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Tissue distribution and fate of persistent organic pollutants in Indo-Pacific humpback dolphins from the Pearl River Estuary, China

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ABSTRACT

Eleven persistent organic pollutant (POP) compounds including ∑PCBs, ∑DDTs, ∑HCHs, aldrin, mirex, endrin, ∑CHLs, dieldrin, HCB, heptachlor and pentachlorobenzene were measured in the kidney, liver, muscle, melon and other tissues of *Sousa chinensis* stranded on the western coast of the Pearl River Estuary in China during 2007–2013. For most parameters of POPs measured, melon tissues contained the highest mean concentrations with the exception of aldrin, which was higher in the kidney and liver tissues. The concentrations of PCBs, DDTs, heptachlor and endrin in the melon tissue exhibited significant correlations with body length, whereas PCBs and heptachlor also displayed significant regression with age. Our studies showed hepatic concentrations of ∑DDTs, ∑HCHs and mirex in *S. chinensis* were generally higher than those found in cetaceans from other geographic locations. The high levels of POP residues in the testis of one male dolphin suggested an increasing risk of infertility in the species.

1. Introduction

The Pearl River Delta region in Guangdong Province is one of the fastest-developing areas in China and Asia. Anthropogenic activities have resulted in the release of unprecedented amounts of persistent organic pollutants (POPs) into the Pearl River Estuary (PRE) during the last three decades (Guan et al., 2007; Guo et al., 2008, 2009; Mai et al., 2005, 2002). These POPs have adverse effects on the immune and reproduction systems of animals (Lahvis et al., 1995). According to the stockholm convention on persistent organic pollutants (POPs) (UNEP, 2001), the production and use of POPs including aldrin, chlordane, dieldrin, endrin, heptachlor, hexachlorobenzene (HCB), mirex, polychlorinated biphenyls (PCBs) and DDT should be eliminated in the assigned countries including China, which ceased DDT production in 2007. Therefore, studies on bioaccumulation and fate of POPs are critical for protecting the local dolphin populations and monitoring the organic contaminants in the PRE ecosystems.

Because they occupying the top trophic level in the marine food chain, long-lived marine mammals can act as a representative indicator for environmental health and contaminant biomonitoring in aquatic ecosystems (Harvell et al., 1999; Lahvis et al., 1995). Indo-Pacific humpback dolphins (*Sousa chinensis*) are one major group of top predators in the PRE and can therefore record POP contamination by bioaccumulation in this region. According to stomach content analyses of stranded Indo-Pacific humpback dolphins in our lab and other studies (Barros et al., 2004), the preferred prey fishes of the dolphins are also consumed by local human populations. Thus, investigation of the POP levels in the dolphins is also important for risk assessment and the protection of human health.

The Indo-Pacific humpback dolphin is protected as one of the first order of rare animals (The National Key Protected Wild Aquatic Animals List) in China and is included in the Convention on International Trade in Endangered Species of Wild Fauna and Flora Appendix I (CITES, 1997). It has also been classified as a 'threatened species' in the International Union for Conservation of Nature and Natural Resources (IUCN) Red List of Threatened Species because it is suffering adverse effects from many human activities. It was estimated that 74.27% of the current population will be lost after three generations (Huang et al., 2012).

Earlier studies have reported high levels of POPs in the blubber of *S. chinensis* individuals from the PRE (Minh et al., 1999; Ramu et al., 2005; Wu et al., 2013), but information on the tissue distribution of POPs in *S. chinensis* is scarce. In ill and starved animals, as the fat reserves in the blubber are mobilized, the concentrations of lipophilic pollutants in other body tissues, particularly the liver, become enriched (Guitart et al., 1996). Therefore, the investigation

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of contaminants in the liver, kidney and other tissues is of more value for toxicological assessment than those in the blubber. In this study, concentrations of various POPs in the liver, melon, kidney, muscle and other tissues of *S. chinensis* from the PRE, China, were determined. Gender and age-related differences were investigated within the species, and tissue-specific PCB congener profiles were estimated.

2. Methods

2.1. Sampling

Stranded Indo-Pacific humpback dolphins were collected between 2007 and 2013 from the PRE (Fig. 1). Tissue samples from the kidney ($n = 4$), liver ($n = 4$), muscle ($n = 4$) and melon ($n = 11$) were taken during necropsies of freshly dead (code 2) or early moderate decomposition (code 3) carcasses. Samples were stored at −20 °C until further analysis. The total body length of the dolphins was measured as the straight-line length from the tip of the upper jaw to the fluke notch. Sex was determined by external and internal examination. Age was estimated by counting the growth layer groups (GLGs) in the dentine of the teeth (Hohn et al., 1989; Myrck et al., 1983). Animals aged 2–7 years are considered to belong to the juvenile category, and animals of ages greater than 7 are considered adult.

2.2. Sample preparation

All of the tissue samples were freeze-dried for 48 h using a Labconco freeze-drying system. The dried samples were then ground with an automatic agate mortar for 10 min. The prepared samples were stored in refrigeration pipes at −80 °C prior to chemical analyses.

Approximately 0.5 g (wet weight) of the tissue sample was mixed with sodium sulfate and added to a pressurized fluid extraction cell along with 2.5 ng of recovery internal standards [13C-chlorobiphenyl (CB) 141]. The powder was completely transferred into a pre-cleaned cellulose thimble and then extracted with 200 ml of a 3:1 (v:v) mixture of hexane and dichloromethane (DCM) for at least 18 hours in a Soxhlet apparatus. The extract was concentrated to 10 ml with a rotary evaporator. An aliquot (1.0 ml) was taken for the gravimetric determination of the lipid content. The rest of the solution was filtered through a disposable syringe filter disc (0.45 μm in pore size) and further concentrated to 2.0 ml for subsequent purification.

The lipidic material (>500 Å) in the samples was fractionated and removed by gel-permeation chromatography (GPC). The GPC column (2 cm inner diameter) was packed with *Bio-Beads S-X3* and washed with a 1:1 (v:v) mixture of hexane and DCM prior to sample fractionation. The extract (approximately 2 ml) was placed on the top of the column, and a 1:1 (v:v) mixture of hexane and DCM was used as a mobile phase at a flow rate of 3 ml min^{-1}. The first 70 ml of the fraction was discarded, and the following 130 ml fraction, which contained PCBs and organochlorine pesticides (OCPs), was collected. The eluent was concentrated to approximately 2.0 ml and divided into two parts for further clean-up steps.

We adopted two types of chromatography columns for further purification according to Mai et al. (2002) due to the different chemical properties of PCBs and OCPs. For PCBs, a glass column (1 cm i.d.) was packaged from bottom to top with 1 cm of anhydrous sodium sulfate, 10 cm of acidic silica gel, and 1 cm of anhydrous sodium sulfate. For OCPs, a glass column (1 cm i.d.) was packaged from bottom to top with 1 cm of anhydrous sodium sulfate, 6 cm of alumina, 10 cm of silica gel, and 1 cm of alumina. Each column was washed with hexane prior to application. An aliquot (1.0 ml) of preliminary cleanup sample extract was placed on the top of each column and eluted with a 70 ml 1:1 (v:v) mixture of hexane and DCM. The eluate was rotary-evaporated to a volume of 5 ml and further concentrated by evaporation with a gentle nitrogen flow to approximately 500 μl for the next instrumental analyses.

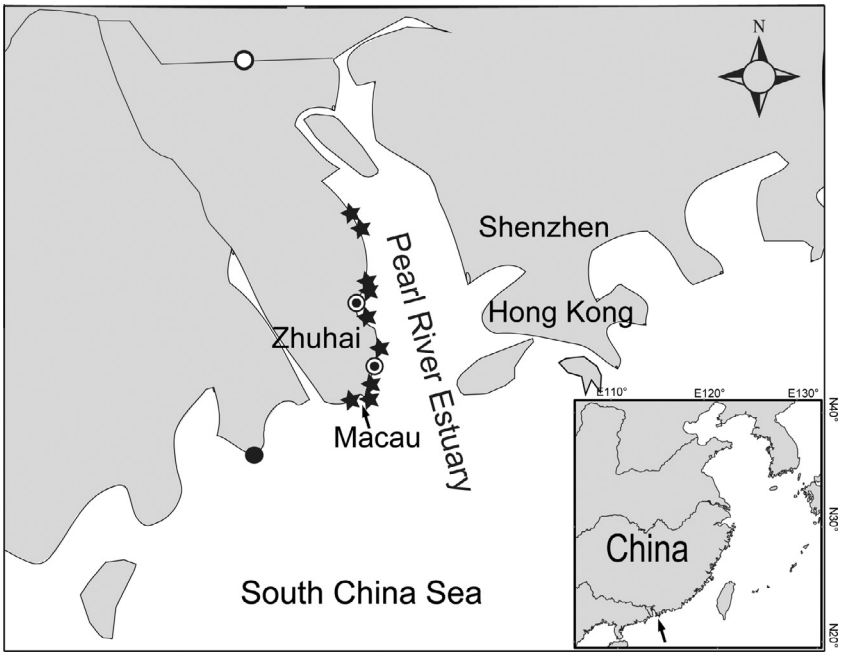

Fig. 1. Sampling locations of stranded Indo-Pacific humpback dolphins from the Pearl River Estuary, China. Five-pointed star (★) denotes the sampled individual whose melon tissue was sampled. Circled dot (⊙) denotes the individual whose melon, liver, kidney and muscle tissues were obtained. Hollowed dot (○) denotes the individual whose liver, kidney and muscle tissues were sampled. The individual humpback dolphin whose muscle, lung, kidney, heart, pancreas, stomach, testis, liver, intestine and blubber tissues were sampled was denoted as solid dot (●).

2.3. Chemical analysis

PCBs and OCPs were analyzed using a gas chromatograph (Agilent 7890, USA) coupled with a mass spectrometer detector (MSD) (Agilent 5975, USA) and a capillary column (DB-5MS with a dimension of 60 m × 0.25 mm × 0.25 μm) (J&W Scientific, USA). The column oven temperature for the detection of OCPs was programmed as follows: 70 °C held for 2 min; increased at 3 °C per minute to 270 °C and held for 5 min; and increased at 5 °C per minute to a final temperature of 300 °C and held for 10 min. The column oven temperature for the detection of PCBs was programmed as follows: 80 °C held for 2 min; increased at 4 °C per minute to 290 °C and held for 6 min; and increased at 20 °C per minute to a final temperature of 310 °C and held for 5 min. The temperatures of the transfer line, injector interface, and ion source were set at 300 °C, 290 °C and 230 °C, respectively. The rate of flow was set at 1.0 ml min^{-1}. Nitrogen was used as the carrier gas.

All tissues were analyzed for 19 PCB congeners (CB 28, 37, 52, 77, 81, 101, 105, 114, 118, 123, 126, 138, 156, 157, 167, 169, 180 and 209), DDTs (*o*,*p*′-DDT, *p*,*p*′-DDT, *o*,*p*′-DDD, *p*,*p*′-DDD, *o*,*p*′-DDE, *p*,*p*′-DDE), HCHs (α-HCH, β-HCH, γ-HCH), CHLs (trans-chlordane and cis-chlordane), mirex, hexachlorobenzene, heptachlor, aldrin, pentachlorobenzene, dieldrin, and endrin. Concentrations are expressed on a wet weight (ww) basis.

2.4. Quality assurance/quality control (QA/QC)

For QA/QC, method blanks, spiked blanks, matrix spikes and 20% duplicated samples were conducted. There were no target analytes detected in the blank samples. For the precision analyses, the relative difference between the duplicate samples was below 15% for all target analytes. A nominal concentration of the target analytes (PCBs and DDTs) was spiked into the sample matrix for determining matrix effects through the calculation of recoveries. The recovery rates for the matrix spike experiments of the target analytes ranged from 79.78% to 118.82%, with a relative significant difference (RSD) of less than 12%. In addition, recovery internal standards (^{13}C–CB 141) were added to all of the samples (including QA samples) to calculate the recovery rates. The recovery rates of PCBs ranged from 85.56% to 111.76% (RSD < 23%).

2.5. Data analysis

Shapiro–Wilks test was performed to examine if data were normally distributed. For the data that are not normally distributed, log-transformation ($Y = \log_{10}X$) was performed before statistical analysis. Since the liver, kidney and muscle tissues were collected from 4 dolphins that were different from the dolphins in which the melon samples obtained, differences in the POP concentrations among different tissues were compared using Student *t*-test. To analyze the differences in the POP concentration in the melon tissues among different age and gender groups, a one-way ANOVA (SPSS 21.0) was performed. The level of statistical significance was set at $p < 0.05$.

3. Results and discussion

3.1. Biological characteristics

Kidney, liver and muscle samples were analyzed from 4 stranded dolphins collected from the Zhuhai coast (2 females, 2 males) in the PRE, and melon samples were analyzed from 11 dolphins (including two individuals in which kidney, liver and muscle samples were obtained) from the same region (3 females, 7 males) (Tables 1 and 2). In addition to kidney, liver and muscle samples, lung, heart, pancreas, stomach, testis, intestine and blubber samples from one male dolphin (ZHSC74) were analyzed to examine the residue profile of POPs in one of the oldest Indo-Pacific humpback dolphins identified so far (40 + y) in the PRE. The ranges of age and length of the dolphins in which kidney, liver and muscle tissues were collected were 9–40 y and 190–269 cm, whereas those of the dolphins sampled for melon tissues were 1–24 y and 107–268 cm, respectively. The majority of the tissue samples were collected primarily from code 3 (moderate decomposition) animals in addition to one freshly dead (code 2) animal (ZHSC74). The dolphins sampled for kidney, liver and muscle tissues were all adult animals, whereas the individuals sampled for melon tissue consisted of 5 adults and 5 juveniles. It is well known that the accumulation of POPs may be influenced by various biological and ecological factors including geographic area, age, sex, tissue type, diet and temporal distribution (Wagemann and Muir, 1984). Therefore, the dolphins sampled for kidney, liver and muscle tissues were grouped into adult males (AM) and adult females (AF), and individuals sampled for melon tissue were grouped into juvenile males (JM), adult males (AM) and adult females (AF).

3.2. POP levels in different tissues

The concentrations of POPs in the kidney, liver, muscle and melon tissues of *S. chinensis* are shown in Table 1. We used wet weight-based concentrations of POPs in this study for a better understanding of the distribution of the OCs between tissues and the importance of the different tissues in storing contaminants. Concentrations according to age (juveniles versus adults) and sex

Table 1
Concentrations of 11 legacy POPs (ng g^{-1} wet weight) in liver, kidney, muscle and melon tissues from Indo-Pacific humpback dolphins from the Pearl River Estuary region.

POPs	Kidney (*n* = 4)			Liver (*n* = 4)			Muscle (*n* = 4)			Melon (*n* = 11)		
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
∑PCBs	115	73.6	10.2–197	419	321	42.0–868	47.7	25.9	5.36–75.0	3030	2320	284–7033
∑DDTs	2090	1260	559–3930	7040	5630	696–16100	4490[a]	2490	1260–7320	97300[a]	71800	7640–236000
∑HCHs	146	36.2	98.8–191	131	54.0	58.6–202	128[a]	48.3	83.2–195	625[a]	178	324–873
∑CHLs	3.62	2.43	1–7.41	6.99	3.33	1.86–10.5	5.43[a]	3.45	1.61–9.98	84.3[a]	70.8	12.3–215
HCB	2.23	0.84	0.86–3.15	4.02	1.55	1.52–5.74	1.69[a]	1.01	0.47–2.94	50.8[a]	47.6	8.17–180
Mirex	20.4	8.92	6.02–30.0	39.1	32.3	8.87–92.9	19.3[a]	17.3	0.40–42.2	312[a]	327	21.0–1150
Heptachlor	1.46	0.83	0.38–2.27	1.48	1.26	0.37–3.43	0.42[a]	0.38	n.d.–0.92	6.83[a]	4.16	1.76–13.6
Aldrin	22.0	24.5	1.04–63.0	16.1	17.3	2.18–44.2	1.7[a]	0.94	0.79–2.99	3.6[a]	3.91	n.d.–12.6
Pentachlorobenzene	0.04	0.07	n.d.–0.17	0.31	0.34	n.d.–0.87	0.01[a]	0.01	n.d.–0.03	10.8[a]	12.2	0.33–44.7
Dieldrin	3.01	2.09	0.77–6.39	3.31	2.29	0.2–6.65	2.53[a]	1.68	0.43–4.55	45.8[a]	46.3	6.7–174
Endrin	5.65	3.05	2.30–9.52	9.78	9.95	0.18–26.5	1.11[a]	0.5	0.57–1.78	76.3[a]	61.3	6.39–182

n.d.: not detected.
[a] One sample was not available.

Table 2
Mean concentrations (ng g^{-1} wet weight) of 11 legacy POPs in liver, kidney, muscle and melon tissues from different age and gender groups of *S. chinensis* from the PRE. Statistical differences of POP concentrations in the melon tissues among the three gender and age groups were determined utilizing a one-way ANOVA, and pairwise comparisons were made using Tukey's Honestly Significant Difference (HSD) test. Different capital letters indicate significant differences in POP concentrations in the melon among age and sex groups.

POPs	Kidney		Liver		Muscle		Melon			
	AM (n = 2)	AF (n = 2)	AM (n = 2)	AF (n = 2)	AM (n = 2)	AF (n = 2)	JM (n = 6)	AM (n = 2)	AF (n = 3)	p (ANOVA)
∑PCBs	140	89.3	715	122	28.25	67.25	1350 A	6320 B	4100 AB	0.008
∑DDTs	1940	2250	11400	2730	7330[a]	3075	60800[a]	128000	138000	0.347
∑HCHs	158	133	130	131	195[a]	95	631[a]	542	669	0.797
∑CHLs	3.03	4.21	9.9	4.1	9.98[a]	3.15	46.1[a]	74.3	155	0.126
HCB	2.75	1.7	5.23	2.83	2.95[a]	1.07	26.1[a]	44	96.6	0.15
Mirex	22.8	18	63.4	15	42.3[a]	7.93	257[a]	403	342	0.892
Heptachlor	1.33	1.6	1.91	1.05	0.92[a]	0.171	4.31[a]	9.58	9.19	0.201
Aldrin	11.9	32	1.89	30.3	3[a]	1.05	1.80[a] AB	0.73 A	8.5 B	0.017
Pentachlorobenzene	0.09	n.d.	0.56	0.05	n.d.[a]	0.015	4.79[a]	9.3	21.8	0.199
Dieldrin	2.45	3.58	5	1.61	4.55[a]	1.52	25.2[a]	36.5	86.3	0.237
Endrin	5.9	5.38	16.5	3.1	1.78[a]	0.77	47.8[a]	83.5	119	0.355

n.d.: not detected.
[a] One sample was not included because its age, body length or gender information was not available.

(adult males versus adult females) can be found in Table 2. For the most residues of POPs measured, the mean concentrations were significantly higher ($p < 0.05$) in the melon, with the exception of aldrin, which was highest in the kidney followed by the liver, melon and muscle. Elevated POP concentrations in the melon have been well described in other studies (Guitart et al., 1996; Yordy et al., 2010) due to its higher fat content and conservative nature as an organ filling a crucial function for sound transmission (Cranford et al., 1996; Koopman et al., 2003, 2002). However, little is known regarding the negative impact of the large burden of POPs in the melon. Recent studies (Li et al., 2013; Mann et al., 2010) have reported the hearing loss in stranded cetaceans, which could play a significant role in some cetacean stranding events. High exposure to POPs could affect the unique fatty acid composition of the melon, which plays an important role in echolocation, and could further cause stranding of cetaceans.

For the other tissues with a lower lipid content (kidney, liver and muscle), no significant differences were found in the mean concentrations of each individual POP, except for the mean concentration of HCB, which was significantly higher ($p = 0.026$) in the liver compared with the kidney. However, the mean concentrations of all 11 POPs (except aldrin) were higher in the liver than in the kidney or muscle (Wagemann and Muir, 1984). The concentrations of majority POPs were higher in the kidney (up to 5-fold) than in muscle except those of ∑DDTs and ∑CHLs, which were 2.2-fold and 1.5-fold higher in the muscle than in the kidney, respectively.

The descending order of highest mean concentrations of POPs was as follows: ∑DDTs, ∑PCBs, ∑HCHs, mirex, ∑CHLs, endrin, dieldrin, HCB, pentachlorobenxene, heptachlor and aldrin in the melon tissues ($n = 11$); ∑DDTs, ∑PCBs, ∑HCHs, mirex, aldrin, endrin, ∑CHLs, HCB, dieldrin, heptachlor and pentachlorobenxene in the liver tissues ($n = 4$); ∑DDTs, ∑HCHs, ∑PCBs, aldrin, mirex, endrin, ∑CHLs, dieldrin, HCB, heptachlor and pentachlorobenxene in the kidney tissues ($n = 4$); and ∑DDTs, ∑HCHs, ∑PCBs, mirex, ∑CHLs, dieldrin, aldrin, HCB, endrin, heptachlor and pentachlorobenxene in the muscle tissues ($n = 4$) (Table 1). It is not surprising that the tissue distribution appeared to vary among the 11 POP compounds given their distinct differences in biochemical properties.

∑DDTs, ∑PCBs and ∑HCHs were the three dominant POPs in these tissues compared with other compounds (Table 1). ∑DDTs had the highest concentrations in all four tissues (kidney, liver, muscle and melon), which were at least 16 times higher than the concentrations of other POPs. Liver and melon had higher concentrations of ∑PCBs compared with ∑HCHs, whereas kidney and muscle exhibited the opposite pattern.

High levels of ∑PCBs and ∑DDTs are likely associated with the suppression of the immune system in the bottlenose dolphin (*Tursiops truncatus*), striped dolphin (*Stenella coeruleoalba*), harbor porpoise (*Phocoena phocoena*), and harbor seals (*Phoca vitulina*) (Aguilar and Borrell, 1994; de Swart et al., 1996; Kuiken et al., 1994; Lahvis et al., 1995; Ross et al., 1995). The mean liver concentrations of DDTs found in *S. chinensis* from the PRE in mainland China (7040 ng g^{-1} ww, ±SD 5630) were lower than those in *S. chinensis* taken from Hong Kong waters from 2000 to 2001 (8990 ng g^{-1} ww, ±SD 4000) (Ramu et al., 2005) and in bottlenose dolphins (7400 ng g^{-1} ww, ±SD 7400) stranded on the Atlantic coast of Florida, USA (Watanabe et al., 2000). However, the DDT values in *S. chinensis* were significantly higher than those in Atlantic spotted dolphins (1570 ng g^{-1} ww, ±SD 125) and pygmy sperm whales (540 ng g^{-1} ww, ±SD 114) from the Atlantic coast of Florida, USA (Watanabe et al., 2000), harbor porpoises from the Black Sea (1150 ng g^{-1} ww, ranging from 607 to 3520 ng g^{-1} ww) (Aguilar and Borrell, 1994), and white-sided dolphins (273 ng g^{-1} ww) and pilot whales (250 ng g^{-1} ww) from the northwest Atlantic (Watanabe et al., 2000) (Table 3). The PRE has been a major mariculture zone in South China. The DDT loading has remained basically unchanged in the PRE, primarily due to the overuse of fish feeds and discharge of antifouling paint from fishing boats (Yu et al., 2011a, 2011b). The enriched ∑DDTs in mariculture sediments in the PRE could eventually accumulate in fishes and longer-lived top predators such as humpback dolphins. Our recent study has shown that ∑DDTs were biomagnified 212-fold in *S. chinensis* compared with prey fishes in the PRE, ranging from 910 to 215,000 ng g^{-1} ww.

The mean concentration of ∑PCBs in *S. chinensis* liver from the PRE (419 ng g^{-1} ww, ranging from 42.0 to 868 ng g^{-1} ww) was 50% lower than that in *S. chinensis* from Hong Kong waters from 2000 to 2001 (929 ng g^{-1} ww, ranging from 67 to 1700 ng g^{-1} ww) (Ramu et al., 2005), and comparable to the value for pygmy sperm whales (*Kogia breviceps*) (560 ng g^{-1} ww, ranging from 290 to 830 ng g^{-1} ww) along the Atlantic coast of Florida, USA (Watanabe et al., 2000). The concentration of ∑PCBs in *S. chinensis* liver was significantly lower than the residue level in bottlenose dolphins, Atlantic spotted dolphins from the Atlantic coast of Florida, USA (Watanabe et al., 2000), and striped dolphins (male or newborn) from the Eastern Mediterranean Sea (Yordy et al., 2010). However, the concentrations of ∑PCBs in *S. chinensis* were apparently higher than the residue content in harbor porpoises from the Black Sea (Aguilar

Table 3
Comparisons of mean hepatic POP concentrations with ranges (ng g^{-1} wet weight) in Indo-Pacific humpback dolphins (*Sousa chinensis*) and other cetaceans from different geographic regions.

Species	Location	PCBs	DDTs	CHLs	HCHs	HCB	Hepatachlor	Mirex	Dieldrin	Reference
Indo-Pacific humpback dolphins	Pearl River Estuary, mainland China	419 (42.0–868)	7040 (696–16,100)	6.99 (1.86–10.5)	131 (58.6–202)	4.02 (1.52–5.74)	1.48 (0.37–3.43)	39.1 (8.87–92.9)	3.31 (0.2–6.65)	This study
Indo-Pacific humpback dolphins	Hong Kong	929 (67–1700)	8990 (5100–14,500)	107 (54–115)	76.5 (66–82)	43.9 (18–61)	n.a.	n.a.	n.a.	Ramu et al. (2005)
Bottlenose dolphin	Atlantic coast of Florida, USA	92,000 (1600–290,000)	7400 (120–20,000)	1800 (45–5800)	17 (n.d.–58)	25 (1–75)	n.a.	n.a.	n.a.	Watanabe et al. (2000)
Atlantic spotted dolphin	Atlantic coast of Florida, USA	13,000 (7900–19,000)	1600 (1400–1700)	460 (440–480)	6.1 (4.5–7.7)	22 (15–29)	n.a.	n.a.	n.a.	Watanabe et al. (2000)
Pygmy sperm whale	Atlantic coast of Florida, USA	560 (290–830)	540 (400–680)	50 (27–73)	1.1 (1.1–1.1)	5.5 (1.4–9.7)	n.a.	n.a.	n.a.	Watanabe et al. (2000)
Striped dolphins (male)	Southeastern Mediterranean Sea	5413 (856–17,270)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Storelli et al. (2012)
Striped dolphins (female)	Southeastern Mediterranean Sea	583 (199–976)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Storelli et al. (2012)
Striped dolphins (newborn)	Southeastern Mediterranean Sea	4750 (822–8678)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Storelli et al. (2012)
Harbor porpoises	Black Sea	346 (171–825)	1150 (607–3520)	n.a.	n.a.	59.7 (40–75.8)	n.a.	n.a.	n.a.	Weijs et al. (2010)
White-sided dolphin	Northwest Atlantic	370	273	93.9	11.2	15.8	1	1.6	19.4	Weisbrod et al. (2001)
Pilot whale	Northwest Atlantic	184	250	55.9	5.6	14.7	4	2.1	5.2	Weisbrod et al. (2001)
Franciscana dolphin	Southeastern and Southern Coast of Brazil	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	4.8	n.a.	De la Torre et al. (2012)

n.a.: not analyzed.

and Borrell, 1994) and white-sided dolphins and pilot whales from the northwest Atlantic (Watanabe et al., 2000) (Table 3).

The mean concentration of CHLs in *S. chinensis* liver in the PRE was 6.99 ng g^{-1} ww (ranging from 1.86 to 10.5 ng g^{-1} ww), which was apparently lower than those in the liver of cetaceans from other geographic locations (Table 3). For HCHs, the mean concentration (131 ng g^{-1} ww) in *S. chinensis* liver from the PRE was apparently higher than the liver residue level of *S. chinensis* from Hong Kong waters (76.5 ng g^{-1} ww) (Table 3). A similar pattern was also found for the residue of mirex, for which the mean concentration in the liver (39.1 ng g^{-1} ww) was significantly higher than in white-sided dolphins (1.6 ng g^{-1} ww) and pilot whales (2.1 ng g^{-1} ww) from the northwest Atlantic (Watanabe et al., 2000) as well as Franciscana dolphins from the southeastern and southern coast of Brazil (4.8 ng g^{-1} ww) (De la Torre et al., 2012). As shown in Table 3, HCB (4.02 ng g^{-1} ww) and dieldrin (3.31 ng g^{-1} ww) in *S. chinensis* were both lower than other reported concentrations in cetacean species from various regions (ranging from 5.5 to 59.7 ng g^{-1} ww for HCB and from 5.2 to 19.4 ng g^{-1} ww for dieldrin) (Aguilar and Borrell, 1994; Watanabe et al., 2000), whereas hepatachlor (1.48 ng g^{-1} ww) was at a level similar to the values reported in white-sided dolphins (1 ng g^{-1} ww) and pilot whales (4 ng g^{-1} ww) from the northwest Atlantic (Watanabe et al., 2000). Aldrin, dieldrin and endrin are all cyclodiene insecticides that are generally much more toxic than DDT. Compared with dieldrin, the less persistent parent compound (aldrin) and the related highly toxic form (endrin) were found at elevated levels in the liver, indicating recent anthropogenic emissions of aldrin and endrin (Table 1).

3.3. Correlation of tissue POP levels with gender, age and body length

More than a 100-fold difference in the concentrations of POPs was observed among the sampled individuals (e.g., the concentrations of pentachlorobenxene in the melon ranged from 0.33 to 44.7 ng g^{-1} ww), highlighting the numerous biotic factors influencing POP accumulation (e.g., age and gender).

The concentrations of $\sum$PCBs ($p = 0.008$) and aldrin ($p = 0.017$) in the melon tissues were significantly different between the adult groups and the juvenile group (Table 2). The mean concentrations of all POPs were apparently higher in the adult group (AM or AF) than in the juvenile group (JM), although the differences are not statistically significant.

Among the 11 compounds of POPs (∑PCB, ∑DDTs, ∑HCHs, ∑CHLs, mirex, HCB, heptachlor, aldrin, pentachlorobenzene, dieldrin, and endrin) measured in the melon of 11 dolphins, the concentrations of only four contaminants (∑PCB, ∑DDTs, heptachlor and endrin) exhibited significant correlations with dolphin body length (Fig. 2) or age (Fig. 3). Of all POPs, heptachlor accumulation in dolphin liver tissue was observed to have the highest correlations with age and body length (r^2 at 0.879 and 0.816, respectively). ∑PCB was also positively associated with age and body length (r^2 at 0.751 and 0.688, respectively). In terms of ∑DDTs and endrin, significant correlations were observed only in the relationship with body length (r^2 at 0.55 and 0.536, respectively).

Although no significant difference was found between genders, the AM group generally had higher residue levels of POPs in the kidney, liver and muscle than the AF group, presumably due to the off-loading phenomenon of contaminants during gestation and lactation (Gardner et al., 2007; Marsili and Focardi, 1997). However, in the melon, the AF group had apparently higher levels of all POPs compared to the AM group, except for PCBs, mirex and heptachlor (Table 2), which were very different from the age-related patterns observed in kidney, liver and muscle. Previous studies (Marsili and Focardi, 1997) showed that organochlorine contaminant levels in the melon of female striped dolphins also decreased with age (Marsili and Focardi, 1997). Based on that, Koopman et al. (2003) suggested that although the melon lipids are not mobilized into the circulation in response to energetic demands, contaminants within the melon are also subject to lactation-associated mobilization despite a lack of lipid use from this tissue. However, the concentrations of most contaminants in the melon of adult female *S. chinensis* showed even higher level than those of adult male *S. chinensis*, indicating that the results in this study contradict with those obtained from Marsili and Focardi (1997), which could be attributed to other factors involved (e.g., preference of prey fishes, geographic locations, body condition or individual differences, etc.). It is well known that the distribution of highly lipophilic POPs among different tissues is generally related to tissue lipid content (Yordy et al., 2010). Therefore, it was not surprising that although the melon is subjected to the mobilization and off-loading of contaminants in pregnant dolphins, residue levels of POPs in the melon were not easily changed, because contaminants will redistribute to the melon in accordance with its stable and high lipid content. However, the conclusion needs to be further confirmed by future studies.

3.4. Congener profile of PCBs

CB 153 was the predominant PCB congener in the kidney, liver, muscle and melon tissues of *S. chinensis*. This result is congruent with the PCB congener profiles reported for the blubber of *S. chinensis* and the Indo-Pacific finless porpoise (*Neophocaena phocaenoides*) in Hong Kong waters (Minh et al., 1999), in the tissues of various marine mammal species (Aguilar and Borrell, 1994; de Swart et al., 1996; Ross et al., 1995), and also in fish tissues (Kuiken et al., 1994). The concentration of CB 153 was followed by those of CB 138 and CB 180 (Fig. 4).

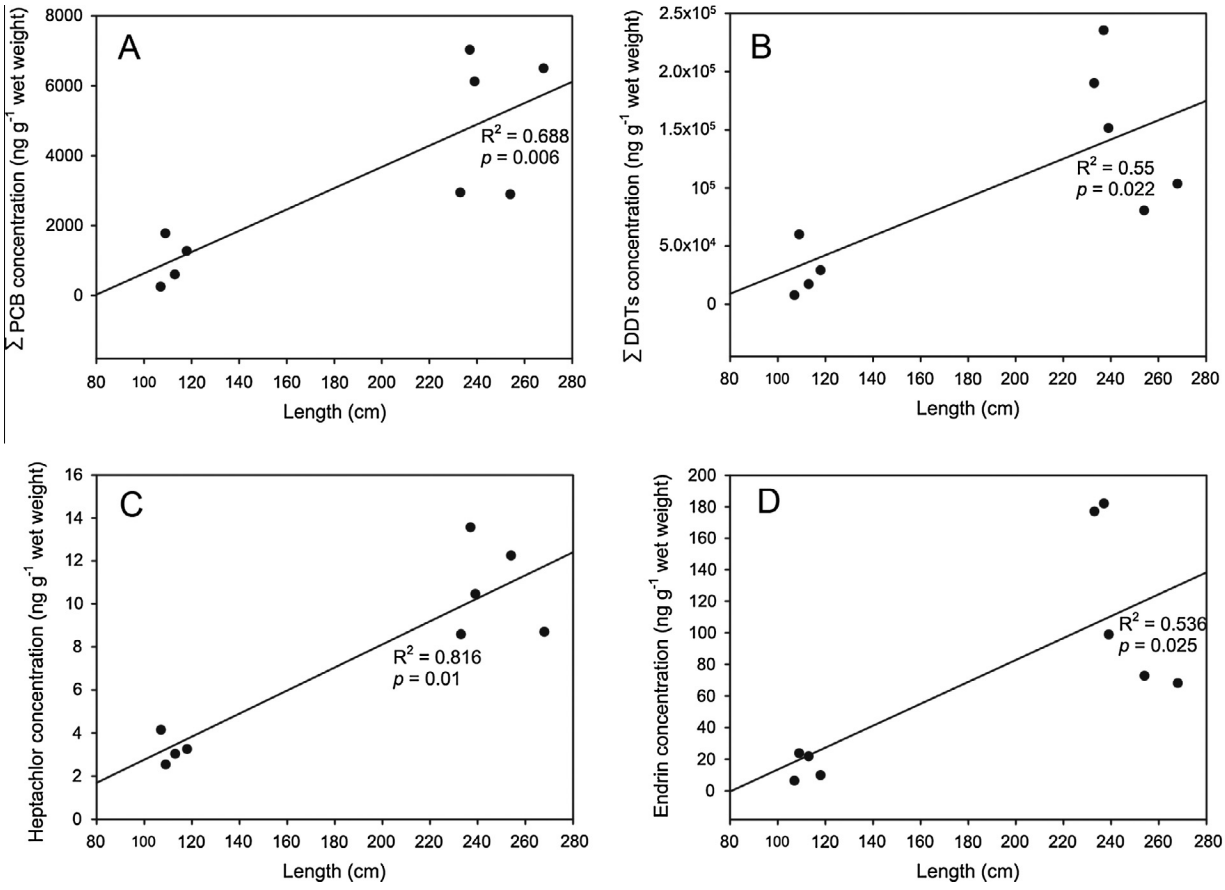

Fig. 2. Linear regressions between melon residue concentrations of PCBs, heptachlor, DDTs, endrin and body length of Indo-Pacific humpback dolphins from the Pearl River Estuary. Concentrations are in ng g^{-1} wet weight, with length in centimeters. (A) Relationships between concentrations of ∑PCBs and length. (B) Relationships between concentrations of ∑DDTs and length. (C) Relationships between heptachlor concentrations and length. (D) Relationships between endrin concentrations and length.

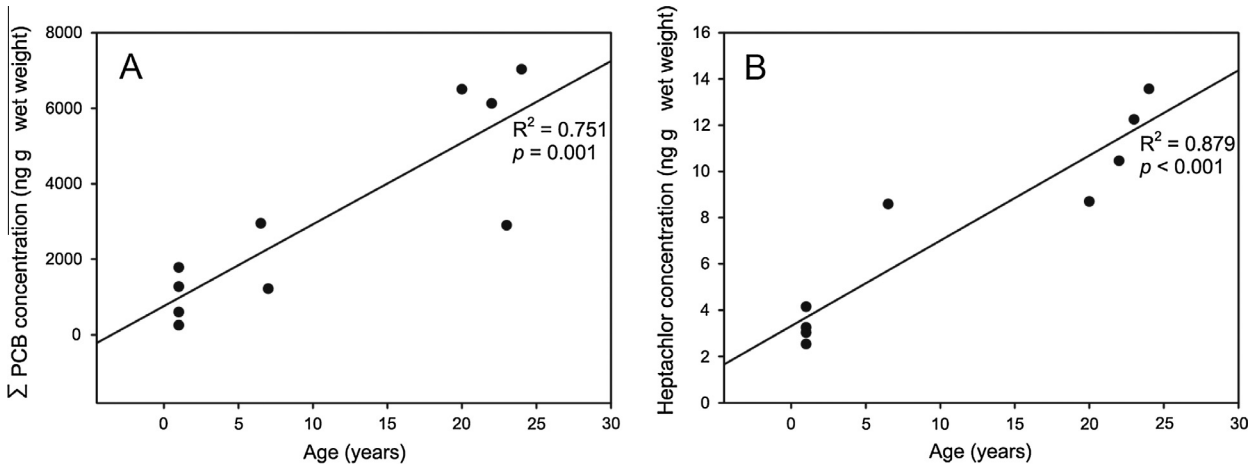

Fig. 3. Linear regressions between melon residue concentrations of PCBs, heptachlor, DDTs, endrin and age of Indo-Pacific humpback dolphins from the Pearl River Estuary. Concentrations are expressed as ng g^{-1} wet weight. (A) Relationships between concentrations of ∑PCBs and age. (B) Relationships between heptachlor concentrations and age.

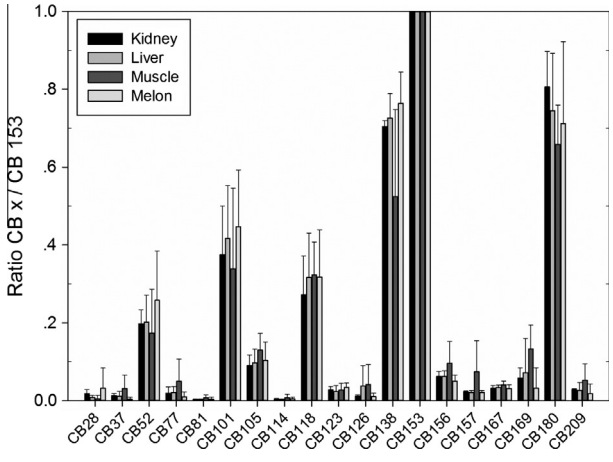

Fig. 4. Concentration ratios of each individual PCB congener over CB 153 in the kidney, liver, muscle and melon tissues collected from stranded Indo-Pacific humpback dolphins from the Pearl River Estuary. Error bars represent the standard deviations (SD).

To better show the differences in the accumulation pattern of each PCB congener among the four tissues, the ratio of each of the 19 PCBs to CB 153 were used to construct relative PCB profiles (Fig. 4). Compared with other tissues, melon had a lower proportion of highly chlorinated PCB congeners (CB 156, CB 157, CB 167, CB 169, CB 189 and CB 209), which represented the unique PCB profile in melon. PCBs are strongly associated with lipid. The fatty acid composition of the melon is unique, consisting of short- and medium-chain (C5–C12), branched, saturated fatty acids specialized for sound transmission (Cranford et al., 1996).

In comparison with the earlier study between 1993 and 1997 (Minh et al., 1999), an apparent increase in the proportion of lower-chlorinated PCB congeners was observed. In fact, the sediment in the PRE was enriched with a higher proportion of lightly chlorinated PCB congeners due to the downward mobility of lower-chlorinated PCBs (Mai et al., 2005), which is in agreement with the PCB profiles in *S. chinensis*. Aroclor 1242 and 1254 were the major compounds of PCBs produced and used in China (Breivik et al., 2002; Zhao et al., 2005). Aroclor 1242 is primarily composed of low-chlorinated (3 Cl and 4 Cl) PCBs, whereas CBs 101, 118 and 138 are indicative of Aroclor 1254. These PCB compounds were all found at relatively higher proportions in the tissues of *S. chinensis*, indicating the role of Indo-Pacific humpback dolphins as important indicators for assessing ocean and human health.

3.5. Distribution of 11 POP compounds in various tissues of the oldest sampled dolphin individual (ZHSC74)

As shown in Table S1, 11 POP compounds were assessed in the lung, kidney, heart, pancreas, stomach, testis, liver, intestine and blubber tissues from an Indo-Pacific humpback dolphin approximately 40 years old. The blubber, liver and testis had elevated levels of most POP compounds compared with other tissues (Table S1). The highest level of aldrin was found in the testis. Its concentration was at least 3-fold higher than the other tissues, representing an alarming threat to the reproductive function of this individual.

In conclusion, our study provides the first comprehensive characterization of the residue profiles of 11 POPs in various tissues of *S. chinensis* from the PRE in mainland China, explores the major contaminants with the potential to cause health problems in stranded dolphins, and is critical for future risk assessment studies and monitoring anthropogenic impacts over time.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.marpolbul.2014.07.007.

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