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Organochlorine and Trace Element Contamination in Bottlenose Dolphins (*Tursiops truncatus*) from the South China Sea

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The cosmopolitan bottlenose dolphin (*Tursiops truncatus*; Montagu, 1821) has been recorded in both the coastal and offshore waters of the South China Sea including Indonesia, Singapore, Malaysia, the Philippines, Thailand, Vietnam, China, Taiwan and Hong Kong (Yang, 1976; Leatherwood *et al*., 1984, 1992; Tas'an and Leatherwood, 1984; Wang, 1984; Parsons *et al*., 1995; Smith *et al*., 1995; Zhou *et al*., 1979; Perrin *et al*., 1996). Although the species is not normally resident in Hong Kong waters, between 1974 and 1998, there were reports of one live-captured animal (Hammond and Leatherwood, 1979), two live strandings and nine stranded carcasses (Parsons *et al*., 1995; Parsons, 1998a,b; Parsons and Jefferson, 2000). Of the latter animals, three were necropsied and samples were taken for ecotoxological analysis. All three animals had been dead for 1–2 days prior to sampling (decomposition code 3; Geraci *et al*., 1983).

At present, all bottlenose dolphins are conservatively assigned to *T. truncatus* (Montagu, 1821). However, two forms of bottlenose dolphins have been recognized in the Indo-Pacific. One form is primarily coastal, with spotting on the ventrum and a long, slender snout. The other form is primarily believed to inhabit offshore waters; is larger, un-spotted and more heavy-bodied than the coastal form; and possesses a shorter, stubbier rostrum. There is growing evidence that these two forms of bottlenose dolphins may in fact be two different species: the coastal *Tursiops aduncus* and the more robust offshore-dwelling *T. truncatus* (Pilleri and Gihr, 1972; Ross, 1977, 1984; Zhou and Qian, 1985; Zhou, 1987; Gao *et al*., 1995; Wang, 1999; Wang *et al*., 1999).

The bottlenose dolphins examined in the current study were all heavy-bodied, un-spotted, with a short rostrum (Parsons *et al*., 1995). After an analysis of genetic samples from these animals, they were all deemed to be members of the species *T. truncatus* (Wang, 1999). An analysis of the bottlenose dolphins' stomach contents revealed a large proportion of pelagic deep-water prey species, confirming that the animals' primary habitat was offshore (Barros *et al*., 2000).

This study reports upon levels of organochlorine and trace element contaminants in these offshore bottlenose dolphins from the South China Sea and compares them with contaminant levels recorded in the same species from other locations in the world.

Figure 1 shows the stranding sites for the three bottlenose dolphins detailed in this study. The methodologies for sample collection and analysis are outlined in Parsons and Chan (1998), Parsons (1999a) and Parsons *et al*. (1999). For the determination of trace element concentrations, blubber, liver and kidney samples were dissected from the carcasses with a stainless steel scalpel. Subsequently, exposed and possibly contaminated surfaces were removed with teflon-coated scissors. The samples were packaged in Tekmar whirl pack bags and frozen at −20°C.

The frozen tissue samples were homogenized in a Heward 80 stomacher and freeze-dried in a Hetosicc FD3 freeze drier. Approximately, 0.5 g of dried tissue was weighed, and then digested in 5 ml of concentrated nitric acid for 24 h at room temperature, followed by a second 24 h at 100°C. The digests were then filtered and made up to 50 ml with double de-ionized distilled water. Trace metal levels were determined by inductively coupled plasma emission spectroscopy on a Perkin–Elmer 400 spectrograph, with a Perkin–Elmer AS-90 automated sampler. Quality assurance included the analysis of a Standard Reference Material (bovine liver, Standard Reference Material 1577b from the US National Institute of Standards and Technology). Metal concentrations in this were found to conform with the certified values (see Parsons *et al*., 1999).

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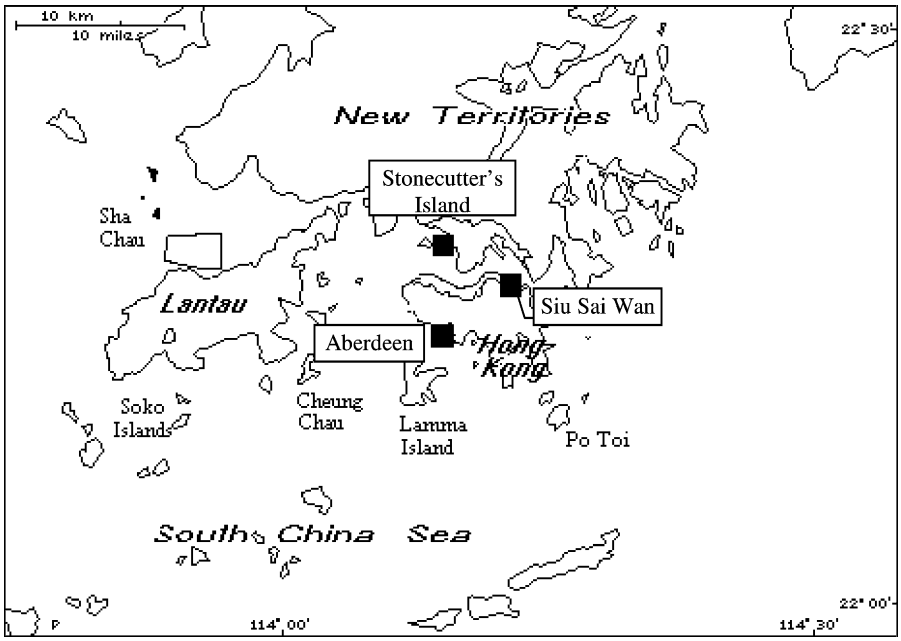

Fig. 1 Map of Hong Kong showing the locations of the three bottlenose dolphin strandings analysed in the study.

Blubber samples to be tested for organochlorine concentrations were removed from the dolphin carcass with stainless steel instruments, wrapped in aluminium foil, packed in Tekmar whirl pack bags and kept deep frozen at −20°C until analysis.These samples were shipped frozen to Montreal for analysis. For the determination of organochlorine pesticides and PCBs in dolphin blubber, a sub-sample of approximately 10 g of blubber was taken and this was prepared and analysed according to the procedure outlined in Chan *et al.* (1996). This consists of homogenising and grinding with anhydrous sodium sulphate, then eluting with a methylene chloride/hexane solution in a 1:1 ratio. The samples were spiked with 5 µl of surrogate internal standard solution containing ^{13}C-labeled PCB (IUPAC Nos. 3, 77, 202 and 209), $^{13}C_{12}$-*p*,*p*′-DDT, and $^{13}C_6$-γ-HCH. Each sample was applied to an SX-3 gel permeation column connected to a Beckmann Gold high-pressure liquid chromatography system for the separation of fat from the fraction containing organochlorine compounds. This was then applied to a florisil column and two fractions were collected: the first (eluted with 40 ml 15:85 methylene chloride/hexane) contained PCB congeners, hexachlorobenzene, *p*,*p*′-DDE, heptachlor, mirex and photomirex; the second (eluted with 60 ml 1:1 methylene chloride/hexane) contained HCH isomers, chlordanes, *p*,*p*′-DDT, heptachlor epoxide and dieldrin. The fractions were spiked with the volumetric internal standard d_{12}-chrysene and prepared for gas chromatography with 100 µl isooctane.

A total of 63 PCBs and 17 chlorinated pesticides were screened in the samples. The characterization of the two fractions was carried out using a Varian Saturn III gas chromatography–ion trap mass spectrometer. A J&W Scientific DB-5MS capillary column (30 m × 0.25 mm internal diameter and 0.25 µm film thickness) was used and the samples were loaded onto a Varian 8200CX autosampler; 1 µl injections were made using the sandwich injection technique. Internal standard PCB solutions (CLB-1-A, B, C, and D) were obtained from the National Research Council of Canada (Halifax, Nova Scotia). The lipid compositions of samples were ascertained using a Soxtec high temperature solvent extraction system, with petroleum ether as the extraction solvent.

The concentrations of trace elements determined in this study are summarized in Table 1. There was considerable variation between the individuals and tissues sampled. For example, the female stranded on 4 December 1994 exhibited the greatest concentration of lead in kidney tissue, whereas the female stranded on 13 July 1995 contained only a trace amount of lead in its liver (0.92 µg g^{-1} dry weight), non-detectable levels in kidney tissue, yet high concentrations of lead in blubber tissue (11.7 µg g^{-1} dry weight).

The only trace element in a concentration high enough to be of a toxicological significance was mercury, in the liver of the animal stranded on 22 November 1994 (299 µg g^{-1} dry weight). Coastal cetacean species in Hong Kong have been noted to possess markedly higher levels of mercury contamination than were discerned for these offshore bottlenose dolphins (up to 906 µg g^{-1} dry weight; Parsons, 1999a), which is presumably due to their accumulation of mercury from contaminated prey species (Parsons, 1999b). In general, the metal levels of the bottlenose dolphin tissues sampled in this study were comparable to concentrations reported from elsewhere in the world (Table 2).

TABLE 1

Trace metal concentrations (μg g^{-1} dry weight) in bottlenose dolphin tissues from the South China Sea.[a]

Necropsy date	Location	Length (cm)	Age (GLGs)	Sex	Tissue	As	Cd	Co	Cr	Cu	Hg	Mo	Ni	Pb	Se	Sn	Zn
25/11/94	Siu Sai Wan, Hong Kong Island	293	30	Male	Liver	20.3	2.35	<0.9	7.82	9.39	299	1.56	<0.9	5.48	40.7	2.35	54.0
					Kidney	<0.9	5.27	<0.9	<0.9	4.39	<0.9	<0.9	<0.9	<0.9	<0.9	<0.9	37.8
					Blubber	12.6	<0.9	<0.9	<0.9	<0.9	<0.9	0.9	0.9	15.3	2.69	<0.9	8.98
4/12/94	Stonecutter's Island	244	6	Female	Liver	1.73	0.87	<0.8	<0.8	12.1	<0.8	0.87	<0.8	<0.9	19.1	<0.8	84.1
					Kidney	1.84	12.9	<0.8	0.92	8.32	<0.8	<0.9	<0.8	6.47	<0.8	<0.8	53.6
					Blubber	<0.8	<0.8	<0.8	<0.8	0.85	<0.8	<0.8	0.85	<0.8	7.67	<0.8	7.67
13/7/95	Aberdeen Harbour, Hong Kong Island	233.5	4–5	Female	Liver	23.0	0.92	<0.8	<0.8	8.27	<0.8	0.92	0.92	0.92	34.9	0.92	49.6
					Kidney	4.29	16.3	<0.8	<0.8	8.59	<0.8	0.86	0.86	<0.8	9.44	1.72	60.1
					Blubber	<0.8	<0.8	<0.8	<0.8	15.0	<0.8	<0.8	0.83	11.7	nd	0.83	10.82

[a] Age is measured in growth layer groups (GLGs; Perrin and Myrick, 1980).

TABLE 2

Trace metal concentrations in bottlenose dolphin tissues from other regions of the world.[a]

Location	Reference	Tissue	Hg	Se	Cr	Zn	Pb	Ni	Cd	Cu
USA	Geraci, 1989	Liver	nd–110	–	–	16–210	nd–3.1	–	nd	0.08–28
	Wood and van Vleet, 1996	Liver*	–	–	–	24–216	–	–	nd–0.5	2.0–70
		Kidney*	–	–	–	22–44	–	–	nd–1.6	1.8–8.4
UK	Law *et al.*, 1991, 1992	Liver	20–21	–	<0.06	32–42	<0.07	<0.06	0.07–0.12	5.7–8.3
Australia	Kemper *et al.*, 1994	Liver	0.14–10.2	–	–	–	0.05–1.0	–	0–10	–
		Kidney	–	–	–	–	<1	–	0–25.5	–
		Muscle	0.22–0.77	–	–	–	0.05–0.10	–	0.05–0.10	–
		Blubber	0.01–3.81	–	–		0.05–3.41	–	0.06–0.11	–
Japan	Arima and Nagakira K, 1979	Muscle	51.8	13.9	–	–	–	–	–	–
Taiwan	Lee and Moke, 1995	Liver	–	–	53.3	89.0	1.32	-	16.43	23.4
		Muscle	–	–	33.8	71.5	2.2	-	0.4	5.6
Hong Kong	This study	Liver	nd–90	5.9–12.2	nd–2.3	14.9–25.2	nd–1.64	nd–0.28	0.26–0.7	2.5–3.6
		Kidney	nd	nd–2.4	nd–0.23	9.45–15	nd–1.62	nd–0.22	1.3–4.1	1.1–2.1
		Blubber	nd	nd–6.1	nd	6.1–8.7	nd–12.2	0.66–0.72	nd	nd–12

[a] All values are shown in μg g^{-1} wet weight. Hong Kong and asterisked American data were converted into wet weight-based values using conversion factors of: liver 30%, kidney 25% and blubber 80%; nd: not detected.

The concentrations of organochlorine compounds in the bottlenose dolphin tissues were more significant toxicologically (Tables 3 and 4). Wagemann and Muir (1984) considered concentrations of DDT in excess of 50 μg g^{-1} to constitute a serious hazard to cetaceans. This level was exceeded in two of the three animals sampled. The low ∑DDT concentration recorded in the male stranded on 22 November 1994 was, however, somewhat surprising. Typically, organochlorine levels in male dolphins accumulate with age, whereas in females, a large proportion of the contaminant load is transferred during pregnancy and lactation (Subramanian *et al.*, 1987; Cockcroft *et al.*, 1989; Morris *et al.*, 1979). However, PCBs, lindane, chlorobenzene and dieldrin were noted in progressively higher concentrations in older animals, as expected.

The PCB concentrations reported in this study (Tables 3 and 4) are largely unremarkable and are lower than concentrations reported for many other bottlenose dolphin populations (Table 5). This presumably reflects a low rate of production of PCBs in the countries adjacent to the South China Sea.

By contrast, the concentrations of DDT in the dolphins studied here were relatively high and are comparable to concentrations reported for cetaceans from highly contaminated areas. The levels of DDT in these bottlenose dolphins were lower, however, than those in coastal cetacean species studied in Hong Kong (up to 380 μg g^{-1} lipid weight for Indo-Pacific hump-backed dolphins, and up to 309 μg g^{-1} lipid weight for finless porpoises; see Parsons and Chan, 1998). This is unsurprising, as coastal cetaceans would be expected to display higher levels of organochlorine contamination than the offshore animals examined in the current study, due to their greater proximity to the anthropogenic sources of these pollutants (Parsons and Chan, 1998). When comparing the concentrations of DDT to those of its metabolites, this distinction between the offshore and coastal species is again apparent. The mean ratio of DDE:DDD:DDT in offshore bottlenose dolphins was 77%:15%:8%, i.e. the majority of total DDT was in the form of DDE. For coastal species, the ratios of DDE:DDD:DDT were 46%:35%:19% and 35%:40%:25% for Indo-Pacific hump-backed dolphins and finless porpoises, respectively (Parsons and Chan, 1998). The relatively high proportions of unmetabolized DDT in the coastal species suggests a closer proximity to the source of DDT (spatially, temporally and trophically) by comparison to the bottlenose dolphins examined in the current study.

Little is known about DDT usage in East Asia (Phillips and Tanabe, 1989). Japan and Hong Kong banned the use of DDT in 1970 and 1988, respectively. China imposed restrictions upon DDT utilization in 1983 (Wolfe *et al.*, 1984), although the use of DDT and of other organochlorine pesticides is undoubtedly continuing within the country.

TABLE 3

Organochlorine concentrations (μ g g^{-1} lipid weight) in the blubber of bottlenose dolphins from the South China Sea.[a]

Necropsy date	25-11-94	4-12-94	13-7-95
Location	Siu Sai Wan, Hong Kong Island	Stonecutter's Island	Aberdeen, Hong Kong Island
Specimen length (cm)	293	244	233.5
Age (GLGs)	30	6	4–5
Sex	Male	Female	Female
Blubber thickness (mm)	$6_d3_L5_v$	$9_d11_L8_v$	$13_d9_L11_v$
% Lipid (wet weight)	28.22	50.61	26.00
∑PCB	12.83	16.49	8.26
Pentachlorobenzene	< 0.01	0.01	< 0.01
Hexachlorobenzene	0.52	0.32	0.11
α-HCH	0.01	0.01	0.02
β-HCH	0.55	0.33	0.09
γ-HCH	< 0.01	0.03	0.01
∑HCH	0.56	0.37	0.12
Aldrin	< 0.01	< 0.01	0.03
Dieldrin	0.76	0.10	0.08
∑Dieldrin	1.29	0.20	0.18
Heptachlor epoxide	0.53	0.10	0.07
Oxychlordane	< 0.01	0.18	0.12
trans-Chlordane	< 0.01	0.03	0.03
cis-Chlordane	0.01	0.13	0.11
trans-Nonachlor	0.14	1.06	0.71
cis-Nonachlor	0.01	0.15	0.15
∑Chlordane	0.17	1.54	1.11
∑Lindane	0.56	0.37	0.12
∑Mirex	0.01	0.12	0.09
p,p′-DDE	11.90	114.41	49.11
p,p′-DDD	1.15	17.58	17.68
p,p′-DDT	0.68	17.29	6.76
∑DDT	13.73	149.28	73.68

[a] Measurements for blubber thickness note whether measurements were taken dorsally (d) laterally (L) or ventrally (v).

TABLE 4

PCB congener concentrations (μg g^{-1} lipid weight) in the blubber of bottlenose dolphins from the South China Sea.

Congener (ICES number)	No. of chlorine atoms in congener	Necropsy date		
		25-11-94	4-12-94	13-7-95
15	2	0.02	< 0.01	< 0.01
18	3	< 0.01	< 0.01	< 0.01
31	3	< 0.01	0.02	0.01
40	4	< 0.01	< 0.01	< 0.01
44	4	0.04	0.03	0.02
49	4	0.31	0.06	0.04
52	4	0.69	0.33	0.15
54	4	< 0.01	< 0.01	< 0.01
60	4	0.03	0.01	< 0.01
70	4	0.08	0.07	0.05
74	4	0.18	0.16	0.10
77	4	0.01	0.03	< 0.01
86	5	0.01	0.02	< 0.01
87	5	0.08	0.09	0.08
95 + 121	5	0.34	0.20	0.09
99	5	0.69	0.46	0.18
101	5	0.54	0.64	0.31
103	5	0.03	0.01	0.01
105	5	0.12	0.20	0.13
110	5	0.01	0.04	0.04
114	5	0.01	0.03	0.02
118	5	0.48	0.76	0.01
128	6	0.18	0.37	0.23
129	6	0.01	0.02	0.01
137	6	0.08	0.13	0.08
138 + 158	6	0.17	0.44	0.94
141	6	0.07	0.05	0.07
143	6	0.04	< 0.01	< 0.01
151	6	0.42	0.46	0.41
153	6	2.22	4.12	1.99
154	6	0.01	0.06	0.04
156 + 157	6	0.01	0.04	0.03
159	6	< 0.01	0.05	< 0.01
163	6	0.65	0.47	0.29
170	7	0.71	0.66	0.30
171	7	0.16	0.14	0.07
172	7	0.10	0.17	0.08
173	7	0.01	< 0.01	< 0.01
174	7	0.09	0.19	0.13
177	7	0.13	0.25	0.15
178	7	0.11	0.19	0.11
180	7	1.83	1.67	0.70
182 + 187	7	0.65	1.17	0.58
183	7	0.28	0.55	0.28
185	7	0.02	0.04	0.02
189	7	0.03	0.08	0.03
191	7	0.02	0.03	< 0.01
194	8	0.25	0.21	0.10
195	8	0.03	0.04	0.03
196 + 203	8	0.27	0.25	0.13
199	8	0.39	0.33	0.16
201	8	0.06	0.05	0.03
202	8	0.06	0.04	0.02
205	8	< 0.01	0.01	< 0.01
206	9	0.06	0.02	0.02
207	9	0.03	0.01	0.01
208	9	0.01	0.01	< 0.01
209	10	0.02	0.01	0.01
∑PCB		12.83	16.49	8.26

This study demonstrates the presence of significant levels of anthropogenic chemicals in the tissues of bottlenose dolphins from the South China Sea. As the rate of industrial development in south-east Asia increases, so too will the pollution of the South China Sea, and hence the contamination of the cetaceans dwelling within its waters.

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TABLE 5

A comparison of organochlorine levels (μg g^{-1} wet weight) detected in the blubber of bottlenose dolphins from various regions of the world.

Location	∑PCB	∑DDT	Reference
UK	11.1	4.65	Wells *et al*., 1994
Netherlands	29–41	13.90–26.90	Koeman *et al*., 1972
Italy	584	170	Corsolini *et al*., 1995
South Africa	14	20	Cockcroft *et al*., 1989
	[a]nd–10.02	0.03–26.25	Cockcroft *et al*., 1991
Australia	0.06	0.18–2.41	Kemper *et al*., 1994
India	0.52	4.6	Tanabe *et al*., 1993
East coast USA	180	40	Geraci, 1989
East coast USA	16.6[b]	7. 1[b]	Salata *et al*., 1995
West coast USA	435	2120	O'shea *et al*., 1980
South Atlantic	3.3	13	De Kock, 1989
South China Sea	4.71 (2.15–8.35)	32.86 (3.87–75.55)	This study

[a] nd: not detectable.
[b] Estimated wet weight value using a hypothetical lipid content of 46% (Corsolini *et al*., 1995).

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Baseline Study of Submerged Marine Debris at Beaches in Curaçao, West Indies

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Pollution of the oceans by debris is a growing but already serious problem world-wide (Nollkaemper, 1994; Clunie and Hendricks, 1995). Several recent studies indicate that beach usage is an important determinant of beach contamination by debris, and hence that recreational beach usage may be a significant source of litter to the marine environment (e.g. Faris and Hart, 1995; Frost and Cullen, 1997; Debrot *et al*., 1999). However, while a significant amount of literature is dedicated to beach litter, data are sparse on riverine litter, and almost non-existent with respect to submerged marine litter (Williams *et al*., 1993). Some studies have recently been completed with regard to submerged litter on the continental shelf (e.g. Galgani *et al*., 1995a,b; Galil *et al*., 1995), but no reports exist on submerged litter in the shallow marine environment at recreational beaches. Since the recreational usage of beaches is increasing on the Caribbean island of Curaçao, beach litter may become a significant source of pollution on the adjacent coral reef. The objective of this study was therefore to quantify the extent and nature of submerged debris at beaches in Curaçao. These data form a baseline for monitoring submerged marine debris on the shallow reef