of a solid phase 2-g silica glass extraction column (Isolute, International Sorbent Tech., Hengoed Mid Glamorgan, UK). Columns were eluted with 3.5-mL hexane (fraction 1), followed by 6.5 mL of 60:40 hexane/DCM and 8 mL DCM (fraction 2) and 5 ml 50:50 acetone/DCM (fraction 3). Fraction 2 (containing BFRs) and fraction 3 (containing ClOPFRs) were reduced and transferred to 2 mL maximum recovery vials and reduced to dryness. Samples were reconstituted with 100 μL of methanol containing 800 ng of decachlorodiphenyl ether (DCDE) (AccuStandard, Inc., New Haven, CT, USA) as an internal standard. Analytes in these purified extracts were chromatographically separated by ultra-performance liquid chromatography (UPLC) (Acquity

UPLC, Waters Corporation, Milford, MA, USA), operated in the gradient mode (100% methanol (A1) and 100% water (B1)), equipped with a C_{18} UPLC analytical column (Acquity UPLC BEH C_{18}, 1.7 μm, 2.1 × 150 mm, Waters Corp.). Analytes were ionized by atmospheric pressure photoionization (APPI), the dopant (acetone) was introduced (150 μL/min) by a liquid chromatography pump (LC-20AD, Shimadzu Corporation, Kyoto, Japan), and product ions were detected by triple quadrupole mass spectrometer (MS) (3200 QTrap, AB Sciex, Framingham, MA, USA) by negative ionization operated in the Multiple Reaction Monitoring (MRM) mode. Quantitation ions for BFRs and BDE-166 were m/z 79($[^{79}\mathrm{Br}]^-$) and 81($[^{81}\mathrm{Br}]^-$) and m/z 35($[^{35}\mathrm{Cl}]^-$) and 37($[^{37}\mathrm{Cl}]^-$) for ClOPFRs, *d*TDCPP and DCDE, respectively. Quantitation was established using a five-point calibration curve ($R^2 > 0.995$) for each of the targeted HFRs (analytical standards from AccuStandards, Inc., New Haven, CT, USA). Further details of the UPLC-APPI/MS analysis can be found in Schreder and La Guardia (2014) [26] and La Guardia and Hale (2015) [24].

2.4. Quality Control Overview

Complete synopsis of all quality assurance and quality control (QA/QC) procedures and their results are listed within the original manuscripts [23,24]. Briefly, surrogate recoveries for the air samples from the gymnasiums and coaches' residences from 69% to 97% for BDE-166, from 75% to 121% for *d*TDCPP, and for laboratory blanks, 92% and 75%, respectively. The laboratory blanks did not contain analytes above their limits of detection. Surrogate recoveries for samples collected at the residences/offices were 85% to 115% for BDE-166, 52% to 123% for *d*TDCPP, and 98% and 82% in laboratory blanks respectively. Surrogate recoveries for travel blanks and field samples ranged from 52% to 70% and 61% to 123%, respectively. These blanks also did not contain the targeted analytes above the limits of detection. Each sample was surrogate recovery corrected.

2.5. Statistical Analysis

A one-way ANOVA of means with a Tukey's Honest Significant Difference (HSD) post-hoc test was conducted to compare concentrations of ∑ClOPFRs, ∑PBDEs, and ∑TBB + TBPH in the three environments tested. Data were tested for normality and equality of variance, and significance was set at $p < 0.05$. Because normality cannot be reliably checked with small sample size, caution should be used when interpreting these results; however, visual examination of the probability plots did indicate that the data more closely approached a normal distribution without log transformation. Regression analysis was conducted to assess the association between physical chemical properties, specifically $\log K_{oa}$, and the effect of basing the inhalation exposure estimate on both the inhalable and the respirable particulate fractions. Statistical analyses were performed using Minitab 16.1.0 (Minitab Inc., State College, PA, USA) and Stata v.13 (Stata Corp. LLC, College Station, TX, USA).

3. Results and Discussion

3.1. Comparison of HFR Air Exposure between Different Indoor Environments

La Guardia and Hale (2015) [24] observed Penta-BDEs, TBB, TBPH, and ClOPFRs in both respirable (<4 μm, nominal) and inhalable (>4 μm, nominal) air particulate fractions collected at gymnasiums and at the residences of gymnastics coaches using personal exposure air monitors (IOM samplers). $\Sigma_{\text{inhalable + respirable}}$ ClOPFRs contributed >65% to the ∑HFR ($\Sigma_{\text{inhalable + respirable}}$ HFR: Penta-BDEs, TBB, TBPH, and ClOPFRs) air particulate burdens in the gymnasiums and coaches' residences (Figure 1). Schreder et al. (2016) [23] also observed ClOPFRs in respirable and inhalable air fractions collected by office-workers wearing IOM samplers during a 24-h work day. Air collection areas included the workers' homes, offices, and while commuting between their homes and work. ΣClOPFRs were an even greater contributor to the office-workers' airborne burden, contributing >97% to the ∑HFRs (Figure 1, Penta-BDEs, TBB, and TBPH, results not previously reported, and Table 1). The ∑HFR air burdens within the gymnasiums (835 $\mathrm{ng \cdot m^{-3}}$) were 20% greater than at the coaches' residences (664 $ng \cdot m^{-3}$) and nearly double those collected at the residences/offices (440 $ng \cdot m^{-3}$) (Table 1). The functionality of the indoor space and the amount of HFR-containing products within contribute to indoor HFR dust burdens [1]. This may account for the higher gymnasium HFR air burdens, due to the presence of large amounts of polyurethane foam to reduce impact related injuries [24]. Treated foam products may also form HFR residuals on clothing and personal products used at the gym. These items, if also returned to the coaches' residences, may have influenced the higher HFR levels observed at their residences compared to levels at the residences/offices. Also, lower HFR air levels observed by Schreder et al. (2016) [23] may be associated with a larger sample size and inclusion of non-residential spaces. However, ClOPFRs were the major contributor to the $\sum$HFRs in each of the three test environments; their levels were similar ($p > 0.05$) in the three indoor environments (means 544,592 and 428 $ng \cdot m^{-3}$, respectively). The inhalable fraction (>4 μm) carried >92% of the $\sum$HFRs, while the respirable (<4 μm) carried <8% (Figure 2). Overall, the inhalable ClOPFR fraction contributed >66% to the $\sum$HFRs. Of the ClOPFRs, TCPP in the inhalable fraction was the major contributor to the ΣClOPFR profile at the coaches' residences, contributing 91% at the coaches' residences (536 $ng \cdot m^{-3}$) and 87% (371 $ng \cdot m^{-3}$) in the residence/office samples (Table 1). According to the U.S. EPA Chemical Data Reporting [27]. TCPP production within the U.S. has been on the rise since the mid-1980s. It was equal to that of TDCPP between 1994 and 2006 at 14,000 $tonnes \cdot year^{-1}$, but nearly tripled by 2012 to 38,000 $tonnes \cdot year^{-1}$. TCPP production now exceeds both TCEP and TDCPP, as policies limiting the usage of the latter two compounds have taken effect [23]. However, within the gymnasiums a majority of the polyurethane foam products were installed prior to 2006. Thus, prior market conditions may account for higher TDCPP levels ($\sum$TDCPP, 278 $ng \cdot m^{-3}$) contributing 51%, with TCPP ($\sum$TCPP, 266 $ng \cdot m^{-3}$) contributing nearly half (49%) to the gymnasiums' $\sum$ClOPFR profile (Table 1).

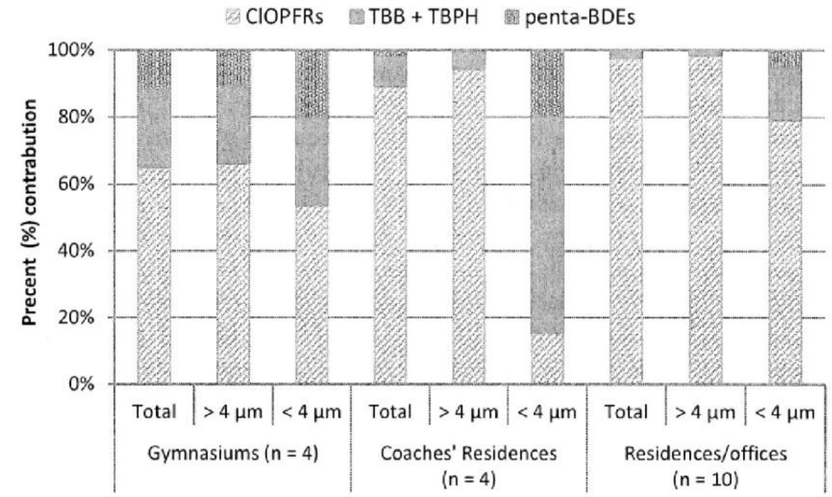

Figure 1. Normalized contributions of various halogenated flame-retardant (HFR) classes to total HFRs (>4 μm + <4 μm), inhalable (>4 μm), and respirable (<4 μm) air particulates within each indoor environment. ClOPFRs, chlorinated organophosphate-FRs; TBB, 2-ethylhexyl 2,3,4,5-tetrabromobenzoate; TBPH, bis(2-ethylhexyl) 3,4,5,6-tetrabromophthalate; penta-BDE, pentabromodiphenyl ether.

Table 1. Halogenated flame retardants (HFRs) inhalable (>4 μm) and respirable (<4 μm) air particulate fraction concentrations ($ng \cdot m^{-3}$) of each indoor environment.

	[1] Gymnasiums (n = 4)				[1] Coaches' Residences (n = 4)				[2] Residences/Offices (n = 10)			
	Inhalable (>4 μm)		Respirable (<4 μm)		Inhalable (>4 μm)		Respirable (<4 μm)		Inhalable (>4 μm)		Respirable (<4 μm)	
HFRs	**Range (%RD)**	**Mean [3]**	**Range (%RD)**	**Mean [3]**	**Range (%RD)**	**Mean [3]**	**Range (%RD)**	**Mean [3]**	**Range (%RD)**	**Mean [3]**	**Range (%RD)**	**Mean [3]**
BDE-47	10.1–82.7 (100%)	33.0	nd–5.70 (75%)	3.06	0.20–2.30 (100%)	1.45	nd–8.50 (75%)	3.34	nd–1.17 (40%)	0.23	nd–1.39 (40%)	0.22
BDE-85	1.50–3.00 (100%)	2.18	nd (0%)	0.05	nd–2.00 (25%)	0.54	nd (0%)	0.05	nd (0%)	0.05	nd (0%)	0.05
BDE-100	4.40–15.4 (100%)	8.03	0.60–5.50 (100%)	2.38	0.50–1.90 (100%)	1.35	nd–1.60 (75%)	1.04	nd–0.19 (20%)	0.07	nd–1.02 (40%)	0.32
BDE-99	18.5–58.5 (100%)	35.4	nd–9.60 (75%)	5.54	nd–4.30 (50%)	1.23	nd–11.1 (50%)	3.10	nd–0.39 (50%)	0.17	nd–1.59 (70%)	0.55
BDE-153	1.30–6.10 (100%)	3.90	nd–6.70 (50%)	1.95	nd–0.70 (50%)	0.28	nd–3.00 (50%)	1.15	nd (0%)	0.05	nd (0%)	0.05
Penta-BDE	36.9–164 (100%)	82.5	1.80–20.7 (100%)	13.0	3.50–7.10 (100%)	4.84	nd–24.1 (75%)	8.68	nd–1.54 (70%)	0.57	nd–3.74 (70%)	1.19
∑Penta-BDE [4]	95.5				13.5				1.76			
TBB	46.4–220 (100%)	143	nd–20.2 (75%)	11.4	2.60–55.4 (100%)	22.0	nd–55.2 (75%)	21.4	nd–23.3 (30%)	3.23	nd–18.4 (40%)	2.99
TBPH	4.90–71.9 (100%)	34.3	nd–12.8 (75%)	5.41	nd–18.3 (75%)	8.61	nd–18.6 (50%)	6.93	nd–25.6 (20%)	2.97	nd–3.44 (50%)	0.57
TBB + TBPH	51.3–184 (100%)	178	nd–31.0 (75%)	16.8	2.60–73.7 (100%)	30.6	nd–73.8 (75%)	28.3	nd–48.9 (30%)	6.20	nd–21.9 (50%)	3.56
∑TBB + TBPH [4]	195				58.9				9.76			
TCEP	nd (0%)	0.05	nd (0%)	0.05	nd (0%)	0.05	nd (0%)	0.05	nd–77.8 (89%)	19.1	nd (0%)	0.75
TCPP	136–525 (100%)	266	nd (0%)	0.05	209–1360 (100%)	536	nd (0%)	0.05	16.0-1180 (100%)	371	nd–28.6 (60%)	12.3
TDCPP	125–397 (100%)	244	32.0–36.5 (100%)	34.3	32.0–69.2 (100%)	50.1	nd–26.4 (25%)	6.64	nd–82.2 (33%)	19.1	nd–20.9 (50%)	4.97
ClOPFRs	376–650 (100%)	510	32.0–36.5 (100%)	34.4	241–1420 (100%)	585	nd–26.4 (25%)	6.74	61.1–1180 (100%)	410	nd–34.4 (80%)	18.0
∑ClOPFRs [4]	544				592				428			
HFRs	608–888 (100%)	771	33.8–83.0 (100%)	64.2	272–1500 (100%)	620	nd–124 (75%)	43.8	61.5–1180 (100%)	417	5.80–37.8 (100%)	22.7
∑HFRs [4]	835				664				440			

[1] La Guardia, M.J.; R. C. Hale 2015 [24]. Halogenated flame-retardant concentrations in settled dust, respirable and inhalable particulates and polyurethane foam at gymnastic training facilities and residences. *Environ. Int. 79*, 106–114. [2] Schreder, E. D.; N. Uding; M. J. La Guardia 2016 [23]. Inhalation a significant route for chlorinated organophosphate flame retardants. *Chemosphere 64*, 181–186. [3] Values reported as non-detect (nd) were replaced with $\frac{1}{2}$ of the reported detection limit when calculating mean values for statistic evaluation. [4] Sum of the inhalable + respirable air particulate fractions. %RD = % rate of detection.

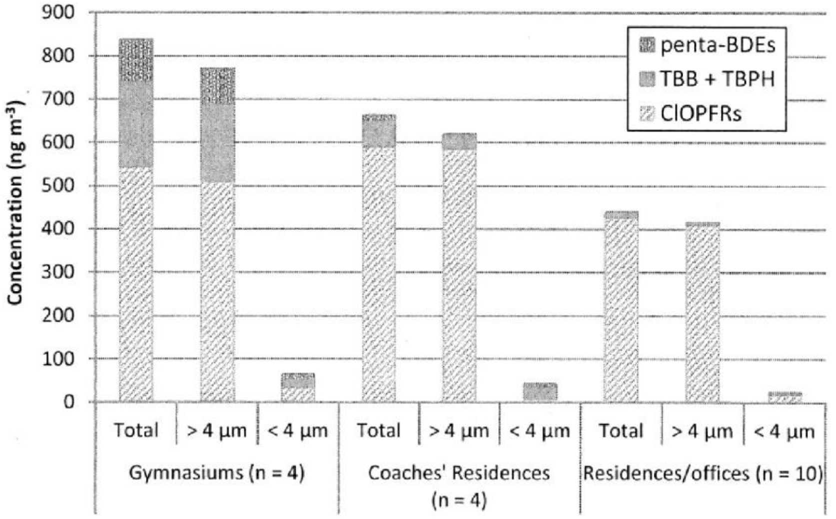

Figure 2. HFR concentrations (ng·m^{-3}) of total (>4 µm + <4 µm), inhalable (>4 µm), and respirable (<4 µm) air particulates within each indoor environment.

Unlike ClOPFRs, constituent profiles of Penta-BDEs, TBB, and TBPH were similar between the gymnasiums, coaches' residences, and residences/offices. However, their total levels (i.e., respirable + inhalable) at the gymnasiums (mean ∑Penta-BDEs 95.5 ng·m^{-3} and ∑TBB+TBPH 195 ng·m^{-3}) substantially exceeded ($p < 0.05$) those detected at the coaches' residences (13.5 and 58.9 ng·m^{-3}, respectively) and in the residence/office environments (1.76, and 9.76 ng·m^{-3}, respectively) (Table 1). Total air contributions of the Penta-BDE replacements ∑TBB + TBPH were approximately 2 to 6 times higher than the ∑Penta-BDEs within each of the three environments, with > 52% of the ∑TBB + TBPH air contributions coming from the larger inhalable fraction (Figure 2). The Penta-BDEs and TBB + TBPH inhalable concentrations at the gymnasiums (82.5 and 178 ng·m^{-3}, respectively) also exceeded ($p < 0.05$) those detected at both the coaches' residences (4.84 and 30.6 ng·m^{-3}) and residences/offices (0.57 and 6.20 ng·m^{-3}) (Figure 2). La Guardia and Hale (2015) [24] observed several Penta-BDEs (i.e., BDE-99, -100, and -153) and TDCPP in the gymnasiums' respirable (<4 µm) air fraction. These HFRs, plus BDE-47 and -85, were also detected in the gymnasiums' inhalable air fractions, each at higher concentrations than those detected in the coaches' residences' respirable and inhalable air samples. Inhalable levels of BDE-100, TBB, and TDCPP in the gymnasiums were also statistically greater ($p < 0.05$) than levels within the coaches' residences. A statistical difference was also observed for the respirable Penta-BDEs fraction between the gymnasiums and residences/offices, 13.0 and 1.19 ng·m^{-3}, respectively. Given the presence of large amounts of uncovered foam in the gymnasiums, our finding of higher flame retardant concentrations is not surprising, but the size of the data set limits the strength of conclusions that can be drawn. Higher Penta-BDE levels at the gymnasiums may be associated with the historic presence of abundant PBDE-containing products. However, flame-retardants such as those contained in Firemaster 550® (i.e., TBB and TBPH), marketed as a Penta-BDE replacement product, are not only being detected but were found to exceed Penta-BDE levels in these indoor environments. This suggests that replacement HFRs follow the same release and exposure pathways as PBDEs and their toxicological consequences merit evaluation.

3.2. HFR Daily Airborne Exposures Equations

The U.S. Centers for Disease Control and Prevention (CDC), Agency for Toxic Substances and Disease Registry (ATSDR) suggests the following equation (Equation (1)) when estimating exposure dose (D) (ng·kg^{-1}·day^{-1}) resulting from contact with contaminated air:

$$D = (C * IR * AF * EF)/BW \tag{1}$$

where C is the contaminant concentration ($ng \cdot m^{-3}$), *IR* is the intake rate of the contaminated medium ($16\ m^{-3} \cdot d^{-1}$ for adults [28]), *AF* is the bioavailability factor, *EF* is the exposure factor, and *BW* is body weight (70 kg for adults [28]). If exposure is intermittent or irregular, the *EF* can then be calculated to average the doses over the exposure interval. In many instances, *EF* will equal 1, representing a constant exposure to the contaminant. This approach was adapted by Nouwen et al. (2001) [29] to calculate daily health risk to dioxin air emissions from municipal waste incinerators, assuming an *AF* of 100%. They also replaced *EF* with an alveolar fraction (*fr*) default value of 0.75; i.e., 75% of the inhaled contaminant will be retained and taken up in the lung's alveolar region (Equation (2)).

$$D_{fr} = (C * IR * AF * fr)/BW \tag{2}$$

Using this approach, Chen et al. (2011) [30] assessed PBDE in the atmosphere at an e-waste recycling site and observed inhalation exposure exceeded the maximum allowed daily intake level of $0.26\ ng \cdot kg^{-1} \cdot d^{-1}$ for BDE-99, established by the Dutch National Institute for Public Health and the Environment (RIVM) [31]. However, contributions from inhaled particulates may influence these estimates, as the size of the airborne particulate matter (PM) will affect its fate within the respiratory tract, thereby affecting bioavailability of associated contaminants. PM with aerodynamic diameters <2.5 μm ($PM_{2.5}$) can penetrate deep inside the lung's gas exchange region, while larger sized PM may reside in the head or tracheobronchial region of the respiratory tract [32]. Lyu et al. (2016) [33] collected size-resolved air particulates (<0.4 to >9.0 μm) at an urban site in Shanghai, China, between 2012 and 2013. Deposition fluxes indicated that only 11% of the PBDE-laden particles would likely enter the alveolar region. Yang et al. (2014) [34] suggested that >50% of organophosphate flame-retardants were associated with suspended particulate matter of a size indicating deposition in the lung's head region, "indicating a comparatively lower exposure risk" (p. 69). Our previous reports indicated that >92% of the flame-retardant airborne burden was associated with larger particulates (>4 μm) [23,24]. It follows that not only should exposure via the lungs' alveolar exchange region be considered when evaluating health risk exposure to air particulates, but also exposure resulting from swallowing the non-respirable fraction and its subsequent uptake through the digestive tract. This more comprehensive approach is also supported by the European Commissions' (EC) Registration, Evaluation, Authorization and Restriction of Chemical (REACH) program [35].

The bioavailability of several PBDEs in the digestive tract was previously evaluated by feeding Sprague-Dawley rats PBDE-laden house dust Standard Reference Material (SRM) 2585 (National Institute of Standards and Technology (NIST), Gaithersburg, MD, USA). Results indicated a >80% absorption efficiency for PBDE with six or less bromines, down to approximately 40% efficiency for the full brominated BDE-209 [36]. Fang et al. (2014) [37] also evaluated the bioavailability of 20 flame retardants in SRM 2585 and 17 house dust samples collected between 2006 and 2010 using an in vitro Tenax bead-assisted sorptive method. Tenax beads have been validated as an effective material to evaluate bioaccessibility of polyaromatic hydrocarbons (PAHs) and pesticides in soils and sediments [38]. Using Tenax beads and simulated digestive fluids, Fang et al. (2014) [37] observed a >80% bioaccessibility for the ClOPFRs, >55% for PBDEs with six or less bromines (35% for BDE-209), and <50% for TBB (49%) and TBPH (29%) (Table S1). We created an exposure estimate using these values: the inhalable air fraction (*AF*) was substituted with the mean digestive tract bioaccessibility (*fd*) factor (Table S1), as suggested by Fang et al. (2014) [37] (D_{inh}, Equation (3)). This assumed these larger air particulates will be trapped on the mucosa lining of the respiratory tract, swallowed, and absorbed through the digestive tract. To represent the bioavailability factor (*AF*) of the respirable air particulates entering the alveolar gas-exchange region of the lung, a value of 100% was assumed for equation D_{res} (Equation (4)). Both exposure routes (D_{res}, D_{inh}) are then combined to estimate the total air exposure (D_{r+i}) resulting from uptake through the alveoli and digestive tract (Equation (5)).

Inhalable (>4 μm) air exposure (D_{inh}):

$$D_{inh} = (C * IR * fd * EF)/BW \tag{3}$$

Respirable (<4 μm) air exposure (D_{res}):

$$D_{res} = (C * IR * AF * EF)/BW \tag{4}$$

Total (<4 μm + >4 μm) air exposure (D_{r+i}):

$$D_{r+i} = D_{res} + D_{inh} \tag{5}$$

3.3. *Analysis of HFR Daily Airborne Exposures, Derived by Alveolar and Digestive Tract Bioavailability*

Using the Nouwen et al. (2001) [29] equation (Equation (2)), daily airborne exposures were first evaluated for each HFR in the three indoor environments (i.e., gymnasiums, coaches' residences, and residences/offices) previously sampled by La Guardia and Hale (2015) [24] and Schreder et al. (2016) [23]. These values were then compared to exposure values prepared by the total respirable + inhalable air exposure equation (D_{r+i}) (Equation (5)) (Table 2). All exposures were evaluated based on a 24-h exposure rate (*EF*, default value of 1) using the mean air concentration (*C*) for each of the detected HFRs. Using the total air exposure equation (Equation (5)), the average multiple uptake exposure rates (D_{r+i}) of the three indoor environments for both Penta-BDEs and ClOPFRs were estimated to be an average of 10% higher (ratios of D_{r+i}/D_{fr} ranged between 0.96–1.17 and 1.09–1.11, respectively (Table 2)) compared to values derived from the alveolar fraction (D_{fr}) alone. In contrast, exposure to the more hydrophobic HFRs, TBB + TBPH was 20% lower with the D_{r+i} approach, (D_{r+i}/D_{fr} range 0.66–0.94, Table 2). The most significant difference was for TBPH, which was 41% less on average compared to values derived from the alveolar fraction used by Nouwen et al. (2001) [29] and Chen et al. (2011) [30] (Equation (2)). Because physical chemical properties, in particular log octanol-air partitioning coefficient (K_{oa}), can be expected to relate to particle partitioning, we assessed the relationship between the D_{r+i}/D_{fr} ratio and log K_{oa}. With the revised D_{r+i} formula, a general increase in estimated exposure levels was observed for HFRs with a log $K_{oa} < 12$, while a decrease was observed for those with a log $K_{oa} > 12$ (Figure 3). Regression analysis showed a strong curvilinear association ($p = 0.007$), with a lower ratio among compounds with higher log K_{oa}.

Daily exposures, as estimated by the revised D_{r+i} formula, were still dominated by the ClOPFRs, D_{r+i} range 79.9–111 $\text{ng}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$. Penta-BDE exposure at the gymnasiums (15.7 $\text{ng}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$) was 5 times higher than at the coaches' residences (2.72 $\text{ng}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$) and >40 times higher than the residences/offices (0.36 $\text{ng}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$) (Table 2). Exposure to the Penta-BDE replacements TBB + TBPH via air particulates exceeded those of the Penta-BDEs at both the residences/offices (1.35 and 9.45 $\text{ng}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$, respectively) and the coaches' residences (0.36 and 2.72 $\text{ng}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$, respectively (Table 2)). Expected total intakes of individual HFRs expressed by either exposure equation were below their corresponding chronic oral reference dose (R*f*D) for PBDEs established by the U.S. EPA [39], TBB, TBPH, and ClOPFRs published by Hardy et al. (2008) [40] and Ali et al. (2012) [41], respectively (Table S2). However, the U.S. EPA recommended R*f*D values are based on relatively old toxicological studies, and other studies have suggested lower R*f*D standards for BDE-99 (0.26 $\text{ng}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$) [31]. Half of this value was detected at the residences/offices (D_{r+i}, 0.15 $\text{ng}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$) and was exceeded 3 to 25 times within the coaches' residences and gymnasiums, with respective estimates of 0.89 and 6.53 $\text{ng}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$. The use of HFRs in consumer products may be declining after the passage of California's revised TB 117-2013 [4], which modified flammability standards for upholstered furniture. However, other products in the indoor environment contribute to flame retardant exposure. For example, building products can also contain HFRs at percent by weight (e.g., TCPP in spray foam insulation and hexabromocyclododecane (HBCD) in insulation boards), these are not covered by this new regulation. These treated HFR-materials will remain in usage for decades, and may increase in abundance as more efficient building insulation is needed to further reduce the use of greenhouse gas emitting fossil fuels for heating. In addition, electronics products typically contain percent levels of flame retardants and also contribute to indoor exposures [42,43].

Table 2. Daily inhalation exposure dose by alveoli fraction (D_{fr}) and alveoli plus digestive tract (D_{r+i}) ($ng \cdot kg^{-1} \cdot bw \cdot d^{-1}$) and their ratio ($D_{r+i}/D_{fr}$).

	Gymnasiums (n = 4)			**Coaches' Residences (n = 4)**			**Residences/Offices (n = 10)**			D_{r+i}/D_{fr}
Analytes	D_{fr}	D_{r+i}	D_{r+i}/D_{fr}	D_{fr}	D_{r+i}	D_{r+i}/D_{fr}	D_{fr}	D_{r+i}	D_{r+i}/D_{fr}	Mean (n = 3)
BDE-47	6.18	6.21	1.00	0.82	1.01	1.23	0.08	0.09	1.13	1.12
BDE-85	0.38	0.33	0.87	0.10	0.09	0.90	0.02	0.02	1.00	0.92
BDE-100	1.78	1.70	0.96	0.41	0.43	1.05	0.07	0.08	1.14	1.05
BDE-99	7.02	6.53	0.93	0.74	0.89	1.20	0.12	0.15	1.25	1.13
BDE-153	1.00	0.94	0.94	0.25	0.30	1.20	0.02	0.02	1.00	1.05
Penta-BDEs	16.4	15.7	0.96	2.32	2.72	1.17	0.31	0.36	1.16	1.10
TBB	26.5	18.6	0.70	7.44	7.36	0.99	1.07	1.05	0.98	0.89
TBPH	6.81	3.27	0.48	2.66	2.10	0.79	0.61	0.31	0.51	0.59
TBB + TBPH	33.3	21.9	0.66	10.1	9.45	0.94	1.67	1.35	0.81	0.80
TCEP	0.26	0.31	1.19	0.26	0.31	1.19	3.40	3.66	1.08	1.15
TCPP	45.7	49.4	1.08	92.0	99.4	1.08	65.7	71.5	1.09	1.08
TDCPP	47.7	54.1	1.13	9.82	11.1	1.13	4.13	4.76	1.15	1.14
ClOPFRs	93.7	104	1.11	102	111	1.09	73.3	79.9	1.09	1.10
[1]∑HFRs	143	141	0.99	115	123	1.07	75.2	81.6	1.09	1.05

[1] ∑HFRs = Penta-BDEs + TBB + TBPH + ClOPFRs.

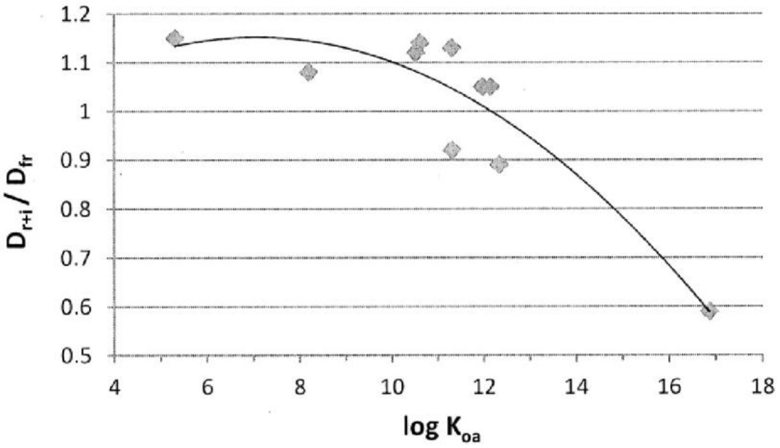

Figure 3. Association of daily inhalation dose exposure comparisons (D_{r+i}/D_{fr}) with log octanol-air partitioning coefficient (K_{oa}). D_{r+i} = ∑respirable + inhalable fraction (Equation (5)). D_{fr} = alveoli fraction (Equation (2)). Regression analysis, curvilinear relationship ($p = 0.007$).

4. Conclusions

In these studies, a wide range of former and currently marketed HFRs used to treat polyurethane foam was detected in indoor air. Two particulate air fractions (respirable: <4 μm and inhalable: >4 μm) were collected during each sampling. Multiple HFRs were detected in both fractions. The inhalable fraction carried the bulk of the ∑HFRs, with ∑ClOPFR contributing 65% to 97% to the total. ∑TBB + TBPH air contributions were approximately 2 to 6 times higher than the ∑Penta-BDEs within each indoor environment, indicating that these Penta-alternatives are also escaping products and entering the breathing zone in indoor environments. The gymnasiums' ∑Penta-BDEs and ∑TBB + TBPH inhalable concentrations exceeded those detected at the coaches' residences and residences/offices. Individual flame retardant levels of BDE-100, TBB, and TDCPP were also significantly higher. A statistical difference was also observed for the respirable Penta-BDEs fraction between the gymnasiums and residences/offices. In our exposure analysis considering airborne particulate size, deposition site, and HFR bioavailability, we found that a refined model creating separate estimates for exposure to smaller particulates, via the lung, and larger particulates, via ingestion, altered total inhalation exposure estimates. Estimated daily inhalation exposures were greater for HFRs with a log $K_{oa} < 12$ (i.e., Penta-BDEs and ClOPFRs) and lower for those with a log $K_{oa} > 12$ (i.e., TBB and TBPH). The most significant change was for TBPH, averaging a 41% decrease in bioavailability. Although inhalation exposure levels did not exceed lowest observed adverse effects levels established by the U.S. EPA, inhalation is just one contributor to total exposure, and some have suggested more stringent standards. These lower exposure levels were exceeded at the coaches' residences and the gymnasiums for BDE-99. Therefore, future work should continue to focus on uptake exposure and health effects of HFRs, as new and recycled HFRs continue to be introduced into consumer products with the potential to be released to the indoor environment throughout the products' life-cycles.

Supplementary Materials: The following are available online at www.mdpi.com/1660-4601/14/5/507/s1, Table S1: HFR nomenclature, octanol air partitioning coefficients (K_{oa}) *, digestive tract bioaccessibility †, and air particle sorption models ‡, Table S2: HFR corresponding chronic oral reference dose (*RfD*) values, $ng \cdot kg^{-1} \cdot d^{-1}$. Figure S1: MultiDust® foam disc Certificate of Conformity, performance criteria 85%, ±10%.

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Conflicts of Interest: The authors declare no conflict of interest.

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