PBDE Concentrations and Congener Profiles among Different Turtle Species, Locations, and Sample Types

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Introduction

Reptiles are greatly underrepresented in the field of ecotoxicology, as evidenced by less than 2% of this literature focusing on this taxon (Sparling et al. 2000). Polybrominated diphenyl ethers (PBDEs) are one class of organohalogen contaminant that may pose a threat to reptile species, and only recently have researchers begun to monitor these compounds in snapping turtles (Letcher et al. 2006), other freshwater turtles (Moss 2005), and sea turtles (Keller et al. 2005, Carlson et al. 2005, Swarthout 2006, Stewart et al. 2006, Carlson in prep.). This presentation will review and compare the concentrations and patterns of PBDE congeners in several turtle species and sample matrices and demonstrate that some reptile sample sets contain unique PBDE profiles.

Materials and Methods

Whole blood, plasma, or egg samples have been collected from six turtle species in the southeastern region of the U.S. Sample information is summarized in Table 1. All samples were spiked with internal standards prior to extraction. Blood and plasma samples were extracted with a mixture of hexane and dichloromethane using either liquid:liquid extraction or a microwave assisted extraction, and formic acid was used as a denaturant. Adipose and egg samples were extracted using pressurized fluid extraction with dichloromethane. Lipid content was determined gravimetrically from subsamples of extracts. Extracts were cleaned up using at least two of the following techniques: size exclusion chromatography, alumina columns, and/or acidified silica columns. PBDEs were quantified using gas chromatography/mass spectrometry (GC/MS). Often two injections were performed, one used a 30 m or 60 m DB-5MS column and electron impact ionization, and the other used a 15 m DB-5MS column and negative chemical ionization. Calibration standards contained at least 14 PBDE congeners (17, 28, 47, 66, 71, 85, 99, 100, 138, 153, 154, 183, 190, and 209), but often contained more congeners which were mainly undetectable in all samples. Blanks, homogenized turtle plasma and egg control materials, and National Institute of Standards and Technology (NIST) Standard Reference Materials (SRMs), including SRMs 1946 (Lake Superior Fish Tissue) and 1589a (PCBs, Pesticides, PBDEs, and Dioxins/Furans in Human Serum), were processed alongside each batch of samples for quality control.

Table 1. Turtle samples analyzed in the NIST laboratory for PBDEs.

Species	Sample Type	Age Class	Sex	Year	Location	N	Study
Loggerhead sea turtle	plasma	juvenile	mixed	1998-2006	Core Sound, North Carolina (NC)	45	Carlson in prep.
Loggerhead sea turtle	plasma	juvenile	mixed	2003	offshore South Carolina to Florida (SC-FL)	29	Carlson et al. 2005
Loggerhead sea turtle	eggs	--	--	2002	Cape Lookout, North Carolina (NC)	9 clutches	Keller et al. 2005; Alava et al. 2005
Loggerhead sea turtle	eggs	--	--	2002	East & West coasts of Florida	30 clutches	Keller et al. 2005; Alava et al. 2005
Leatherback sea turtle	whole blood	nesting adults	female	2003	Juno Beach, Florida	6	Stewart et al. 2006
Leatherback sea turtle	eggs	--	--	2003	Juno Beach, Florida	6 clutches	Stewart et al. 2006
Kemp's ridley sea turtle	whole blood	juvenile	mixed	2001-2002	Gulf of Mexico and S. Carolina	49	Swarthout 2006
Green sea turtle	whole blood	juvenile	mixed	2001-2002	Gulf of Mexico	9	Swarthout 2006
Common musk turtle	plasma	adult	male	2004	Tennessee River Gorge	5	Moss 2005
Common musk turtle	plasma	adult	female	2004	Tennessee River Gorge	5	Moss 2005
Cumberland slider turtle	plasma	adult	male	2004	Tennessee River Gorge	5	Moss 2005
Cumberland slider turtle	plasma	adult	female	2004	Tennessee River Gorge	5	Moss 2005

Results and Discussion

Concentrations of ΣPBDEs for each sample set are shown in Table 2. The two freshwater turtle species (common musk turtles, *Sternotherus odoratus*, and Cumberland sliders, *Trachemys scripta troosti*) had higher blood concentrations than the sea turtle species. Within the sea turtle species, Kemp's ridley and leatherback turtles had higher blood concentrations (pg/g wet mass) than loggerhead and green turtles. This is somewhat surprising, because chlorinated contaminant concentrations are typically higher in loggerhead turtles than leatherback and green turtles because of trophic differences. Eggs had higher concentrations than blood on a pg/g wet mass basis, but lower levels when normalized to lipid content. ΣPBDE concentrations in turtle blood were similar or higher than those measured in North American human blood (median=61 ng/g lipid in 2000-2002, Sjödin et al. 2004). The turtle egg ΣPBDE concentrations were much lower than those measured in gull eggs from the Great Lakes (mean=3080 ng/g lipid, Norstrom et al. 2002).

Table 2. Total PBDE concentrations in turtle samples analyzed in the NIST laboratory.

	pg/g wet mass		ng/g lipid	
Samples	mean	SD	mean	SD
Loggerhead plasma NC	81.9	156	26.1	45.2
Loggerhead plasma SC-FL	152	183	99.2	97.5
Loggerhead eggs NC	1040	1090	13.5	14.3
Loggerhead eggs FL	138	168	1.74	2.20
Leatherback blood	218	193	49.6	47.3
Leatherback eggs	842	631	16.9	12.7
Kemp's ridley blood	219	263	95.7	105
Green turtle blood	156	214	64.9	101
Musk turtle plasma male	1240	463	545	232
Musk turtle plasma female	809	507	123	39.4
Slider plasma male	885	550	448	477
Slider plasma female	460	329	230	234

Some of these turtle sample sets had unique PBDE congener profiles (Figure 1) not seen in other species. In the majority of reported profiles in wildlife and humans, PBDE 47 is the predominant congener, followed by 99, 100, 153, and 154 (reviewed by Hites, 2004). This typical pattern was seen in loggerhead plasma collected from SC-FL, loggerhead eggs from FL, and Kemp's ridley blood from the Gulf of Mexico. However, the loggerhead plasma and eggs, both from NC, showed higher proportions of PBDE 100 and 154. Additionally, the freshwater turtle species from Tennessee showed PBDE 100 as the predominant congener (this peak was confirmed to be a penta-BDE by GC/time of flight-MS). The reasons for these unique patterns are unknown, but it appears that they are due to geographical differences more than species differences. For example, two species of turtles inhabiting the same region in Tennessee show the same pattern. Also, plasma samples from the same species (loggerhead sea turtle), of the same age class, from two different regions (NC vs. SC-FL) show differing patterns. These loggerheads might be indicating the PBDE contamination profile of their local summer foraging grounds or perhaps these two turtle aggregations have different migratory pathways or foraging strategies that might also influence the different PBDE profiles. An additional possible reason for these unusual profiles is that different reptile species may have different abundances/activities of biotransformation enzymes that allow them to debrominate or hydroxylate certain congeners more easily than others. Future studies should investigate geographical differences in prey selection and PBDE profiles in preferred prey, as well as biotransformation of PBDEs in reptiles.

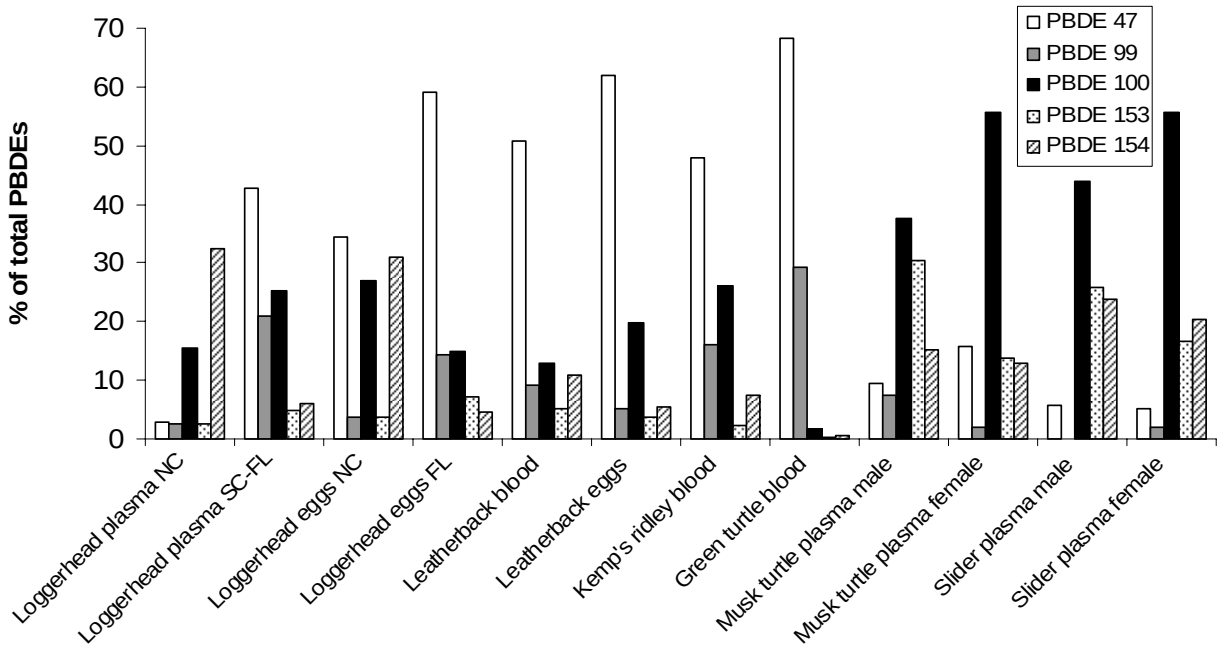

Figure 1. Comparison of PBDE congener profiles in different sample types from several turtle species captured at many locations. NC=North Carolina; SC-FL=South Carolina to Florida.

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Disclaimer: Certain commercial equipment or instruments are identified in the paper to specify adequately the experimental procedures. Such identification does not imply recommendations or endorsement by the NIST nor does it imply that the equipment or instruments are the best available for the purpose.

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