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Polybrominated Diphenyl Ethers in American Eels *(Anguilla rostrata)* from the Delaware River, USA

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The Delaware River, like other rivers that bisect urbanized and industrialized lands, receives substantial loadings of contaminants through numerous point and nonpoint inputs such as urban and agricultural run-off, industrial discharges, and atmospheric deposition. The river also has significant in-place repositories of contamination within its sediments. Although many research studies and monitoring efforts have characterized the magnitude and extent of contaminants such as heavy metals, PCBs and organochlorine pesticides (e.g., Sheldon and Hites, 1978; Kennish and Ruppel, 1996; Ashley et al., 2003a), less information regarding other contaminant classes such as polybrominated diphenyl ethers (PBDEs) exists.

Certain structures (referred to as congeners) of PBDEs have been and are currently used as flame retardants in products ranging from computers to textiles (Raloff, 2003). Production and use of these chemicals has been largely concentrated in industrialized countries however, due to their persistence and volatility, PBDEs are globally ubiquitous (e.g., Betts, 2002; Ikonomou et al., 2002) and have been shown to bioaccumulate in aquatic organisms (e.g., Boon et al., 2002).

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The objective of this research was to assess the levels of PBDEs in American eels (*Anguilla rostrata*) collected from the Delaware River Estuary. American eels are thought to have limited home ranges (Parker, 1995; Morrison, 2001) and consequently have been shown to be reliable bioindicators of polychlorinated biphenyl (PCB) pollution within water bodies such as the Delaware and Hudson Rivers (Ashley et al., 2003b). Because PBDEs have similar structures and lipophilic natures compared to PCB congeners, it was hypothesized that eels may also be reliable indicators of localized PBDE contamination within the Delaware River and its tributaries. This study is the first to report PBDE concentrations in American eels from this ecosystem.

Materials and Methods

In 1998, American eels were captured (using eel pots or electroshocking) from various locations within the Delaware River Estuary (Fig. 1) and subsequently analyzed for mercury, PCBs and other organochlorine pesticides (NJDEP, 2000; Ashley et al., 2003b). The unused homogenized filets (skin-off) from these eels were subsequently archived in individual sealed jars at –20°C. In late 2005, seventeen of these homogenates were thawed, re-extracted, and subsequently re-analyzed for PCBs and analyzed for the first time for PBDEs as part of this study. These quantifications of PCB levels compared very well to the values obtained years prior with differences observed for each sample on par with the differences we often observe for replicate PCB analysis (relative percent differences from 2 to 30%). From these comparative data, it is reasonable to suggest that the process of long-term

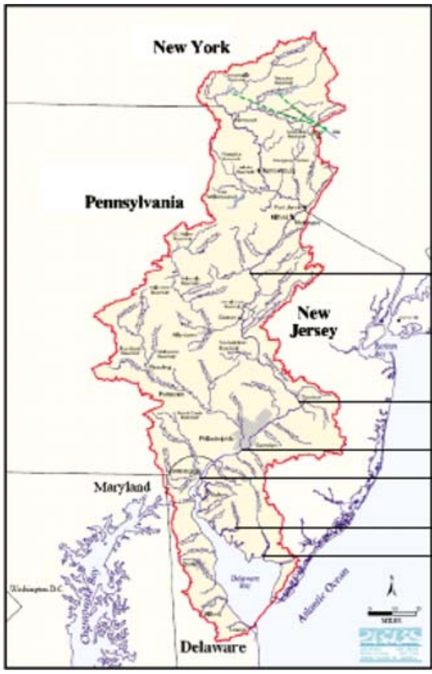

Fig. 1 Collection sites of American eels within the Delaware River Estuary

archiving at low temperatures did not have an affect on the samples' PCB levels. Because PCB and PBDE congeners have similar structures and similar physicochemical properties, PBDE loss through long term archiving is hypothesized to be minimal as well. Length and weight measurements of all collected eels were made upon collection while lipid content was determined gravimetrically prior to PCB and PBDE analysis in 2005. Identification of sex was not consistently performed and is not reported.

Subsamples (several grams wet weight) of homogenized eel tissue were extracted using previously published methods (Ashley et al., 2000; Stapleton et al., 2004). Briefly, thawed archived samples were mixed with Na_2SO_4 to eliminate water and were extracted with dichloromethane using a Soxhlet extractor for 18 hours. The extracts were sub-sampled for gravimetric lipid determination. Lipids were removed from sample extracts by gel permeation chromatography (GPC) using DCM (J.T. Baker, 99.5% purity) as the mobile phase. The collected fraction containing analytes was concentrated by roto-evaporation and an N_2 stream. Solid–liquid chromatography using florisil (J.T. Baker, 60–100 mesh) was performed as an additional clean-up step using petroleum ether (J.T. Baker, 99.5% purity) as the eluant.

Internal standards were added to all the samples and calibration standards prior to PCB and PBDE analysis. For PCBs, 2,3,6-trichlorobiphenyl (PCB 30; Accustandard, 99% purity) and 2,2′,3,4,4′,5,6,6′-octachlorobiphenyl (PCB 204; Accustandard, 99% purity) were used while for PBDE quantification, 4′-fluoro-2,3,3′,4,5,6-hexabromodiphenyl ether (FBDE 160; Chiron, Norway, 99% purity) was used. One hundred and eight PCB congeners (Accustandard, 95–99% purity), either singly or coeluting, were analyzed using a Hewlett Packard 5890 gas chromatograph equipped with a ^{63}Ni electron capture detector and a 5% phenyl methylpolysiloxane column at the Academy of Natural Sciences (Ashley et al., 2003b; Swackhamer, 1987). Twenty-eight singly or coeluting PBDE (BDE 17, 25, 28+33, 30 47, 49, 66, 71, 75, 85+155, 99, 100, 116, 119, 138, 153, 154, 156, 181, 183, 190, 191, 203, 205, 206, 209; Accustandard, 95–99% purity) were quantified at Duke University using a gas chromatography (GC) with a mass spectrometer (MS) operated in negative chemical ionization mode (NCI).

On-column injection was employed. A 0.25 mm x 15 m fused silica capillary column coated with a 5% phenyl methylpolysiloxane column (DB-5MS; 0.25 μm film thickness) was installed in the GC and connected to the MS. The oven temperature program was held at 80°C for 2 min followed by a temperature ramp of 12°C/min to 140°C, followed by a second temperature ramp of 5°C/min to a final temperature of 280°C, which was held for an additional 20 min. The auxiliary temperature was maintained at 280°C. For all BDE congeners except BDE 209, ion masses 79 and 81 (bromide ions) were monitored as quantitative and qualitative ions. For BDE 209, ions 487 and 409 were monitored. Values for total PBDEs (t-PBDE) and total PCBs (t-PCBs) in this study are defined as the sum of all individual or coeluting PBDE and PCB congeners, respectively, that were analyzed.

Halogenated analyte loss through analytical manipulations was assessed by the addition of surrogate PCB congeners 14, 65 and 166 prior to extraction by Soxhlet apparatus. These surrogates were not industrially prepared and therefore are not present in the environment. Average recoveries of these three congeners were 103%, 109%, and 109%, respectively. Matrix blanks were generated to monitor possible laboratory contamination and to calculate the detection limits for PCBs and PBDEs. Chromatograms of most blanks were void of significant peaks suggesting that little contamination through laboratory exposure occurred. The matrix blank-based detection limits for t-PCBs and t-PBDEs were 21 ng/g and 2.0 ng/g, respectively.

National Institute for Standards and Technology (NIST) Standard Reference Material (SRM 1946, Lake Superior Fish Tissue) was used to evaluate extraction efficiency and analytical accuracy. For PCBs, the majority (70%) of congeners fell within 10% of published values (after correction for surrogate loss). For PBDEs congeners, determined concentrations were 60 to 130% of the NIST certified values.

Results and Discussion

Lipophilic organic contaminant concentrations in fish may be expressed both on a wet weight (ng/g wet) and lipid normalized (ng/g lipid) basis (Table 1). The majority of PCB congeners in American eels (>95%) were well above detection limits. However, for PBDEs, approximately 60% of the congeners analyzed fell below the limits of quantification or were undetected altogether. Total PCB concentrations (ng/g wet), as determined in this study from analyses of archived samples, ranged from 70 (Trenton) to 4090 (Deepwater). Two of the seventeen eels exceeded the U.S. Food and Drug Administration's do not eat guideline of 2000 ng/g wet weight. Total PBDE concentrations ranged from 1 to 408 ng/g wet weight. Values for t-PBDEs were consistently an order of magnitude less than their counterpart PCB levels. To date, there are no published consumption guidelines for t-PBDEs.

Based on location caught, the American eels were grouped into five regions from the northern Delaware River to the head of the estuary: DE Water Gap, Trenton, Ft. Mifflin, Deepwater, and the DE Bay tributaries (Cohansey and Maurice Rivers) (Table 1). Total PCBs and t-PBDEs (ng/g wet weight basis) varied widely within and between collection sites. For PCBs, wet weight concentrations were fairly consistent between the four 'northern' regions of the Delaware River system but the Bay tributaries harbored eels with much lower concentrations. The same trend was observed for t-PBDEs however two of the four eels captured at Trenton had concentrations about four times higher than eels from other regions. Differences in wet weight concentrations may often be attributed to differences in individual lipid contents. Lipid contents varied from 1% to 19% (Table 1). Upon lipid normalization, some of the spatial trends observed with wet weight levels changed but did not disappear. For PCBs, Trenton and Bay tributary eels had the lowest concentrations, while the Water Gap, Ft. Mifflin and Deepwater eels were similar (with one notably high concentration eel collected from Ft. Mifflin). High lipid-normalized concentrations arose in those eels that had moderate wet weight concentrations but whose lipid contents were relatively low. For t-PBDEs, a DE Water Gap eel contained over 5500 ng/g lipid. Again, this eel had relatively low lipid content which translated to a large normalized concentration.

To assess the extent that lipid content determines the body burden of PCBs and PBDEs, contaminant classes were correlated to corresponding lipid contents. For both t-PCBs and t-PBDEs, correlations with lipid content were very low (r^2 = 0.28 and 0.11, respectively) and not significant (at $p = 0.05$) suggesting that lipid variation was not a large determinant of contaminant burden. Linear regressions between each contaminant class and lengths of eels and weights of eels were equally low (r^2 values all less than 0.1) suggesting these characteristics were not large factors in determining contaminant loads in these particulars eels either. Correlating t-PCBs with t-PBDEs did produce a significant positive correlation (r^2 = 0.82) when two eels from Trenton were not included in the regression analysis. With the exception of these two eels, this trend suggests

Table 1 Summary of t-PCB and t-PBDE concentrations on a wet weight and lipid normalized basis for American eels collected from various regions of the Delaware River and its tributaries (Cohansey and Maurice Rivers)

Location caught	Lipid content (%)	t-PBDE (ng/g wet)	t-PCBs (ng/g wet)	t-PBDE (ng/g lipid)	t-PCBs (ng/g lipid)
DE Water Gap	2.0	25.6	894.6	1312	45764
DE Water Gap	9.5	124.4	2073.2	1303	21717
DE Water Gap	1.2	68.8	376.5	5652	30910
Trenton	16.5	373.7	1170.9	2271	7117
Trenton	12.9	1.3	69.6	10	540
Trenton	13.6	3.1	229.3	23	1692
Trenton	15.1	407.9	746.9	2709	4961
Ft. Mifflin	7.8	67.3	1261.4	862	16140
Ft. Mifflin	1.3	21.9	942.1	1735	74482
Ft. Mifflin	9.3	46.2	1405.9	495	15061
Deepwater	4.8	71.2	1862.6	1498	39193
Deepwater	18.6	157.2	4091.7	845	21994
Deepwater	8.6	77.7	1318.2	905	15351
Cohansey R.	12.9	3.6	223.3	28	1733
Cohansey R.	6.6	3.3	169.1	51	2579
Cohansey R.	2.3	1.2	147.2	51	6374
Maurice River	9.1	11.0	426.7	121	4689

that the two contaminants are likely delivered to the aquatic system via similar processes. The two anomalous eels had high t-PBDE concentrations yet relatively low t-PCB levels. This may suggest that although the two contaminant classes have similar sources to most of the portions of the Delaware River (at least those studied), t-PBDE sources may be elevated at Trenton. Moreover, looking at the lipid normalized concentrations for t-PBDEs, there seems to be a slight but steady increase in concentration with northward collection sites, a trend that was mirrored in t-PBDE levels in osprey eggs (Toschik et al., 2005). However, given the limited number of eels caught at each location (n = 3 or 4), additional studies including a larger set of eels would have to be conducted before definitively drawing statistically-based conclusions from the trends observed in this preliminary study.

Both PCB and PBDE congener patterns remained relatively invariant over the spatial scales studied. That is, congener profiles of Maurice and Cohansy Rivers' eels compared very closely to those eels captured in the northern reaches of the Delaware River at DE Water Gap, and sites between these two end members. The most abundant brominated diphenyl ether (BDE) congeners detected in American eels, in order of largest contributor to smallest, were 47 < 100 < 154 < 119 and 49. Coeluting PDE congeners 28+33, 66, 75, 99, 153, 154, and 155 were often detected but at very low concentrations.

The fully brominated conformation of the diphenyl ether (BDE 209) was not detected in any of the eel samples. Stapleton et al. (2004a) found transformation of BDE 209 to lesser brominated products in the common carp. Moreover, through additional dietary exposure studies, BDE 99 was rapidly debrominated to BDE 47, which accumulated in carp tissue (Stapleton et al., 2004b). The high relative abundance of BDE 47 and low to nondetectable levels of BDE 99 and 209 suggests that American eels have, like carp, the potential for metabolic debromination. Preliminary data (Ashley, unpublished data) on PBDE levels and patterns in Delaware River Estuary sediment confirms that 209 is the dominate congener in this matrix. Eels are likely exposed to this congener but do not accumulate it because of biotransformation processes.

The coeluting PCB congener groups 153+132+105 and 163+138 were the dominant PCB congeners in all eel samples. These were the dominant congeners in the industrial mixtures of Aroclor 1254 and 1260. Their appearance in these samples and in those from other studies (e.g., Ashley et al., 2003b) reconfirms the persistent nature of these congeners.

Despite the fact that analyses of biota for t-PBDEs levels has increased over the past five years, this relatively new class of compounds has yet to be fully characterized in many fish species. Not surprisingly, this data set represents one of the few which highlights the levels of t-PBDEs in biota within the Delaware River Estuary. American eels from the heavily urbanized and industrialized Passaic River have recently been analyzed as part of a New Jersey Department of Environmental Protection's (NJDEP) survey (Ashley, unpublished data) with t-PBDEs comparable to the ranges observed in this study (Table 2). More data has been garnered on European eels. Concentrations on a lipid normalized basis were comparable to this study (Table 2). For example, a study in Holland conducted from 1983–1989 revealed t-PBDE concentrations ranging from <50 to 1,700 ng/g lipid (de Boer, 1990). The highest reported literature values comes from a report by Goemans and Belpaire (2004) who found up to 32,000 ng/g lipid in eels collected from Belgium. The numbers of eels analyzed in this study were comparable to other American and European studies but the need to more fully evaluate these contaminant levels is warranted to more fully and statistically evaluate the factors determining their levels. Moreover, it is prudent to recognize that reported biotic concentrations of total PBDEs will vary simply due to the definition of total PBDEs in each study. However, in these studies, the predominant congeners found were consistently similar to those found in this study reflecting the widespread usage of the technical formulations used as flame retardants. However, with the U.S. now following western European bans on the pentaBDE and octaBDE commercial formulations, and the fact that fishes may selectively debrominate some congeners, differences in world-wide congener patterns may begin to be discerned.

Table 2 Comparison of ranges of t-PBDE concentrations in American and European eels

Location	t-PBDE concentrations	Units	n	Reference
The Netherlands	<50 to 1,700	ng/g lipid	34	de Boer, 1990
Belgium	2 to 14	ng/g wet	4	Covaci et al., 2005
Germany	3.6 to 21.4	ng/g lipid	5	Lepom et al., 2002
Belgium	~50 to 32,000	ng/g lipid	18	Geomans and Belpaire, 2004
Passaic River	192 to 1,357	ng/g lipid	11	Ashley, unpublished data
Delaware River	10 to 5,652	ng/g lipid	17	This study

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