Polybrominated Diphenyl Ethers (PBDEs) in Blubber of Free-Ranging Bottlenose Dolphins (*Tursiops Truncatus*) from Two Southeast Atlantic Estuarine Areas

Patricia A. Fair · Gregory Mitchum · Thomas C. Hulsey · Jeff Adams · Eric Zolman · Wayne McFee · Ed Wirth · Gregory D. Bossart

Abstract Blubber tissue samples from bottlenose dolphins collected during the summers of 2003 and 2004 were screened for 13 (17, 28, 47, 66, 71, 85, 99, 100, 138, 154, 153, 183, 190) polybrominated diphenyl ethers (PBDEs) from dolphin populations in the Indian River Lagoon, FL ($n = 58$) and the Charleston Harbor estuary, SC ($n = 53$). Within each population, we investigated contaminant levels of PBDEs and the effects of factors including age, sex, the interaction of age and sex, and location. Six PBDE congeners (28, 47, 99, 100, 153, and 154) were routinely detected in all samples using gas chromatography/mass spectometry methods. Significantly higher ($p \leq 0.0001$) mean ΣPBDE blubber concentrations were observed for Charleston dolphins ($\overline{X}$ = 5,860 ng/g lipid; range = 429–22,780 ng/g lipid) when compared to Indian River Lagoon dolphins ($\overline{X}$ = 1,260 ng/g lipid; range = 195–3,790 ng/g lipid). PBDE 47 was the major congener representing ~61% of the ΣPBDE in both dolphin populations, followed by BDE100, BDE154, BDE99, BDE153, and BDE28, respectively. Significantly higher ($p < 0.0001$) mean ΣPBDE were observed in adult male dolphins compared to pregnant and adult female dolphins at both sites, with gender differences two-fold in the Indian River Lagoon and twelve-fold for Charleston. For Charleston dolphins, the juveniles in addition to the adult males also had significantly higher levels compared to pregnant and adult females. This study establishes baseline levels of PBDEs in bottlenose dolphins for these two areas and is the first assessment of PBDEs in free-ranging dolphins. The levels of PBDEs in Charleston dolphins represent some of the highest measured in marine mammals and warrants further investigation of these emerging, bioaccumulative chemicals and their potential deleterious effects.

Keywords Brominated flame retardants · Polybrominated diphenyl ethers · PBDEs · Bottlenose dolphin · *Tursiops truncatus*

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Polybrominated diphenyl ethers (PBDEs) are a class of brominated flame-retardant chemicals used widely in consumer products. PBDEs are primarily derived from three technical mixtures, penta-BDE, octa-BDE, and deca-BDE (Hardy 2002). Although penta-BDE and octa-BDE mixtures are being phased out, their widespread use in consumer products has made them ubiquitous contaminants, and the deca-PBDE formulation continues to be produced in the United States (Alcock et al. 2003; EPA 2006). These chemicals are increasingly being detected in the environment and wildlife worldwide, including remote regions (DeWit 2002; Muir et al. 2006) and have also been found in nearly all humans examined (Hites 2004; Sjodin et al. 2003). Several recent reviews have focused on the presence of PBDEs in the environment (Darnerud 2003; Darnerud et al. 2001; DeWit 2002; Hakk and Letcher 2003; Hardy

2002; Houde et al. 2005; Law et al. 2006). Trends in PBDEs vary depending on the type of sample and the region of sampling (DeWit 2002). Although studies from different regions have recently reported leveling or decreasing trends in a number of different biota (Johansen et al. 2004; Kajiwara, N. et al. 2004; Kierkegaard et al. 2004), PBDE concentrations in North America follow an increasing trend for all types of sample. Significantly increasing trends of PBDEs include measurements in human serum from the United States (Sjodin et al. 2003) and lake trout from Lake Ontario, where concentrations are more than 300 times what they were only 20 years ago (Luross et al. 2000). Body burdens of PBDEs have been dramatically increasing in American wildlife (Luross et al. 2000; Norstrom et al. 2002; She et al. 2002) and humans (Betts 2002; Schecter et al. 2004).

These emerging PBDE chemicals are persistent, toxic, bioaccumulative, and widely distributed environmentally. Because they are persistent in the environment, reservoir sources of PBDEs are likely to be present long into the future. Recent U.S. market basket surveys found that on a lipid basis, fish contained the highest PBDEs, followed by meat and dairy products (Schecter et al. 2004; Schecter et al. 2006). Although there are multiple and variable sources of PBDE for human exposure (Schecter et al. 2004; Wu et al. 2005), dietary exposure through fish represent only a percentage of the daily PBDE dietary intake estimate for humans (Hakk and Letcher 2003). In contrast, fish constitute the major food source for dolphins.

The high level of PBDE contamination in human populations, and wildlife is cause for concern because these chemicals have been shown to be toxic in laboratory studies. Results from experimental PBDE exposure studies include cancer (NTP 1986), reproductive and developmental toxicity (Stoker et al. 2004), endocrine disruption (Hallgren and Darnerud 2002), and central nervous system effects (Eriksson et al. 2002; Viberg et al. 2003). The increasing global environmental presence of PBDEs has heightened interest in these compounds, resulting in several recent toxicological reviews (Birnbaum and Staskal 2004; Darnerud 2003; DeWit 2002; Gill et al. 2004; Hardy 2002; McDonald 2002). However, complete toxicological evaluation is not available and little is known about PBDE toxicity and metabolism in cetaceans.

Marine mammals have been found to harbor some of the highest concentrations of persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs), PBDEs, and pesticides (Aguilar et al. 2002; Boon et al. 2002). They are top-level predators and have extensive fat stores that can accumulate these lipophilic pollutants. Many organic contaminants have the potential to induce toxicological impacts in wildlife and humans (Jones and de Voogt 1999) and their high accumulation in aquatic mammals have also recently been associated with adverse health effects (Jepson et al. 2005; Reijnders 1986). Whereas many persistent and ubiquitous organic pollutants such as PCBs are a legacy of the past, brominated flame retardants are widely used and rapidly increasing in the environment.

In recent years, PBDEs have been found in several marine mammal species (Aguilar et al. 2002), including: northern fur seals (*Callorhinus ursinus*) from the Pacific coast of Japan (Kajiwara et al. 2004), polar bears (*Ursus maritimus*) from Alaska, the Canadian Artic, Greenland, and Svalbard (Muir et al. 2006), harbor seals (*Phoca vitulina*) from San Francisco Bay (She et al. 2002), harbor porpoises (*Phocoena phocoena*) from British Columbia (Ikonomou et al. 2002), Irrawaddy dolphins (*Orcaella brevirostris*) from India (Kannan et al. 2005), and cetaceans from Hong Kong (Ramu et al. 2005). Reports of concentrations of PBDEs in bottlenose dolphins (*Tursiops truncatus*) have been limited and analyses have been conducted primarily on tissues obtained from stranded animals (Johnson-Restrepo et al. 2005; Kuehl and Haebler 1995; Kuehl et al. 1991; Tuerk et al. 2005).

Stranded animals are not entirely representative of free-ranging populations, as they might have died from natural causes such as diseases and starvation, they present skewed age composition (Aguilar et al. 2002), and changes in tissue composition can occur rapidly after death and might bias contaminant loads (Borrell and Aguilar 1990). A recent review on contaminants in cetaceans (Houde et al. 2005) compared the advantages and limitations of studying stranded and free-ranging delphinid populations and indicated that the collection of samples from free-ranging marine mammals provides a more realistic estimation of the contaminant loads of wild animals. To our knowledge, the current study represents the first assessment of PBDEs in free-ranging dolphins from two southeastern U.S. sites and will enable comparisons with data obtained from stranded animals.

Bottlenose dolphins inhabit temperate coastal environments and are the most common cetacean in the coastal waters of the southeastern U.S. The widespread coastal distribution of bottlenose dolphins and their role as apex predators support their relevance as important sentinel species for biomonitoring spatial and temporal trends in contaminants (Bossart 2006; Fair and Becker 2000; Reddy et al. 2001; Wells et al. 2004). Both the Charleston, South Carolina, USA (CHS) and Indian River Lagoon, Florida, USA (IRL) sites have long-term and ongoing photo-identification studies and evidence for long-term site fidelity (Mazzoil et al. 2005; Speakman et al. 2006; Zolman 2002). Bottlenose dolphins living in these locations are likely affected by local anthropogenic activities and might serve as indicators of coastal pollution. It is important to determine the distribution and exposure levels of these emerging

and bioaccumulative chemicals in order to understand the potential risks posed by these chemicals and to mitigate sources of exposure. The present study was conducted to determine body burdens of PBDEs in free-ranging bottlenose dolphins at two sites along the southeast Atlantic coast.

Materials and Methods

Sample Collections

Capture–release studies were conducted in the estuarine waters of Charleston, SC, USA (W 32°46′51″N, 79°55′33″W; Fig. 1) during August of 2003 and 2004. The CHS site included the Charleston Harbor, as well as the lower portions the Ashley, Cooper, and Wando rivers, and associated creeks, and the Stono River Estuary. In June of 2003 and 2004, capture–release studies were conducted in the Indian River Lagoon, FL, USA in two separate areas (Fig. 2) extending from the north near Merritt Island, FL 80°47′46″W to south in the St. Lucie Inlet 27°47′41″N. The northern area included the Mosquito Lagoon, portions of the Indian and Banana Rivers north of latitude 28°15′0″N; the southern portion included the St. Lucie Inlet, the north and south forks of the St. Lucie River, and the Indian River south of latitude 27°25′0″N. This study was part of the Bottlenose Dolphin Health and Risk Assessment (HERA) Project, aimed at assessing the health status of dolphins in these two areas. Dolphins were captured using established techniques (Fair et al. 2006). Once restrained, dolphins were placed on a processing vessel where veterinarians conducted health exams and collected tissue samples. A sterile scalpel and forceps, prerinsed with hexane and methanol, were used to surgically remove a blubber biopsy sample (5 cm × 3 cm, full depth) from a location ~10 cm posterior and ~10 cm below the posterior insertion of the dorsal fin. The site was prepared with surgical scrub (2% chlorhexidine gluconate) followed by an alcohol-soaked gauze pad. A local anesthetic (2% lidocaine) was injected in an ''L'' block array. The site received a final rinse with methanol prior to the biopsy procedure. Using precleaned and sterilized scalpel and forceps, the blubber tissue sample was immediately separated from the epidermis and placed in a precleaned Teflon® vial and stored on the boat in a liquid-nitrogen vapor cooler. Samples were stored at –80°C until analyzed.

Age was determined by counting the postnatal dentine layers (Hohn et al. 1989) in a tooth extracted during the sampling procedure. A total of 111 blubber samples were collected over the course of the study: 58 from IRL dolphins (31 in 2003 and 27 in 2004) and 53 from CHS dolphins (38 in 2003 and 15 in 2004). All animal capture and sampling protocols were conducted under National Marine Fisheries Permit No. 998-1678-00 and approved by the Harbor Branch Oceanographic Institution Institutional Animal Care and Use Committee (IACUC).

Contaminant Analyses

Each blubber sample was analyzed for 13 PBDE congeners (17, 28, 47, 66, 71, 85, 99, 100, 138, 154, 153, 183, and 190). Total PBDEs were calculated as the sum of the lipid-normalized congeners. All solvents used during this extraction process were High Purity Solvents for Gas Chromatograph (GC) and Pesticide Residue analysis from

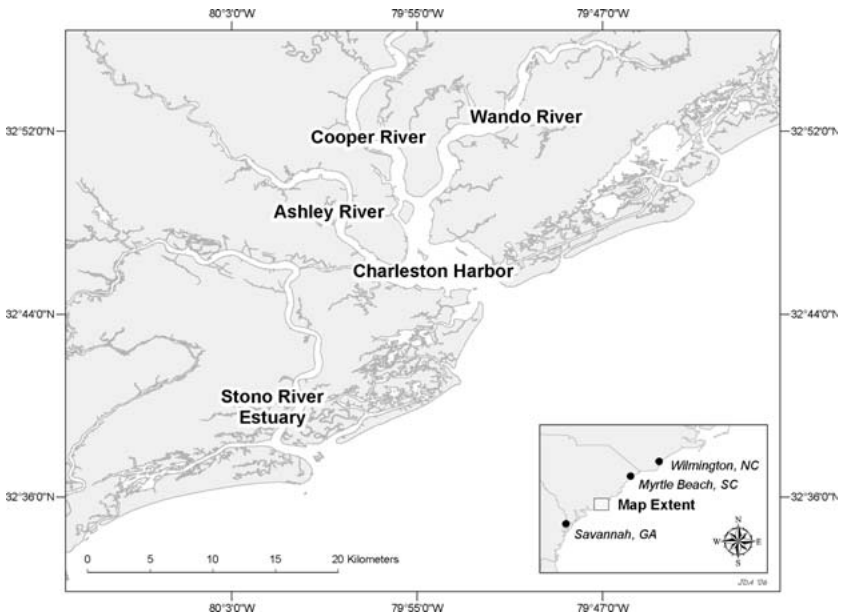

Fig. 1 Charleston, SC study site

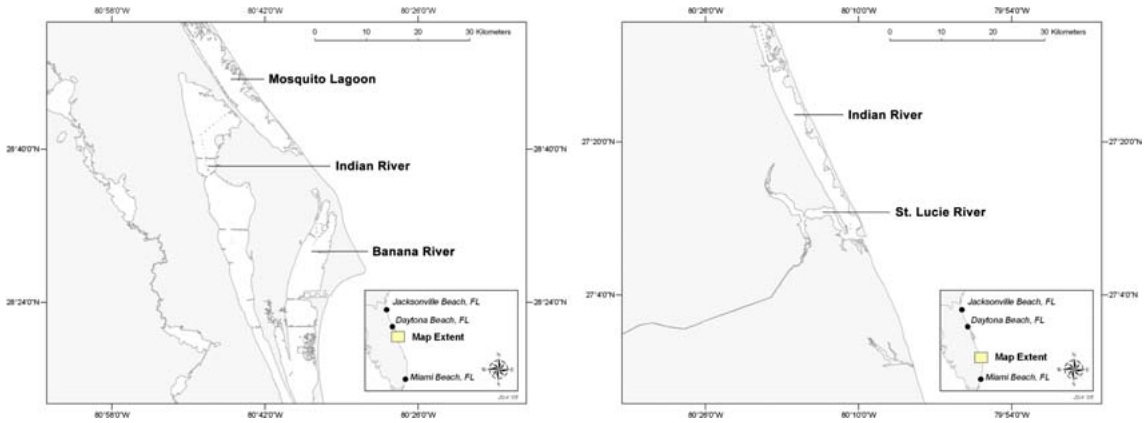

Fig. 2 Northern (left) and southern Indian River Lagoon, FL study sites

Burdick and Jackson (Muskegon, MI, USA). Individual samples (~1 g full depth; confirmed by the presence of attached muscle) were placed in a solvent-washed Petri dish and weighed. The samples were diced into thin pieces and placed into individual 33-mL extraction cells filled with anhydrous sodium sulfate and spiked with a known concentration of C^{13}-labeled BDE28 and BDE100 (Cambridge Isotope Laboratories, Andover, MA, USA) as internal standards. Methylene chloride (Pesticide Residual Grade; Burdick and Jackson Inc.) was used to rinse biopsy residues into the extraction cells and as the extraction solvent for the high-pressure Accelerated Solvent Extractor (ASE) (Dionex^{TM} Corporation, Sunnyvale, CA, USA) run at 1300 psi and 110°C. Each extraction cell was flushed with solvent five times during the ASE protocol. The methylene chloride extracts were then passed through a funnel containing anhydrous sodium sulfate to remove any residual water and brought volumetrically to 100 mL for lipid determination. Lipids were measured gravimetrically in duplicate by removing 5-mL aliquots from the 100-mL volumetric flask into preweighed aluminum pans. The lipid samples were dried at 68°C for 24 h and reweighed. Remaining extracts were reduced to 1 mL under nitrogen (TurboVap II; Zymark Corp., Hopkinton, MA, USA), diluted with methylene chloride, and placed on an AccuPrep Gel Permeation Preparative Liquid Chromatograph (J2 Scientific, Columbia, MO, USA) packed with 70 g of SX3 biobeads swelled with methylene chloride. The sample was introduced via an automated injection system with a flow rate of 5 mL methylene chloride/min and the contaminant fraction was collected from 31.8 to 46.7 min. Each extract was then reduced to 1 mL under nitrogen and passed though a 1-g florisil column on a Zymark SPE Workstation (Zymark Corp., Hopkinton, MA, USA). The florisil column was eluted with 30 mL of a 20% ethyl/petroleum ether solution to separate PBDEs from polar interfering components. The samples were reduced in volume under nitrogen and reconstituted in iso-octane for analysis.

The extracts were analyzed using an Agilent 6890 Gas Chromatograph (Agilent Technologies Inc., Palo Alto, CA, USA) equipped with an Agilent 5973 mass selective detector operated in the electron impact mode. Congeners were identified and quantified by internal standard using selective ion monitoring after being separated on a 30-m DB-5 column with an inner diameter of 0.25 mm and thickness of 0.25 μm, and a flow rate of 1 mL/min. The oven temperature parameters were as follows: initial temperatures of 80°C with a temperature ramp of 10°C /min up to 165°C, followed by a 1.5°C/min increase to 200°C, and a final ramp at 8°C /min to 315°C. Using this process, method detection limits were generally between 1 and 4 ng/g wet mass for each PBDE congener.

Quality Assurance

Data quality was ensured by tracking a series of reagent blanks and reagent spikes through the quantitation process. For each sample set (IRL 2003, IRL 2004, CHS 2003, and CHS 2004), there were a series of at least two blanks and spikes analyzed for quality assurance. None of the 13 PBDEs quantified in this study were detected in any of the reagent blank samples. Spiked samples consistently averaged between 89% and 94% recovery for total PBDE, with the minimum and maximum percent recovery for any one congener ranging from 69% to 111%.

Statistical Analyses

The wet weight contaminant concentrations were divided by the amount of lipid in the samples, and lipid-normalized values were used in all statistical analysis. For statistical

Table 1 Summary of mean (± standard deviation), minimum and maximum PBDE concentrations (ng/g lipid) in blubber biopsies from free-ranging dolphins sampled in Charleston, SC (CHS) and in the Indian River Lagoon, FL (IRL)

Location	*n*		Lipid %	BDE28	BDE47	BDE99	BDE100	BDE153	BDE154	ΣPBDE
CHS										
All dolphins	53	*X* ± SD	36 ± 9.2	42 ± 25.6	3630 ± 2742.3	326 ± 60.7	1150 ± 949.7	203 ± 160.7	511 ± 380.4	5860 ± 4285.2
		min	16	4	158	41	43	31	50	429
		max	62	168	14450	1410	5380	668	1560	22783
Adult males	31	*X* ± SD	33 ± 8.7	40 ± 16.2	4110 ± 1920.6	341 ± 168.5	1380 ± 661.2	268 ± 157.8	687 ± 357.9	6830 ± 2970.9
Age		min	16	4	1040	96	314	73	164	1711
(*X* = 17; SD = 5.7)		max	62	83	7970	719	2980	668	1560	13167
Adult females	5	*X* ± SD	36 ± 2.5	23 ± 5.5	246 ± 69.2	62 ± 18.3	66 ± 23.4	61 ± 29.0	106 ± 72.5	565 ± 198.3
Age		min	31	16	158	41	43	36	48	429
(*X* = 23; SD = 4.6)		max	37	29	349	89	102	110	226	904
Pregnant Females	4	*X* ± SD	34 ± 12.0	30 ± 2.9	1130 ± 1059.2	137 ± 96.4	270 ± 267.4	59 ± 16.5	116 ± 66.5	1740 ± 1501.7
Age		min	23	27	525	71	126	45	66	923
(*X* = 20; SD = 10.8)		max	51	34	2710	278	671	81	212	3990
Juvenile males	5	*X* ± SD	39 ± 9.6	54 ± 36.0	5120 ± 5257.3	480 ± 527.1	1628 ± 2110.1	178 ± 190.5	394 ± 305.8	7850 ± 8398.7
Age		min	25	28	1860	161	411	61	145	2680
(*X* = 6; SD = 1.7)		max	51	114	14460	1411	5384	515	905	22800
Juvenile females	8	*X* ± SD	44 ± 8.4	57 ± 47.9	4220 ± 3004.3	430 ± 345.9	1070 ± 751.6	126 ± 107.2	355 ± 295.7	6260 ± 4517.0
Age		min	26	19	1270	102	260	31	75	1756
(*X* = 5; SD = 1.1)		max	52	168	10600	1147	2380	365	994	15600
IRL										
All dolphins	58	*X* ± SD	37 ± 9.9	25 ± 28.6	757 ± 525.0	79 ± 42.7	248 ± 190.2	50 ± 30.5	92 ± 63.7	1260 ± 814.6
Age		min	16	3	83	4	4	16	17	196
(*X* = 15; SD = 4.2)		max	65	229	2330	252	860	204	300	3790
Adult males	25	*X* ± SD	34 ± 11.1	22 ± 9.4	1040 ± 529.1	89 ± 43.1	353 ± 195.9	59 ± 25.4	125 ± 65.9	1690 ± 838.1
Age		min	16	5	352	4	4	25	34	463
(*X* = 15; SD = .2)		max	65	42	2330	193	860	117	301	3790
Adult females	14	*X* ± SD	38 ± 9.3	33 ± 57.0	367 ± 266.2	68 ± 57.0	118 ± 83.8	47 ± 46.8	64 ± 49.2	696 ± 428.5
Age		min	22	3	83	20	25	16	18	196
(*X* = 13; SD = 5.7)		max	50	229	894	253	249	204	219	1410
Pregnant females	2	*X* ± SD	43 ± 3.8	24.9 ± 7.6	421 ± 106.8	66 ± 14.3	130 ± 62.5	40 ± 6.6	57 ± 10.4	739 ± 179.7
		min	40	20	346	56	86	35	49	612
		max	45	30	497	76	174	44	64	866
Juvenile males	11	*X* ± SD	38 ± 6.0	21 ± 7.3	617 ± 300.3	67 ± 21.0	178 ± 77.8	38 ± 12.8	58 ± 17.7	979 ± 411.8
Age		min	30	15	319	41	103	25	37	594
(*X* = 7; SD = 1.7)		max	47	41	1320	105	340	70	90	1920

Table 1 continued

Location	*n*		Lipid %	BDE28	BDE47	BDE99	BDE100	BDE153	BDE154	ΣPBDE
Juvenile females	6	$X \pm$ SD	40 ± 11.8	21 ± 7.0	945 ± 690.6	87 ± 35.7	287 ± 276.9	43 ± 25.0	94 ± 88.7	1480 ± 1109.1
Age		min	27	11	123	25	32	16	17	224
(X = 5; SD = 0.8)		max	59	29	2210	136	829	89	270	3560

Note: ΣPBDE value represents congeners found above the detection value

evaluation of the total PBDE data, only congeners having values greater than the minimum detection limit (MDL) were included. The Student's *t*-test (for comparison of two categories) and analysis of variance (ANOVA: for greater than two categories) were used to examine the statistical significance in differences between means. Interpretation of statistical significance testing should consider the small population sizes as a result of stratification by age, gender, and site. Descriptive data include mean, standard deviation, range, median, standard error, and 95% confidence intervals stratified by site, age, and gender. Spearman rank correlation tests were used to examine the potential linear association between PBDEs in dolphin blubber and dolphin characteristics (*e.g.*, age and gender).

Results and Discussion

Blubber Lipid Content

Total lipid content (% wet weight) averaged 36% (SD ± 10) for all dolphins from both sites, although individual blubber samples were highly variable in lipid content, ranging from 16% to 65% (Table 1). Lipid content was not likely a factor in PBDE concentration because only a weak correlation ($r = 0.03$, $p = 0.72$) was found between PBDE concentrations and lipid. There were also no differences in blubber thickness in animals from the two sites as measured by ultrasound. Measurements of brominated compounds in sea lions also found no correlation of lipid content with any brominated compound (Stapleton et al. 2006). The mean lipid percent was very similar for each of the age/gender groups, ranging from a low of 34% in IRL adult males to a high of 44% in CHS juvenile females. The IRL and CHS dolphins had lower mean values than those reported previously for samples collected from stranded dead bottlenose dolphins, which averaged 68% (range: 67–78%) for adult and 74% (range: 64–80%) for juveniles in the inner blubber layer (Struntz et al. 2004) and 70% for inner and 60% for outer layers (Koopman 2001). Blubber lipid content varies depending on such factors as season, geographic location, nutritional, and disease conditions, in addition to sampling technique. Previous studies have reported that dart biopsies of wild cetaceans showed lower lipid values than from necropsy samples (Krahn et al. 2001, 2004). Possible reasons suggested for these differences were as follows: lipid seeping from the blubber structural matrix as the dart is removed from the animal, lipid washing away when the dart falls into the water before being retrieved, and uneven sampling biasing collection of greater epidermas and connective tissues. Samples in our study were collected via surgical biopsy and were not subject to many of the bias associated with dart biopsies. To our knowledge, there have been no comparative studies on lipid variation found between samples collected from surgical biopsies with either dart biopsies or stranded dolphins. Although all methods have inherent biases, surgical biopsy techniques offer many advantages in which the sample is obtained directly from living tissue and followed by immediate sectioning and freezing. Comparisons of sampling techniques, including remote and surgical biopsy with samples collected from stranded animals, might provide further insight on the effects of sampling techniques and how closely they might reflect true blubber lipid values.

PBDE Concentrations in Dolphin Blubber

The six major PBDE congeners (28, 47, 99, 100, 153, and 154) predominant in the commercial penta-BDE formulation were detected in all blubber samples. The other seven PBDE congeners (17, 66, 71, 85, 138, 183, and 190) were not found at levels above the detection limit. Descriptive statistics were calculated for the major individual congeners and are presented in Table 1. There was no interannual influence on the observed PBDE concentration at either site. The mean concentrations of the six primary ΣPBDE congeners measured in the blubber of CHS dolphins ($\overline{X}$ = 5,860 ng/g lipid; SD = 4,290) were significantly higher ($p \leq 0.0001$) than the IRL dolphins ($\overline{X}$ = 1,260 ng/g; SD = 979) (Table 1 and Fig. 1). The highest ΣPBDE value found in a CHS dolphin was 22,780 ng/g lipid, whereas the highest concentration found in an IRL dolphin was 3,790 ng/g, a six-fold difference. The lower and upper 95%

confidence intervals for ΣPBDE in CHS were 4,680–7,050 ng/g lipid and 1,050–1,470 ng/g lipid in IRL dolphins.

The summed mean concentration of PBDE congeners (5,860 ng/g) found in blubber tissue of CHS dolphins exceeded mean values reported in reviews for many marine mammal species (Hites 2004; Houde et al. 2005) and bottlenose dolphins from the U.S. east coast and Gulf of Mexico as well as that reported in other dolphin species (Kuehl and Haebler 1995; Kuehl et al. 1991; Tuerk et al. 2005). Body burdens of PBDEs in adult CHS male dolphins were twice the concentration measured in male dolphins during an unusual mortality event (UME) in the Gulf of Mexico (Kuehl and Haebler 1995), although specific comparisons are difficult as the PBDE congeners analyzed were not listed. Comparison of PBDE measurements in biota are often difficult due to the rapid increases of PBDE levels during the last 20 years (DeWit 2002; Law et al. 2006) and differences in the number of individual congeners measured. A common suite of BDE congeners recently proposed by Law et al. (2006) might facilitate PBDE data comparisons and elucidate sources. The highest PBDE accumulation for a single animal during the UME measured 16,300 ng/g (DeWit 2002) and this body burden was exceeded by a CHS dolphin that had 22,780 ng/g. One study measuring PBDEs in Asian cetaceans reported a maximum value of 6,000 ng/g lipid in a single Indo-Pacific humpback dolphins (*Sousa Chinensis*) (Kajiwara et al. 2006), which came the closest to the mean value for the CHS dolphins. The PBDE values in CHS dolphins were comparable to that found for harbor porpoises in 1996–1998 (Law et al. 2002), Beluga whales in 1988–1999 (Lebeuf et al. 2004) and long-finned pilot whales in 1996 (Lindstrom et al. 1999). A study analyzing PBDE levels in 12 marine mammal species from Europe also found relatively high concentrations in killer whales and bottlenose dolphins (Law et al. 2006). The mean concentrations of ΣPBDE (1,260 ng/g; range: 195–3,790 ng/g) found for the IRL dolphins were very similar to values reported for stranded dolphins along the east coast of Florida, during the period 2000–2001 ($\overline{X}$ = 1,130 ng/g lipid; range: 200–4,500 ng/g) (Johnson-Restrepo et al. 2005).

Age and Gender Comparisons

The relationships between PBDE levels and age and gender were analyzed for each site. Many factors are known to affect contaminant body burdens such as sampling and analytical techniques, spatial and temporal patterns of variation, and biological characteristics (*e.g.*, age, length, sex), diet and nutritional condition (Aguilar et al. 1999). By sampling both sites during the same time periods and water temperatures and employing the same sampling and analytical protocols, our study was able to control for several potential variables.

Ages were determined from examination of dental enamel and ranged from 2 to 29 years. A wide variety of age categories have been used in bottlenose dolphin studies; typically sexual maturity has generally been categorized from 5 to 12 years for females and 10 to 13 years for males (Mead and Potter 1990). In our study, adults were defined as females age 7 and older and males age 10 and older and juveniles categorized as less than these ages. Age alone was found to have no influence on PBDE concentrations. This finding was consistent with several other studies on PBDE concentration in marine mammals (Kannan et al. 2006; Thron et al. 2004) as well as in humans (Johnson-Restrepo et al. 2005; Mazdai et al. 2003; Sjodin et al. 2004). A possible explanation for the absence of an age-related increase in PBDE concentration might be the relatively recent introduction of PBDEs and/or metabolism and excretion differences. For both sites, significantly lower ($p < 0.0001$) mean ΣPBDE were observed in adult female and pregnant dolphins when compared to male dolphins (Fig. 3). For CHS dolphins, juveniles, in addition to adult males, were significantly higher than pregnant and adult females (Fig. 3). The IRL juveniles did not have significantly higher levels than the adult and pregnant females, which might be due to the lower PBDE levels, higher variability and/or lower sample sizes (Fig. 3).

Differences in PBDE levels between males and females have been observed for beluga whales (Alaee et al. 1999), ringed seals (Ikonomou et al. 2002), and long-finned pilot whales (Lindstrom et al. 1999; van Bavel et al. 1999), suggesting a transfer of PBDEs from females to calves as

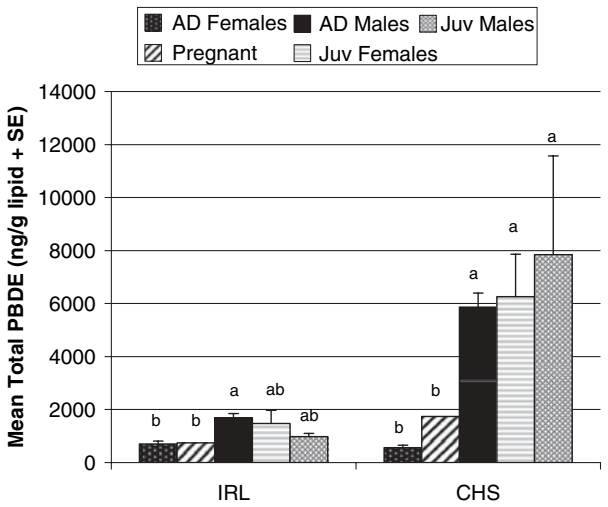

Fig. 3 Mean concentrations of ∑PBDE congeners in blubber tissue from dolphins near Charleston, SC (CHS) and from the Indian River Lagoon, FL (IRL)

occurs in other POPs. Typical accumulation patterns have been observed in beluga whales in which the adult females had lower PBDE concentrations (2 ng/g lipid) compared to males (630 ng/g lipid) (Muir et al. 2006). Elevated PBDE levels in young and juvenile pilot whales from the Faroe Islands support the postnatal transfer of PBDEs (Lindstrom et al. 1999; van Bavel et al. 1999). Other studies in marine mammal species have reported the lowest PBDE concentrations occurring in females, the highest in juveniles, and no difference between males and juveniles (Thron et al. 2004; Tuerk et al. 2005). We observed a similar pattern, with females having the lowest PBDE concentrations compared to adult males and juveniles.

In marine mammals, females reduce their contaminant loads via the transfer of pollutants to their offspring through gestation and lactation (Borrell et al. 1995; Crockcroft et al. 1989), whereas contaminants continue to increase with age in males. Lactation was suggested as the main route of PBDE transfer from mother to offspring in long-finned pilot whales (*Globicephala melas*) (Lindstrom et al. 1999). In mice, the transport of penta-BDE through breast milk was found to be substantial (30–40%) from females to their offspring when administered a dose of BDE47 (DeWit 2002). A high correlation was also found between maternal and fetal PBDE blood levels in humans, indicative of exposure at birth (Mazdai et al. 2003). Evidence that human fetuses in the United States might be exposed to relatively high levels of PBDEs (Mazdai et al. 2003) might also suggest toxicological implications for fetuses and calves of marine mammals because they accumulate much higher body burdens of these persistent chemicals.

PBDE Congener Patterns in Blubber of Dolphins

The relative proportion of total PBDE congeners to the sum of the six individual PBDE congeners observed in blubber tissue for all dolphins from both the IRL and CHS was similar (Fig. 4). BDE47 was the most prominent individual congener. The pooled PBDE geometric mean for all CHS dolphins was 3,630 ng/g lipid (range = 157–14,450 ng/g lipid), whereas IRL dolphins had a PBDE geometric mean of 804 ng/g lipid (range = 82–3,030 ng/g lipid). As the major PBDE congener, BDE47 represented 61% of the total mean PBDE concentration for CHS and IRL dolphins (Fig. 4). The percentage of PBDE congeners shown in Figure 2 for all dolphins was similar among all age and gender categories. This is consistent with several reported studies that also found BDE47 to be the highest congener present in biological tissues (Hites 2004), including marine mammals (ranging from 60% to 75% in Indo-Pacific humpback dolphins from Hong Kong (Kannan et al. 2005). The ranked order of the six congeners: BDE47 > BDE100 >BDE154 > BDE99 > BDE153 > BDE28. These results were comparable to those found in stranded bottlenose dolphins, for which PBDE congeners 47, 100, 154, and 99 accounted for 94% of the total PBDE (Johnson-Restrepo et al. 2005). Our results also show a pattern of PBDE accumulation occurring in the blubber of bottlenose dolphins similar to that found in other marine mammal species (Boon et al. 2002; Kalantzi et al. 2005; Kannan et al. 2005). The same five congeners (47, 100, 154, 153, and 99) also predominate in human tissues, accounting for 90% of the total PBDE body burden (McDonald 2005). It appears that a consistent pattern of major congeners accumulate in marine mammals as well as in humans. One congener, BDE209, not analyzed in this study, was found by Johnson-Restrepo et al. (2005), to be below detectable levels (<0.022 ng/g lipid weight) in blubber of bottlenose dolphins along the Florida coast. They also reported that PBDEs in these dolphins were one to two orders of magnitude greater than those found in lower-trophic-level fish species (range of 8–88 ng/g lipid weight), indicating biomagnification of PBDEs in the marine food web. For comparison, 24 fish species analyzed as part of a US market basket survey had PBDE levels ranging from 11 to 480 ng/g lipid weight (Schecter et al. 2006).

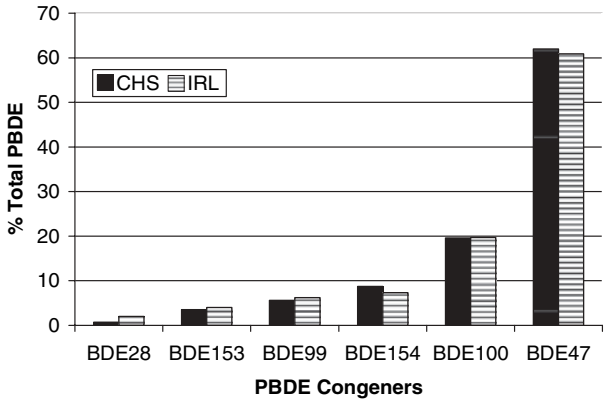

Fig. 4 Relative proportions of PBDE congeners measured in blubber tissue from dolphins in Charleston, SC (CHS) and the Indian River Lagoon, FL (IRL)

The bioaccumulated congeners (47, 100, 154, 153, and 99) found in dolphin blubber tissue are mostly contained in penta-BDE, but recent studies also suggest that some of these congeners might result from debromination of higher brominated congeners (Stapleton et al. 2004). Although BDE 99 is higher than BDE 47 in the penta-formulations, the lower amount of BDE99 found in dolphins is suggestive of increased metabolism and/or elimination of this congener. Although PBDE 153 and 154 are constituents of the penta- and octa-BDE mixtures and might be the source of these congeners in dolphin blubber, it is also possibly the result from debromination of the deca-BDE mixtures. Very

seldom do congener profiles in biota mimic commercial mixtures, and other congeners and ratios are often found (Hites 2004).

Consequences of PBDE Levels in Dolphins

A number of convincing ecological studies provide evidence that PBDEs bioaccumulate (Hites 2004) as well as biomagnify in aquatic ecosystems (Hagland et al. 1997). Some evidence suggests that metabolism of higher brominated congeners might contribute to the accumulation of lower brominated congeners (Stapleton et al. 2004; Tomy et al. 2004). Profiles of PBDE congeners found in wildlife are most likely due to a combination of direct uptake from the environment, debromination of higher congeners to lower brominated congeners, and differential trophic web biomagnifications. The combined effects of biomagnification and bioconversion or biodegradation of PBDE congeners in rising trophic levels might result in body burdens in marine mammals of lower brominated compounds, which are also known to have lower toxicological thresholds (Darnerud 2003).

Multiple health effects have been found in experimental animals following exposure to commercial PBDE mixtures. A critical body burden window was estimated by Birnbaum and Cohen Hubal (2006) to be <0.8 to 8.0 mg/kg based on toxicological effects in mice. Comparisons with the most highly exposed human body burden of 1.5 ng/g lipid (equivalent to 0.3 ng/g or 0.3 mg/kg) they suggest a margin of exposure could be <10. Potential health risks from exposures to PBDEs in mammals include impacts on thyroid hormone levels, neurobiological development, and fetal toxicity/tetratogenicity (Darnerud 2003). Results from one study assessing health risks of PBDEs in grey seals (*Halichoerus grypus*) suggest that these compounds might also act as endocrine disruptors in marine mammals (Hall et al. 2003). There is also evidence to suggest that marine mammals have relatively low activities of drug detoxifying enzymes and are vulnerable to the toxic effects of contaminant exposures (Tanabe et al. 1988). Thus, marine mammals might be at an increased risk of exposure to, and toxic effects from, PBDEs.

Studies have found dramatic increases in PBDE levels in North American wildlife over the last 10–20 years, with levels doubling every 2–5 years depending on species and location (Ikonomou et al. 2002; Lebeuf et al. 2004). Temporal trend analysis of PBDEs in blubber of coastal Florida dolphins estimated a doubling time of 3–4 years (Johnson-Restrepo et al. 2005). It is important to study the time trends for bioaccumulative brominated flame retardants and to assess the role of these ''emerging'' chemical pollutants in the health and recovery of impacted dolphin populations.

The high PBDE body burden found in the CHS dolphins are likely due to exposure to higher environmental levels, which warrants further investigation on possible sources. PBDEs, as a class of emerging environmental contaminants, often enter the environment through urban runoff and sewage outfall (DeWit 2002). Sewage sludge is considered to be one of the primary sinks for PBDEs (Law et al. 2006). The PBDEs found in dolphins from our study likely result from a range of potential sources such as sewage and urban runoff. High PBDE concentrations found in Indo-Pacific humpback dolphins near Hong Kong have also been attributed to sewage discharge into coastal waters (Ramu et al. 2005). Previous studies investigating PBDEs and other POPs found elevated concentrations of these compounds in tissues from beluga whales collected near industrialized areas or those with higher human populations (Alaee et al. 1999; Stern and Ikonomou 2000). Therefore, it is not surprising that the CHS dolphins had mean PBDE levels five times higher than those found in the IRL dolphins. Charleston is an urban estuary with a higher population density and input from four wastewater treatment facilities in the harbor and another six in the surrounding area (SCDHEC 1998). The Indian River Lagoon is a populated coastal region, but it is distributed along an expansive 156-mile estuary containing buffers such as the Merritt Island National Wildlife Refuge and has experienced a reduction of discharge from wastewater treatment since 1996 (Sigua et al. 2000). Considering that dolphins in the Charleston area exhibit high levels of PBDEs, the environment and fish from this area might also have higher PBDE contamination. Food is one of the major routes of intake for PBDE and data from food surveys report that fish have one of the highest overall PBDE levels (Schecter et al. 2004). Thus, dolphins might be exposed to PBDE contaminants primarily via the food chain, as supported by PBDE biomagnifications study in a marine food web of coastal Florida, which included dolphins (Johnson-Restrepo et al. 2005). A recent feeding study with grey seals observed a high absorption (89–99%) of all BDE congeners (Thomas et al. 2005). Results presented here underscore the importance of exploring potential sources of PBDEs and the toxicological risks for dolphins, one of the top predators in this ecosystem. It also highlights the need for further investigation of PBDE contamination in the environment and fish from these areas, particularly fish consumed by both dolphins and humans. The relatively high levels of PBDEs found in blubber from dolphins in the Charleston estuarine areas provides a strong basis for continued monitoring of contaminant loads in these animals and investigation of their potential health effects.

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