Arch. Environ. Contam. Toxicol. 49, 415–420 (2005)
DOI: 10.1007/s00244-005-7078-6

ARCHIVES OF
Environmental
Contamination
and Toxicology

Organochlorine Pesticides, Polychlorinated Biphenyls, and Polybrominated Diphenyl Ethers in Irrawaddy Dolphins from India

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Received: 5 April 2005/Accepted: 12 May 2005

Abstract. The Irrawaddy dolphin (*Orcaella brevirostris*) is at risk of extinction throughout its range as a result of incidental catches, habitat degradation, and pollution. Populations of Irrawaddy dolphins are constrained by the species' narrow habitat requirement—lagoons, estuaries, rivers, and lakes—and are therefore particularly vulnerable to the effects of human activities. In this study, for the first time, concentrations of organochlorine (OC) pesticides, polychlorinated biphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs) were determined in tissues of Irrawaddy dolphins collected from Chilika Lake, India, to understand the status of contamination. Dichlorodiphenyltrichloroethane and its metabolites (DDTs) were the predominant contaminants found in Irrawaddy dolphins; the highest concentration found was 10,000 ng/g lipid weight in blubber. Hexachlorocyclohexanes (HCHs) were the second most prevalent contaminants in dolphin tissues. Concentrations of PCBs, chlordanes, hexachlorobenzene, tris(4-chlorophenyl)methane, and tris(4-chlorophenyl)methanol were in the ranges of few ng/g to few hundreds of ng/g on a lipid-weight basis. In general, concentrations of OC pesticides and PCBs in Irrawaddy dolphins were lower than the concentrations reported for coastal and riverine dolphins collected in Asia. PBDEs were detected in the blubber of Irrawaddy dolphins at concentrations ranging from 0.98 to 18 ng/g lipid weight. BDE congener 47 accounted for 60% to 75% of the total PBDE concentrations. Although these results establish the baseline levels of persistent organic pollutants in Irrawaddy dolphins, efforts should be made to decrease the sources of contamination by DDTs and HCHs in Chilika Lake.

High accumulation of persistent organic pollutants, such as polychlorinated biphenyls (PCBs), in aquatic mammals has been linked to adverse health effects (Reijnders 1986; Kannan *et al.* 1993a; Colborn and Smolen 1996; Jepson *et al.* 2005; Ylitalo 2005). Because of their high trophic level in the food chain and relatively low activities of drug-metabolizing enzymes, aquatic mammals such as dolphins accumulate increased concentrations of persistent organic pollutants (Tanabe *et al.* 1988) and are thereby vulnerable to toxic effects from contaminant exposures. Dolphins inhabiting riverine and estuarine ecosystems are particularly vulnerable to the activities of humans because of the restricted confines of their habitat, which is in close proximity to point sources of pollution. River dolphins are among the world's most seriously endangered species. Populations of river dolphins have been dwindling and face the threat of extinction; the Yangtze river dolphin (*Lipotes vexillifer*) in China and the Indus river dolphin (*Platanista minor*) in Pakistan are already close to extinction (Renjun 1990; Perrin *et al.* 1989; Reeves *et al.* 1991; Reeves and Chaudhry 1998). In addition to habitat degradation (such as construction of dams; Reeves and Leatherwood 1994), boat traffic, fishing, incidental and intentional killings, and chemical pollution have been threats to the health of river dolphins (Kannan *et al.* 1993b, 1994, 1997; Senthilkumar *et al.* 1999). Monitoring of the levels of toxic contaminants in tissues of dolphins is necessary if we are to understand the exposure levels and the risks posed by organic pollutants and mitigate the sources of exposure.

The Irrawaddy dolphin (*Orcaella brevirostris*) is one of the smallest cetaceans found along sea coasts and in estuaries in parts of Southeast Asia, from the Bay of Bengal, throughout the Indo-Malay Archipelago, to northern Australia. The habitat of the Irrawaddy dolphin includes the large river and estuarine systems of the Irrawaddy, the Mahakam, and the Mekong. The Irrawaddy dolphin is also found in Chilika Lake (also referred as Chilika Lagoon), which stretches along the East Coast of India in the Mahanadi River delta (Figure 1). Chilika Lake is the largest brackish water lake in India and has an area of approximately 1165 km^2 (Figure 1). The Irrawaddy dolphin is listed as an "endangered species" in the International Union for Conservation of Nature and Natural Resources Red Book and is listed in the Convention on International Trade in Endangered Species of Wild Fauna and Flora Appendix II. It has been also included in the list of 'threa-

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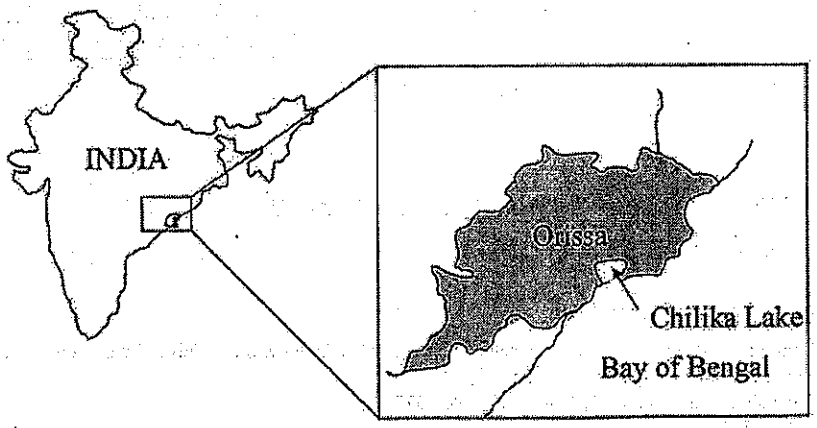

Fig. 1. Map of India showing Chilika Lake, the sampling location of the Irrawaddy dolphins.

tened species' under the Indian Wildlife Protection Act of 1972. Formerly noted as extremely abundant, the population of Irrawaddy dolphin in Chilika Lake was between 20 and 30 animals in the mid-1980s (Reyes 1991; Dhandapani 1992). However, the population was still under study in 1998 (Sahu *et al.* 1998). Surveys conducted in 2000 estimated approximately 40 to 50 individuals in Chilika Lake (Sinha 2004).

Exposure to toxic environmental contaminants can compromise the health and reproductive potential of dolphins (Colborn and Smolen 1996). Nevertheless, in the absence of monitoring data, realistic exposure and hazard assessment of chemical contaminants in dolphins is not possible. Before this study, no tissue residue concentrations of organochlorines (OCs) in Irrawaddy dolphins had been determined. In this study, we measured the concentrations of OC pesticides, PCBs, and polybrominated diphenyl ethers (PBDEs) in tissues of five stranded Irrawaddy dolphins collected from Chilika Lake during 2000 to 2001.

Materials and Methods

Tissues were obtained from five dolphins found stranded, dead, in Chilika Lake, Orissa, in 2000 to 2001. One of the most frequent reasons for dolphin casualties is accidental striking by propellers of small boats. Tissues of a juvenile male dolphin were slightly decomposed. Blubber was taken from all of the individuals, whereas liver, kidney, and muscle tissues were available from only two of the individuals. Sex was determined, and body length was measured. The age of the individuals could not be assessed. However, except for one male dolphin (107 cm), which was a juvenile, all other individuals analyzed were adults. Samples were stored at –20°C until analysis.

Chemical Analysis

OC pesticides PCBs, and PBDEs were analyzed according to methods described elsewhere (Kajiwara *et al.* 2004a; Ueno *et al.* 2004) but with some modifications. For PBDEs, briefly, 5 g tissue samples were ground with anhydrous sodium sulfate and extracted in a Soxhlet apparatus with a mixture of diethyl ether and hexane for 7 to 8 hours. After addition of 5 ng each of internal standards ($^{13}C_{12}$-labeled BDE-3, BDE-15, BDE-28, BDE-47, BDE-99, BDE-153, BDE-154, and BDE-183), an aliquot of the extract was transferred to a gel permeation chromatography column (GPC; Bio-Beads S-X3; Bio-Rad, Hercules, CA; 2 cm i.d. and 50-cm length) for lipid removal. The GPC fraction containing organohalogens was concentrated and passed through 1.5 g activated silica gel (Wakogel S-1; Wako Pure Chemical, Tokyo, Japan) column with 5% dichloromethane in hexane for cleanup. $^{13}C_{12}$-labeled BDE-139 was added to the final solution before analysis by gas chromatography-mass spectrometry (GC-MS). Quantification was performed using a gas chromatographer (Agilent 6980N) equipped with a mass spectrometer (Agilent 5973N) for the determination of mono- to hepta-BDEs. A DB-1 fused silica capillary column (30 m × 0.25 mm i.d. × 0.25-μm film thickness) was used for the separation of mono- to hepta-BDEs. Nine major PBDE congeners (BDE-3, BDE-15, BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, and BDE-183) were quantified in this study. All of the congeners were quantified using the isotope dilution method with responses from corresponding $^{13}C_{12}$-labeled congener. Recoveries of $^{13}C_{12}$-labeled BDE congeners through the analytical procedure ranged between 60% and 120%.

PCBs, dichlorodiphenyltrichloroethane and its metabolites (DDTs), hexachlorocyclohexane isomers (HCHs), chlordane-related compounds (CHLs), and hexachlorobenzene (HCB) were analyzed according to the methods described by Kajiwara *et al.* (2004a). An aliquot of the extract from Soxhlet procedure was subjected to GPC for lipid removal. The GPC fraction containing OCs was concentrated and passed through an activated Florisil column for cleanup and fractionation. Quantification of PCBs and most of the OC pesticides was performed using a gas chromatograph (Agilent 6980N) equipped with a micro-electron capture detector and an auto-injection system (Agilent 7683 Series Injector). The GC column used for the separation of OCs was a fused silica capillary (DB-1; 30 m × 0.25 mm i.d. × 0.25-μm film thickness; J&W Scientific). Identification and quantification of tris(4-chlorophenyl)methane (TCPMe) and tris(4-chlorophenyl)methanol (TCPMOH) were performed using a GC-MS (Agilent 5973N) in selected ion-monitoring (SIM) mode.

Concentration of individual OCs was determined from the sample peak area relative to that of the corresponding external standard. The PCB standard used for quantification was a mixture of 62 PCB isomers and congeners (BP-MS) obtained from Wellington Laboratories, Inc. (Ontario, Canada). Concentrations of PCB isomers and congeners that were represented by individually resolved peaks were summed to obtain total PCB concentrations.

Procedural blanks were analyzed simultaneously with every batch of five samples as a check for interferences or contamination arising from solvents and glassware. Lipid contents were determined by measurement of the total nonvolatile solvent-extractable material in subsamples of the original extracts. The concentrations of organohalogens were expressed on a lipid-weight basis unless otherwise specified.

For quality assurance and control, our laboratory participated in the Inter-laboratory Comparison Exercise for Persistent Organochlorine Contaminants in Marine Mammals Blubber, organized by the Na-

tional Institute of Standards and Technology (Gaithersburg, MD) and the Marine Mammal Health and Stranding Response Program of the National Oceanic and Atmospheric Administration's National Marine Fisheries Service (Silver Spring, MD). A standard reference material (SRM 1945) was analyzed for selected PCB congeners and OC pesticides. Data from our laboratory were in good agreement with those for reference materials. Mean deviation from the certified values was 13% (range 0.5% to 20%) for OC pesticides and 28% (range 1.3% to 57%) for PCB congeners.

Results and Discussion

DDTs were the predominant OCs found in Irrawaddy dolphin tissues. A DDT concentration as great as 10,000 ng/g lipid weight was found in the blubber of an adult male dolphin. HCHs were the second most abundant OCs followed by PCBs. Concentrations of HCB and chlordanes were relatively low. TCPMe and TCPMOH were also detected in several samples. Lipid content in the liver and kidney of the single juvenile male dolphin was 49% and 37%, respectively. The reason for the high content of lipid in this individual was not known. In general, starvation or disease can result in the mobilization of lipid from blubber, which could lead to increased lipid content of liver and/or kidney. However, pathologic investigation was not performed for the dolphins analyzed. Relatively low lipid content in the blubber of several other dolphins (11% to 22%) suggests that these individuals were emaciated.

DDT concentrations in the blubber of Irrawaddy dolphins ranged between 310 and 10,000 ng/g lipid weight. Whereas the lowest concentration (310 ng/g) found in an adult female could be explained by lactational transfer of DDT to offspring, concentrations of DDT in the blubber of other individuals analyzed were >1000 ng/g lipid weight. The occurrence of increased concentrations of DDTs in biological samples from India has been reported earlier (Kannan *et al.* 1995). DDT is still being used for malaria control, although its use was banned for agricultural purposes in 1989. Nevertheless, the concentrations of DDT in Irrawaddy dolphins were approximately 2-fold to 3-fold less than what was reported for bottlenose and spinner dolphins collected during 1990 to 1991 (Tanabe *et al.* 1993) and during 1997 to 1999 (Karuppiah *et al.* 2005) from the Bay of Bengal. Similarly, the DDT concentrations that we measured in Irrawaddy dolphins were 3- to 10-fold lower than the levels in Ganges river dolphins collected during 1992 to 1996 from the Ganges River in India (Senthilkumar *et al.* 1999). Lower concentrations of DDTs in Irrawaddy dolphins than in bottlenose and spinner dolphins from the Bay of Bengal may be related to a difference in trophic status. The prey items of Irrawaddy dolphins include small fish and crustaceans. In addition, the use of DDT in India has decreased from 19,000 tons/y in the early 1990s (Kannan *et al.* 1995) to 7000 tons in 2001 to 2002.

Among DDTs, *p,p'*-DDE was the predominant compound in the tissues of Irrawaddy dolphins (Figure 2). *p,p'*-DDE accounted for 77% of the total DDT concentration in blubber and for 90% in kidney. The technical mixture of DDT contains 80% *p,p'*-DDT and 20% *o,p'*-DDT (Kannan *et al.* 1995). *p,p'*-DDT can undergo degradation in the environment and in biota to *p,p'*-DDE and *p,p'*-DDD, which are relatively stable metabolites. A high proportion of *p,p'*-DDE in biota would suggest exposure to aged residues from the environment and greater metabolic transformation by the organism. In contrast, a high proportion of *p,p'*-DDT in biota would imply recent exposure by and slow metabolism in the animals. The composition of *p,p'*-DDE we found in the Irrawaddy dolphins was relatively higher than what had been found in Ganges river dolphins collected during 1992 to 1996 in India (Kannan *et al.* 1994; Senthilkumar *et al.* 1999). The proportion of *p,p'*-DDT was low (1% to 6%) and comparable with what was reported for coastal (bottlenose and spinner) dolphins collected during 1997 to 1999 from the Bay of Bengal in India (Karuppiah *et al.* 2005). These results suggest a decrease in the recent inputs of DDT into the coastal and estuarine environment in India.

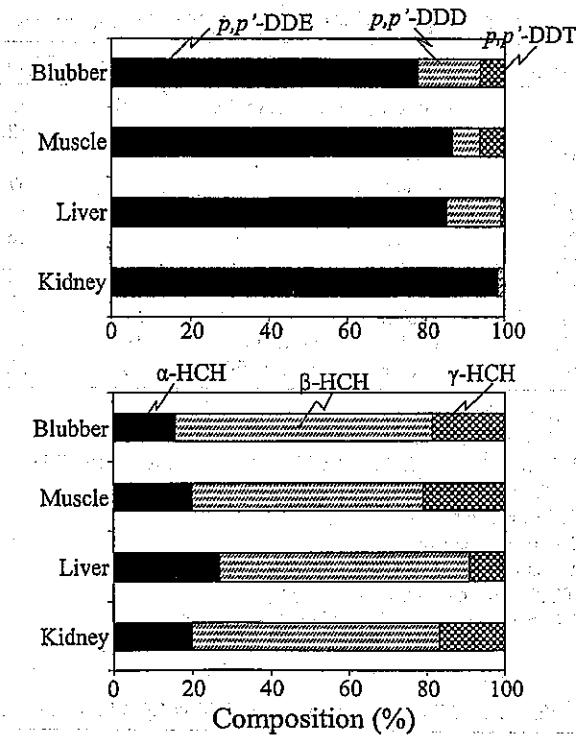

Fig. 2. Composition (%) of DDT compounds and HCH isomers relative to total DDT and total HCH concentrations, respectively, in tissues of the Irrawaddy dolphins from Chilika Lake, India.

HCHs were the second most prevalent OC contaminants in Irrawaddy dolphins. The highest concentration of HCHs was found in the blubber of an adult male dolphin (1200 ng/g lipid weight). Increased concentration of HCHs in Irrawaddy dolphins appears to be characteristic for this species. In particular, a concentration of HCHs that is greater than that of PCBs is different from the pattern found in dolphins from other parts of the world (Kajiwara *et al.* 2004a; Minh *et al.* 2000). Dolphins collected from several coastal locations—including those of other developing countries such as Brazil, Hong Kong, China, and the Philippines—showed greater concentrations of PCBs than HCHs (Kajiwara *et al.* 2004a; Minh *et al.* 2000). The greater concentration of HCHs than of PCBs in Irrawaddy dolphins can be explained by the watershed of Chilika Lake, which is predominantly agricultural rather than urban and industrial, and that the agricultural activities contribute to exposure to OC pesticides.

Table 1. Concentrations of OCs (ng/g lipid weight) in the tissues of Irrawaddy dolphins from Chilika Lake, India

Sample ID	Tissue	Sex	BL (cm)	Lipid (%)	PCBs	DDTs	CHLs	HCHs	HCB	TCPMe	TCPMOH
1B	Blubber	Male (juvenile)	107	53	28	1100	1.7	180	3.6	<2.0	20
1M	Muscle			12	95	3000	4.4	220	8.5	<2.0	49
1L	Liver			49	28	900	1.6	130	4.4	<2.0	18
1K	Kidney			37	36	1100	ND	99	5.9	<2.0	<5.0
2B	Blubber	Female(adult)	213	13	71	2000	2.6	59	5.3	8.6	<5.0
2M	Muscle			16	81	1900	ND	ND	3.9	7.6	37
2L	Liver			4.2	35	430	ND	ND	2.8	<2.0	<5.0
2K	Kidney			5.9	79	1300	ND	ND	2.9	< 2.0	<5.0
3B	Blubber	Female (adult)	226	11	35	310	3.2	91	4.2	<2.0	<5.0
4B	Blubber	Male (adult)	197	17	390	10000	41	1200	17	16	97
5B	Blubber	Female (adult)	201	22	360	7900	40	1000	16	18	130

BL = Body length.
CHLs = Chlordane–related compounds.
DDTS = Dichlorodiphenyltrichloroethane and its metabolities.
HCB = Hexachlorobenzene.
HCHs = Hexachlorocyclohexanes.
ND = Not detected.
PCBs = Polychlorinated biphenyls.
TCPMe = Tris (4-chlorophenyl) methane.
TCPMOH = Tris (4-chlorophenyl) methanol.

Table 2. PBDE concentrations (ng/g lipid weight) in the tissues of Irrawaddy dolphins from Chilika Lake, India

Sample ID	Sex	BL (cm)	Tissue	Lipid (%)	BDE-28	BDE-47	BDE-99	BDE-100	BDE-153	BDE-154	BDE-183	Total PBDE
1B	Male (juvenile)	107	Blubber	53	0.053	0.89	0.12	0.10	<0.05	<0.05	<0.05	1.2
1M			Muscle	12	<0.01	1.9	0.34	0.19	<0.05	<0.05	<0.05	2.4
1L			Liver	49	0.066	1.1	0.15	0.072	<0.05	<0.05	<0.05	1.3
1K			Kidney	37	0.063	1.2	0.70	0.20	<0.05	<0.05	<0.05	2.2
2B	Female (adult)	213	Blubber	13	0.089	1.5	0.45	0.47	<0.05	<0.05	<0.05	2.5
2M			Muscle	16	0.060	1.4	0.44	0.12	0.19	0.093	0.59	2.9
2L			Liver	4.2	<0.01	0.46	0.16	0.083	<0.05	<0.05	<0.05	0.71
2K			Kidney	5.9	0.068	1.1	0.38	0.16	<0.05	<0.05	<0.05	1.7
3B	Female (adult)	226	Blubber	11	0.032	0.66	0.17	0.12	<0.05	<0.05	<0.05	0.98
4B	Male (adult)	197	Blubber	17	3.4	12	0.57	0.91	0.27	0.35	<0.05	18
5B	Female (adult)	201	Blubber	22	2.8	6.3	0.78	0.55	0.26	0.23	<0.05	11

BL = Body length.
PBDE = Polybrominated diphenyl ethers.

India is one of the major users of the technical HCHs; technical HCH has predominantly been used for agricultural purposes (Kannan *et al.* 1995). Although the use of technical HCH (containing 55% to 60% α-, 5% to 14% β-, and 10% to 18% γ-isomers) was banned in 1997, lindane (γ-HCH) has remained in use for malaria control in some areas. The measured concentrations of HCHs in Irrawaddy dolphins were twofold to fivefold lower than in Ganges river dolphins collected during 1992 to 1996 in India (Senthilkumar *et al.* 1999). β-HCH was the predominant HCH isomer that we found in Irrawaddy dolphins (Figure 2). α- and γ-isomers collectively accounted for <40% of the total HCH concentrations in the blubber of Irrawaddy dolphins. A high proportion of β-HCH in dolphins suggests this isomer's resistance to enzymatic and metabolic degradation.

PCBs were found at concentrations ranging from 28 to 390 ng/g lipid weight (Table 1). These concentrations were 10- to 100-fold lower than the concentrations reported in Ganges river dolphins collected during 1992 to 1996 (Senthilkumar *et al.* 1999). Similarly, concentrations of PCBs in Irrawaddy dolphins were lower than the concentrations reported for dolphins from the Bay of Bengal (Karuppiah *et al.* 2005) and from other Asian coasts (Minh *et al.* 2000). A threshold PCB concentration of 8700 ng/g lipid weight has been reported to elicit physiologic effects in aquatic mammals (Kannan *et al.* 2000). Concentrations of PCBs in Irrawaddy dolphins were one to two orders of magnitude lower than the threshold concentration. Because the Chilika Lake watershed is agricultural rather than urban and industrial in nature, PCB concentrations in Irrawaddy dolphins were not expected to be high. Furthermore, contamination of the Indian environment by PCBs is relatively low (Kannan *et al.* 1995).

HCB, chlordanes, TCPMe, and TCPMOH were also found in the tissues of Irrawaddy dolphins, although at concentrations lower than those of DDTs and HCHs. TCPMe and TCPMOH were found in those individuals that had the highest concentrations of DDTs, suggesting that these two compounds originate from DDTs (Watanabe *et al.* 2000). In general, concentrations of TCPMOH were greater than the concentrations of TCPMe.

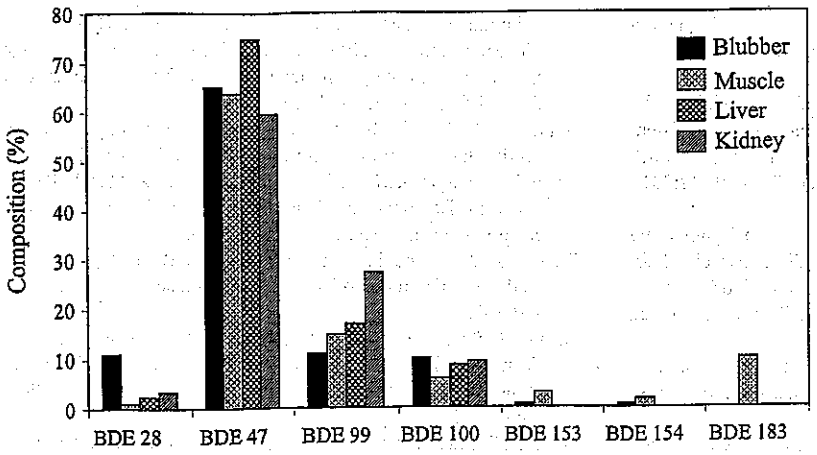

Fig. 3. Contribution (%) of PBDE congeners to total PBDE concentrations in tissues of Irrawaddy dolphins from Chilika Lake, India.

PBDEs, a class of brominated flame retardants, were found in the blubber of Irrawaddy dolphins at concentrations ranging from 0.98 to 18 ng/g lipid weight (Table 2). PBDE congeners were also found in muscle, liver, and kidney tissues of dolphins. This is the first study to report the occurrence of PBDE in biota from India. The concentrations of PBDE in the Irrawaddy dolphins are one to three orders of magnitude lower than concentrations reported for coastal dolphins from the United Kingdom and the United States (Law *et al.* 2005; Tuerk *et al.* 2005), and twofold to fivefold lower than concentrations in northern fur seals collected from Japan in 1999 (Kajiwara *et al.* 2004b). Although lower levels of PBDEs in Irrawaddy dolphins could be explained by the rural and agricultural nature of the Chilika Lake watershed, the detection of these flame retardants in the Indian environment confirms the widespread distribution of these compounds.

Similar to PBDE profiles reported in cetaceans from several other locations, BDE 47 is the major congener, accounting for 60% to 75% of the total PBDE concentrations (Figure 3); it was followed by penta-BDE congeners 99 and 100. This profile suggests sources originating from the use of penta-BDE mixture. The greater proportion of BDE 99 in kidney suggests preferential elimination of this congener through urine compared with the other congeners. The technical penta-BDE mixture has a higher proportion of BDE 99 than BDE 47. However, the lower proportion of BDE 99 than of BDE 47 that is seen in biological matrices, including these dolphin tissues, suggests preferential elimination or metabolic degradation of BDE 99.

In general, the residue levels of organochlorines and PBDEs in Irrawaddy dolphins are lower than the concentrations reported for other cetaceans in the coastal and riverine waters of Asia. Because the habitat of Irrawaddy dolphins is vulnerable to degradation, further efforts to conserve this endangered species should focus on establishing protected areas, raising public awareness, managing fisheries to decrease incidental killings and ensure the sustainability of prey species, and decreasing chemical pollution.

Acknowledgments. This study was supported by Grants-in-Aid for Scientific Research (Grant No. 16201014) from Japan Society for the Promotion of Science and for Young Scientists (Project No. 16780139) and the 21st Century COE Program from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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