METABOLIC FATE OF POLYBROMINATED DIPHENYL ETHERS (PBDES) IN CALIFORNIA KESTREL BLOOD

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Abstract

To explore the metabolic fates of PBDEs in California wild birds, kestrel plasma samples were analyzed for 18 PBDEs and 7 OH-BDE metabolites by using HRGC-HRMS and GC-NCI/MS or EI/MS/MS. The concentrations of Σ_{18}PBDEs ranged from 2.73 to 12.6 ng/g wet wt. (median 4.17). BDE-209 comprised 26% (median) of ΣPBDEs (0.05 to 3.68 ng/g wet wt), followed by BDE-153, 99, 47, 183, 154, and 100, the "terrestrial" pattern seen in California urban raptors. BDE-207, the possible BDE-209 breakdown product, was abundant in almost all samples, and correlated (R^2=0.61) with levels of BDE-209. The ratios of BDE-207:209 (9% on average) were higher than that of Saytex deca-formulation (~0.25%), indicating that the BDE-207 levels observed may result from the debromination of BDE-209. Σ_7OH-BDEs were detected only at trace levels, ranging from ND to 0.34 ng/g wet wt (median 0.05). The ratios of OH-BDE:PBDE were generally low (<2% on average), athough one kestrel sample showed as high as 10%. 4'-OH-BDE-49 and 6-OH-BDE-47 were detected in <50% of kestrel plasma samples. In summary, our data suggest that debromination may be a preferred degradation pathway for BDE-209 in California urban/suburban raptors. Further investigation is underway to better understand the complicated metabolic pathways of PBDEs, particularly BDE-209.

Introduction

Polybrominated diphenyl ethers (PBDEs) were introduced as highly effective flame-retardants. Europe and North America banned production of penta- and octa-PBDE mixtures in the late 1990s[1]. The lipophilicity of these industrial pollutants make them bioavailable, and their environmental persistence allow them to biomagnify up the food chain, leading to concerns regarding potential adverse health effects in top predators, including humans. Deca-BDE (BDE-209) is a major brominated flame retardant still available in world markets in most countries outside of Europe[2]. The commercial mixture containing deca-formula is of particular concern because it is still actively used in consumer products in North America, including California. BDE-209 is of concern since it is bioavailable[3,4] and breaks down to lower PBDEs in abiotic[5] and biotic environments[6]. Peregrine falcon eggs collected from North America contain some of the highest BDE-209 levels yet recorded[4]. PBDEs such as BDE-47, -99, -100, and -153 have been shown in test animals to biotransform into many hydroxylated metabolites (OH-BDEs)[7,8]. OH-BDE metabolites are reported to be endocrine disrupters[9,10], and emerging evidence suggests these metabolites may be more potent endocrine disruptors (especially of the thyroid) than their respective parent compounds[11]. Biotransformation of BDE-209 to the lower BDEs and/or their hydroxylated metabolites[6] is of major interest, with possible significant public and environmental health consequences. We are investigating the debromination/metabolic fates of PBDEs, particularly BDE-209, in California wildlife samples by measuring levels of PBDE congeners and some of their hydroxylated metabolites (OH-BDEs).

Materials and Methods

Plasma samples (0.11-0.39 g) were collected from nine kestrels brought to the Lindsay Wildlife Hospital in Walnut Creek, CA for rehabilitation. The kestrels nested in the San Francisco Bay region, and the peregrines throughout California. Both $^{12}C_{12}$ and $^{13}C_{12}$ PBDE standards were obtained either from Wellington Labs. Inc., Guelph, ON, Canada or Cambridge Isotope Labs. Inc., Andover, MA, USA. The OH-BDE standards of 2'-OH-BDE68, 6-OH-BDE47, 5-OH-BDE47, 4'-OH-BDE49, 5'-OH-BDE100, 4'-OH-BDE103, 5'-OH-BDE99, 4'-OH-BDE101, and ^{13}C-6-OH-BDE47 were purchased from Wellington Laboratory (TerraChem Inc., Shawnee Mission, KS, USA). PBDEs and OH-BDE metabolites were

extracted from plasma using standard extraction and phase-separation techniques[12]. Due to the small volume of plasma sample, the final extracts were concentrated to 10 μL. Levels of PBDEs and OH-BDEs (as MeO-BDEs) were measured using isotope dilution/HRGC-HRMS (ThermoFinngan MAT95, Bremen, Germany) equipped with DB-5MS (15 m × 0.25 mm i.d., 0.1 μm film thickness, J & W Scientific, Folsom, CA) and a Varian 1200 GC-MS (Varian Inc., Walnut Creek, CA) equipped with DB-5MS (30 m × 0.25 mm i.d., 0.25 μm thickness, J & W Scientific, Folsom, CA), respectively. Molecular ions were monitored to identify tri- to penta-BDEs, and M-2Br ions identified hexa- to deca-BDEs. The bromine ions (79/81) were monitored for OH-BDEs. The presence of OH-BDE congeners were confirmed by GC-EI-MS/MS by monitoring other fragmenation ions, particularly 6-OH-BDE47 and ^{13}C-6-OH-BDE47 that coeluted in NCI/MS. Seven tetra-penta OH-BDEs were quantified. Hexa-nona OH-BDEs were monitored in both NCI-MS and EI-MS/MS. The mean (± SE) surrogate recoveries averaged 83±17%. The concentrations of OH-BDEs are presented as ng/g wet wt and, for comparison, PBDEs are also reported on a wet wt basis. Chemical analyses were performed in the ultra clean laboratory of the Department of Toxic Substances Control, Berkeley, CA.

Results and Discussion

We measured 18 PBDEs (tri to deca) and also identified 7 OH-BDE metabolites (tetra to penta) from kestrel plasma. The concentrations of Σ_{18}PBDEs ranged from 2.73 to 12.6 ng/g wet wt. (median 4.17). BDE-209 was the major PBDE (0.05 to 3.68 ng/g wet wt), comprising 26% (median levels) of Σ_{18}PBDEs, followed by BDE-153, 99, 47, 183, 154, and 100 (Table 1). This profile is the "terrestrial pattern" we also see in eggs from California peregrine falcons[4]. One kestrel blood collected from Oakley showed the intermediate pattern between "aquatic" and "terrestrial" profile, with BDE-153>183>154>99>47>209. The potential BDE-209 nona breakdown compound, BDE-207, was consistently abundant in almost all samples. BDE-207 levels correlated with BDE-209 (R^2=0.61). The percent fraction BDE-207/BDE-209 (9% on average, excluding Oakley kestrel which was 126%) was >40-fold higher than that of Saytex deca-formulation in current use (~0.25%)[13]. This suggests that these BDE-207 levels observed in the plasma may result from the debromination of BDE-209.

Table 1. PBDE and OH-BDE concentrations of kestrel plasma collected from northern California, USA.

	PITT	PLEA	OAKL	MORA	MART	CONC1	CONC2	RICH	SANR	Min	Med	Max	Mean	Stdev
PBDE (ng/g wet wt)														
PBDE-32	0.06	0.06	0.04	0.06	0.04	0.25	0.06	0.06	0.05	0.04	0.06	0.25	0.08	0.07
PBDE28/33	0.05	0.05	0.04	0.05	0.03	0.21	0.05	0.05	0.05	0.03	0.05	0.21	0.06	0.06
PBDE-71	0.08	0.07	0.05	0.06	0.03	0.31	0.06	0.06	0.05	0.03	0.06	0.31	0.08	0.08
PBDE-47	0.19	0.18	0.20	0.26	0.15	0.55	0.27	0.39	0.17	0.15	0.20	0.55	0.26	0.13
PBDE-66	0.06	0.06	0.04	0.05	0.03	0.24	0.04	0.05	0.04	0.03	0.05	0.24	0.07	0.06
PBDE-100	0.08	0.13	0.03	0.08	0.06	0.27	0.23	0.29	0.20	0.03	0.13	0.29	0.15	0.10
PBDE-99	0.21	0.35	0.44	0.39	0.24	0.57	0.55	0.81	0.37	0.21	0.39	0.81	0.44	0.19
PBDE-85	0.05	0.05	0.04	0.04	0.03	0.27	0.05	0.05	0.04	0.03	0.05	0.27	0.07	0.08
PBDE-154	0.07	0.17	1.53	0.03	0.05	0.17	0.11	0.19	0.22	0.03	0.17	1.53	0.28	0.47
PBDE-153	0.31	1.11	6.71	0.28	0.19	1.12	0.45	0.62	0.51	0.19	0.51	6.71	1.26	2.07
PBDE-183	0.14	1.30	3.26	0.22	0.08	0.39	0.17	0.15	0.14	0.08	0.17	3.26	0.65	1.05
PBDE-196	0.04	0.04	0.04	0.04	0.03	0.24	0.05	0.05	0.04	0.03	0.04	0.24	0.06	0.07
PBDE-197	0.05	0.39	0.05	0.04	0.04	0.26	0.05	0.05	0.04	0.04	0.05	0.39	0.11	0.13
PBDE-201	0.04	0.04	0.03	0.03	0.03	0.19	0.04	0.04	0.03	0.03	0.04	0.19	0.05	0.05
PBDE-203	0.05	0.05	0.05	0.04	0.03	0.23	0.04	0.04	0.04	0.03	0.04	0.23	0.06	0.06
PBDE-207	0.08	0.08	0.06	0.13	0.06	0.08	0.20	0.06	0.06	0.06	0.08	0.20	0.09	0.05
PBDE-209	2.62	1.45	0.05	2.00	1.80	0.37	3.68	0.43	0.70	0.05	1.45	3.68	1.45	1.20
ΣPBDEs	4.17	5.59	12.64	3.79	2.93	5.72	6.09	3.38	2.73	2.73	4.17	12.64	5.23	3.04
OH-BDEs (ng/g wet wt)														
ΣOH-BDE	0.01	0.04	0.05	0.06	0.01	0.00	0.10	0.34	0.05	0.00	0.05	0.34	0.06	0.10
OH-BDE /PBDE	0.003	0.01	0.004	0.02	0.004	0.000	0.02	0.10	0.02	0.00	0.01	0.10	0.02	0.03

PITT: Pittsburg, PLEA: Pleasanton, OAKL: Oakley, MORA: Moraga, Mart: Martinez, CONC: Concord, RICH: Richmond, SANR: San Ramon

OH-BDE metabolites were detected only in trace levels, ranging from ND to 0.34 ng/g wet wt (median 0.05). This was lower than North America west coast Bald Eaglet plama[14] collected from British Columbia (0.31-0.92), albeit PBDE levels were comparable each other. Apart from the different metabolic capacities between these two species, it could be partly because a greater number (14) of OH-BDE congeners were analyzed in their study. In addition, Bald Eaglet had a higher prevalence of lower PBDEs such as BDE-47,-99, and -100 (no BDE-209), typical "aquatic" profile, possibly resulting in the formation of more tetra OH-BDEs (6'-OH-BDE49 and 6-OH-BDE47) detected predominately in their study. The ratios of OH-BDE:PBDE in this study were generally low (<2% on average), although one kestrel had a ratio of 10%. OH-BDEs and PBDEs were not correlated with each other. These results suggest that OH-BDEs may follow more complicated pathways such as continuous debromination/hydroxylation[15,16,17], and/or diphenyl ether cleavage to form bromophenols[18]. 4'-OH-BDE-49 and 6-OH-BDE-47 were detected in <50% of kestrel plasma samples. The highest OH-BDE level was measured in a Richmond kestrel where 6-OH-BDE-47 predominated, followed by 4'-OH-BDE-49.

In summary, some OH-PBDEs were detected in plasma samples from northern California kestrels. However, the levels were very low, suggesting that debromination may be a preferred degradation pathway for BDE-209 in California urban/suburban raptors. To better understand the complicated metabolic pathways of PBDEs, particularly BDE-209, further investigation is underway for other metabolic breakdown products such as methoxy metabolites (MeO-BDEs), bromophenols, and heavier hydroxylated PBDEs (hexa to nona). The high levels of BDE-209 seen in these urban/suburban wild birds are of concern since the parent compound, as well as the debromination/hydroxylation products (the banned lower BDEs and their metabolites) may be associated with developmental deficits or behavioral abnormalities in the chick or with increase risk of fail-to-hatch eggs or chick mortality.

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