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Polybrominated diphenyl ethers (PBDEs) and organochlorines in small cetaceans from Hong Kong waters: Levels, profiles and distribution

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Abstract

Polybrominated diphenyl ethers (PBDEs) and organochlorine compounds (OCs) were determined in the blubber, liver and kidney of Indo-Pacific humpback dolphins (*Sousa chinensis*) and finless porpoises (*Neophocaena phocaenoides*) stranded in Hong Kong coastal waters during 1995–2001. Among the organohalogen compounds analyzed, DDTs were the most dominant contaminants with concentrations ranging from 9.9 to 470 μg/g lipid wt. PBDEs in Hong Kong cetaceans, which are reported for the first time, were detected in all the samples with values ranging from 0.23 to 6.0 μg/g lipid wt., with a predominance of BDE-47. Results from this study suggest PBDEs should be classified as priority pollutants in Asia. Higher concentrations were found in humpback dolphins than in finless porpoises, and this was attributed mainly to differences in habitat. Elevated residues of PCBs and DDTs in some cetaceans suggest these species may be at risk.

Keywords: Hong Kong; Finless porpoises; Humpback dolphins; PBDEs; Organochlorines; DDTs

1. Introduction

Hong Kong, with a land area of 1042 km^2 and a population of 6.8 million people, is located at the mouth of the Pearl River in the South China Sea (Richardson et al., 2001). Large scale coastal development, coupled with sewage, shipping and industrial discharge ensure that local waters receive a considerable load of trace organic contaminants (Zheng and Richardson, 1999). Numerous investigations conducted in our laboratory have indicated contamination by persistent organochlorines (OCs) in various environmental compartments of Hong Kong, such as sediments (Kannan et al., 1989), mussels (Tanabe et al., 1987; Kannan et al., 1989; Monirith et al., 2003) and cetaceans (Minh et al., 1999). These findings indicate a serious environmental situation in Hong Kong, which is believed to be a consequence of recent urbanization and industrialization. There have been no reports on the widely used flame retardants, polybrominated diphenyl ethers (PBDEs) in Hong Kong ecosystems. Given that high PBDE concentrations are generally found in marine mammals located at the top of the food chain (Boon et al., 2002), it is imperative to investigate the presence of these emerging, bioaccumulative, and possibly deleterious chemicals in Hong Kong cetacean populations. Finless porpoises (*Neophocaena phocaenoides*) and Indo-Pacific humpback dolphins (*Sousa chinensis*) are residents of Hong Kong coastal waters, and as such they may be

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considered indicators of coastal pollution in Hong Kong.

The present study is aimed at understanding contamination by persistent organohalogen compounds in cetaceans found stranded in Hong Kong coastal waters during 1995–2001. Residue levels and accumulation characteristics of PBDEs in the Hong Kong environment are discussed for the first time in comparison to OCs, such as DDTs (dichlorodiphenyl trichloroethane and its metabolites), PCBs (polychlorinated biphenyls), HCHs (hexachlorocyclohexane isomers), HCB (hexachlorobenzene), CHLs (chlordane related compounds), TCPMe [*tris* (4-chlorophenyl) methane] and TCPMOH [*tris* (4-chlorophenyl) methanol]. The data may shed light on the levels of organohalogen pollution in Hong Kong waters and their potential impacts on cetaceans.

2. Materials and methods

2.1. Samples

The fifteen Indo-Pacific humpback dolphin (*S. chinensis*) and seven finless porpoise (*N. phocaenoides*) specimens used in the present study were found dead in several locations in Hong Kong from 1995 to 2001. Biological data of the animals analyzed are given in Table 1. Blubber, liver and kidney samples were excised from dead animals, wrapped in aluminum foil, and kept in a deep freezer at −20 °C until analysis.

2.2. Chemical analysis

Analysis of PBDEs was performed following the procedure described by Ueno et al. (2004) with slight modification. Briefly, 2–8 g of the target sample was ground with anhydrous sodium sulfate and extracted in a Soxhlet apparatus with a mixture of diethyl ether and hexane for 7–8 h. An aliquot of the extract, after adding 5 ng of internal standards ($^{13}C_{12}$-labeled BDE-3, BDE-15, BDE-28, BDE-47, BDE-99, BDE-153, BDE-154, BDE-183, and BDE-209), was added to a gel permeation chromatography column (GPC; Bio-Beads S-X3, Bio-Rad Laboratories, 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 of activated silica gel S-1 (Wako Pure Chemical Industries Ltd., Japan) column with 5% dichloromethane in hexane for cleanup. $^{13}C_{12}$-labled BDE-139 was added to the final solution prior to GC-MSD analysis. Quantification was performed using a GC (Agilent 6980N) equipped with MSD (Agilent 5973N) for mono- to hepta-BDEs, and GC (Agilent 6980N) coupled with MSD (JEOL GCmate II) for deca-BDE, having an electron impact with selective ion monitoring mode (EI-SIM). GC columns used for quantification were DB-1 fused silica capillary (J&W Scientific Inc.) having 30 m × 0.25 mm i.d. × 0.25 μm film thickness for mono- to hepta-BDEs, and 15 m × 0.25 mm i.d. × 0.1 μm film thickness for deca-BDE. Ten major congeners of PBDEs (BDE-3, BDE-15, BDE-28, BDE-47, BDE-99,

Table 1
Concentrations of organohalogens (μg/g lipid wt.) in the blubber of cetaceans collected from Hong Kong coastal waters

ID	Year	Sex	BL (cm)	Lipid (%)	PBDEs	PCBs	DDTs	CHLs	HCHs	HCB	TCPMe	TCPMOH
Finless porpoise												
NP00-28/12	2000	M	>123	40	na	4.7	63	0.34	0.27	0.21	0.076	0.066
NP00-26/12	2000	M	152	34	0.78	7.2	51	0.40	0.17	0.11	0.055	0.063
NP00-25/12	2000	F	145	28	0.47	18	110	0.43	0.10	0.28	0.099	0.044
NP01-20/03	2001	M	121	63	0.23	1.4	26	0.14	0.034	0.087	0.024	0.034
NP01-24/05	2001	M	159	65	0.98	28	260	1.4	0.86	0.16	0.15	0.058
NP01- 12/04	2001	M	163	32	0.84	22	260	1.9	0.31	0.25	0.082	0.066
NP01-15/06	2001	UK	123	27	0.28	1.9	9.9	0.15	0.032	0.075	0.019	0.015
Humpback dolphin												
SC95-2/9	1995	UK	189	10	na	39	380	1.4	0.49	0.36	0.55	0.23
SC97-10/9	1997	M	110	41	na	5.2	27	0.19	0.18	0.21	0.017	0.015
SC97-3/9	1997	M	265	53	0.35	15	62	0.29	0.12	0.26	0.089	0.027
SC97-2/5	1997	UK	105	51	na	12	82	0.49	1.2	0.11	0.037	0.053
SC98-7/8	1998	M	221	13	1.8	3.7	200	1.1	0.57	0.16	0.19	0.089
SC98-3/5	1998	UK	112	38	na	2.8	19	0.21	0.21	0.098	0.0067	0.014
SC98-17/1	1998	UK	238	9.2	na	22	49	0.53	0.16	0.13	0.099	0.024
SC98-17/4	1998	UK	>233	51	na	18	68	0.41	0.18	0.23	0.069	0.037
SC00-30/11	2000	M	231	34	2.6	78	470	3.8	1.0	0.43	0.26	0.25
SC00-14/2	2000	F	252	18	0.67	9.4	51	1.2	0.11	0.15	0.087	0.032
SC01-06/5	2001	M	106	29	0.28	72	150	1.7	2.2	0.35	0.074	0.058
SC01-03/6	2001	M	107	32	na	56	160	2.0	1.7	0.29	0.082	0.12
SC01-06/02	2001	M	160	17	na	38	280	6.5	0.68	0.86	0.20	0.16
SC01-28/06	2001	M	247	18	6.0	83	290	5.9	0.69	0.36	0.45	0.15
SC01-11/2	2001	F	234	75	1.6	17	76	1.4	0.36	0.24	0.086	0.042

UK: unknown; M: Male; F: Female; na: no data available; BL: body length.

BDE-100, BDE-153, BDE-154, BDE-183 and BDE-209) were quantified in this study. All the congeners were quantified using the isotope dilution method to the corresponding $^{13}C_{12}$-labeled congener. Recovery of $^{13}C_{12}$-labeled BDE ranged between 60% and 120%.

OCs including PCBs, DDTs, HCHs, CHLs, HCB, TCPMe and TCPMOH were analyzed following the method described by Kajiwara et al. (2003). Another aliquot of the extract was subjected to GPC for lipid removal. The GPC fraction containing OCs was concentrated and passed through an activated Florisil column for clean-up and fractionation. Quantification of PCBs and most of organochlorine pesticides was performed using a GC (Agilent 6980N) equipped with a microelectron capture detector (micro-ECD) and an autoinjection system (Agilent 7683 Series Injector). The GC column used for OC analysis was a fused silica capillary (DB-1; 30 m × 0.25 mm i.d. × 0.25 μm film thickness, J&W Scientific Inc.). Identification and quantification of TCPMe and TCPMOH were performed using a GC-MSD (Agilent 5973N) in SIM mode equipped with an autoinjection system (Agilent 7683 series injector). The concentration of individual OCs was quantified from the peak area of the sample compared 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 individually resolved peaks of PCB isomers and congeners were summed to obtain total PCB concentrations.

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

3. Results and discussion

3.1. Contamination status

Concentrations of PBDEs and OCs detected in the blubber of finless porpoises and Indo-Pacific humpback dolphins from Hong Kong coastal waters are shown in Table 1. Among the organohalogens analyzed, DDTs ranked first followed by PCBs > CHLs > PBDEs > HCHs > HCB > TCPMe > TCPMOH. This is the first assessment of PBDE contamination in the Hong Kong environment. For all the contaminants, higher concentrations were found in humpback dolphins when compared to finless porpoises. This difference can be accounted for by differences in habitat. Humpback dolphins inhabit the western waters of Hong Kong, which are close to the Shenzhen economic zone, and their habitat is estuarine (Jefferson, 2000). However, finless porpoise inhabit southern and eastern waters of Hong Kong which are more oceanic-influenced (Jefferson et al., 2002a). Similarly, significantly higher concentrations of OCs were observed in Little Egret (*Egretta garzetta*) eggs from Mai Po in the western part of Hong Kong when compared to Night Heron (*Nycticorax nycticorax*) eggs from A Chau in the eastern part of Hong Kong (Connell et al., 2003).

Summed concentrations of PBDE congeners measured in the blubber of finless porpoises and humpback dolphins ranged from 230 to 980 ng/g lipid wt. and 280 to 6000 ng/g lipid wt., respectively (Table 1). It is noteworthy that PBDE residues in cetaceans from Hong Kong were apparently higher when compared to northern fur seals (*Callorhinus ursinus*) from the Pacific coast of Japan (Kajiwara et al., 2004), and bottlenose dolphins (*Tursiops truncatus*) from the South Atlantic US coast (Kuehl et al., 1991). The levels observed in this study were comparable to harbor seals (*Phoca vitulina*) from San Francisco Bay (She et al., 2000) and harbor porpoises (*Phocoena phocoena*) from British Columbia (Ikonomou et al., 2002). The results confirm that developing countries can also be a source for PBDE contamination. Our previous study to elucidate geographical distribution of PBDEs using skipjack tuna showed highest concentrations of PBDEs in samples from the East China Sea (Ueno et al., 2004). The plausible reasons for the high levels of PBDEs observed in this study may be the daily discharge of large quantities of untreated sewage into coastal waters of Hong Kong (Chan and Yung, 1995) as well as export of waste electric products (computers, televisions etc.) used in developed nations to Asian developing countries such as China as trash (Hileman, 2002). These findings are consistent with studies associating high PBDE concentrations with point-sources such as wastewater treatment plant outfalls (Hale et al., 2001).

In Hong Kong cetaceans, DDTs and PCBs were the predominant contaminants. DDT levels were high when compared to Dall's porpoises (*Phocoenoides dalli*) from Japan (Kajiwara et al., 2002) and spinner dolphins (*Stenella longirostris*) from the Philippines and India (Prudente et al., 1997), but were lower than some highly polluted areas in the Mediterranean (Kannan et al., 1993). High DDT levels found in Hong Kong cetaceans are related to DDT utilization in this country until 1989 (Phillips and Tanabe, 1989), and possible continued use in surrounding waters of China. As for PCBs, concentrations were higher than in humpback dolphins from Bay of Bengal, India (Prudente et al., 1997) but lower than in diseased cetaceans from the Mediterranean Sea (Kannan et al., 1993; Corsolini et al., 1995). A large proportion of PCBs still remain in transformers and

capacitors in China (Zhou et al., 2001) and this may be one of the contributing factors to the high levels of PCBs observed in this study.

3.2. PBDE congener profiles

Of the 10 congeners analyzed, a total of eight congeners from di- to hepta-BDE were identified in Hong Kong cetaceans (Fig. 1). BDE-3 (mono-BDE) and BDE-209 (deca-BDE) were not found above the detection limits of the analysis, which were 0.01 and 0.5 ng/g on lipid wt., respectively. Similar PBDE congener patterns for humpback dolphin and finless porpoise were found. BDE-47, BDE-99 and BDE-100 made up 90% on average of the total PBDE load. These results are in agreement with the general PBDE congener pattern reported in biological samples (Ikonomou et al., 2002). It is not known whether China uses OctaBDE and/or DecaBDE. In the present study, BDE-183 could be detected in trace amounts only in a few individuals.

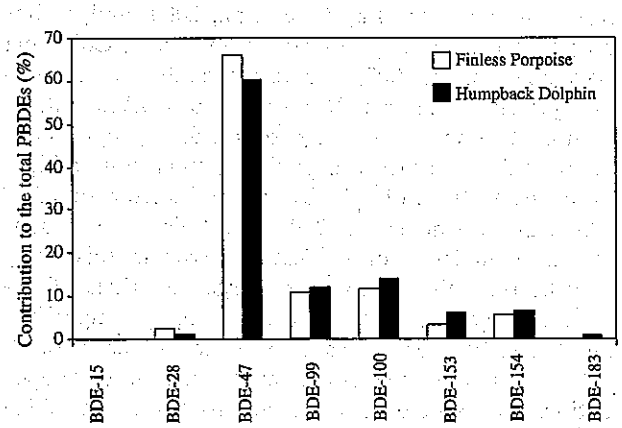

Fig. 1. PBDE congener patterns in finless porpoises and humpback dolphins from Hong Kong coastal waters. Vertical bars represent contribution of each congener to total PBDEs concentration.

Debromination of BDE-183 to lower congeners may be the reason, as experimental studies in fish have shown debromination of BDE-183 to BDE-154 (Stapleton et al., 2004). BDE-209, the major congener of DecaBDE formulation, was not detected in any of the samples, suggesting its relatively low bioaccumulation potential. As of now, the type of commercial PBDE mixture used in and around Hong Kong is not known. Hence, information on this aspect is vital to compare the congener profiles in the present study to that of the commercial mixtures.

3.3. Residue composition pattern of organochlorine pesticides

The residue composition pattern of the various organochlorine pesticides is shown in Fig. 2. Percentages of the main components of DDTs varied between the specimens. The major constituent of DDTs was *p,p'*-DDE, accounting for more than 40% in both humpback dolphins and finless porpoises. Concentrations and proportions of *p,p'*-DDT in both the cetaceans were higher in the present study when compared to finless porpoises from Japan (Arakane et al., 2002), suggesting that DDT is still being used in and around Hong Kong. The wide range of DDT concentrations, and DDT composition in waters and sediments in Daya Bay, Southern China, also suggest recent inputs to the coastal waters (Zhou et al., 2001). The Pearl River Estuary, just west of Hong Kong, drains a vast area of agricultural lands in southern China, and the continuing use of DDT in this area is therefore very much possible. Overall, in all the samples of humpback dolphins and finless porpoises analyzed, the percentage of *p,p'*-DDD was relatively high. Harbour porpoises from the Black Sea, which has a reductive environment also had high proportions of *p,p'*-DDD (Tanabe et al., 1997). The anaerobic conditions prevalent due to the large quantities of sewage discharged as mentioned before may be

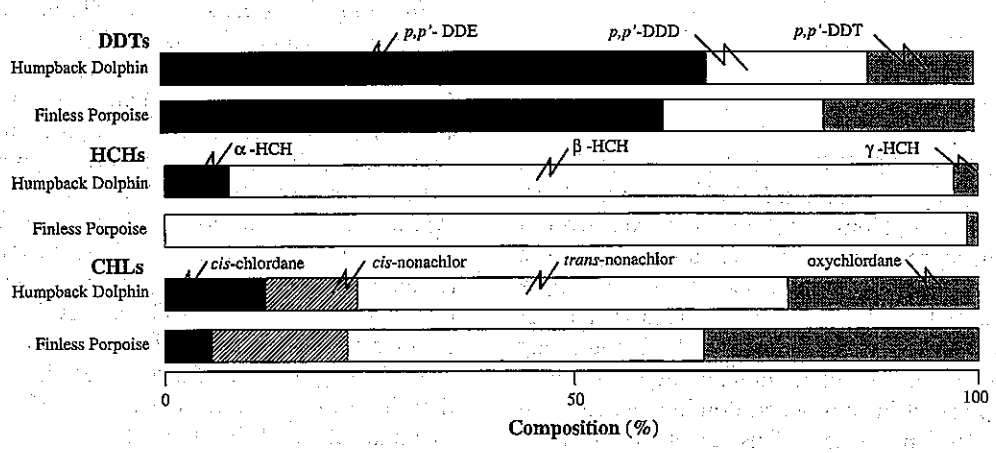

Fig. 2. Percentage compositions of organochlorine pesticides in the blubber of cetaceans from Hong Kong coastal waters.

responsible for high *p,p'*-DDD levels in cetaceans. Sediments and fish analyzed from inland water systems of Hong Kong also showed high *p,p'*-DDD levels (Zhou et al., 1999).

For CHLs, *trans*-nonachlor was the dominant compound, followed by oxychlordane, *cis*-nonachlor and *cis*-chlordane. This pattern is similar to previous studies dealing with marine mammals (Minh et al., 1999; Watanabe et al., 1999). The proportion of *cis*-chlordane in humpback dolphins was more than in finless porpoises, perhaps because humpback dolphins are a coastal species and there might have been fresh inputs of CHLs into the coastal waters from terrestrial environments.

For HCH isomers, β-HCH was the most predominant isomer in both the cetacean samples. Minh et al. (1999) suggested that the predominance of β-HCH among HCH isomers reflects its persistence towards enzymatic degradation.

3.4. Contaminant distribution between tissues

To understand contaminant distribution between tissues, blubber, liver and kidney samples from some individuals of finless porpoises and humpback dolphins were analyzed. Concentrations of organohalogens in the three different tissues of both species are shown in Table 2. The lipid normalized PBDE and OC concentrations in various tissues were highest in blubber when compared to the other tissues, consistent with the previous data reported for OCs (Watanabe et al., 1999). PBDE congener profiles in all three tissue samples analyzed were similar. BDE-47 was the dominant congener and accounted for 70% of the total PBDE load. Similarly, no tissue preference for BDE-47 was found for marine mammals from the North Sea (Boon et al., 2002). For fishes from the lakes of Europe, higher BDE-47 was found in the liver when compared to the muscle (Vives et al., 2004). In the case of DDTs, the proportions of metabolites were higher in the liver and kidney when compared to blubber. *p,p'* -DDT percentage in the liver of both finless porpoise and humpback dolphin was less than 1% (Fig. 3). The liver is the main center for detoxification of xenobiotics and this may be the reason for the lower percentage of *p,p'*-DDT. Secondly, the liver of striped dolphins (*Stenella coeruleoalba*), which comprise mainly phospholipids, had lower proportions of *p,p'*-DDT (Fukushima and Kawai, 1981). Thus the nature and levels of total lipids in a tissue is an important factor influencing the uptake of lipophilic substances. No distinct trend could be found for CHLs and HCHs among the three tissues. For two immature finless porpoise samples, concentrations of organochlorines were higher in liver when compared to blubber. The lipid percentage in liver was also high, which suggests that mobilization of lipids from blubber had taken place. When mobilization of lipids occurs, both lipids and liphophilic pollutants are drawn to the liver for catabolism; therefore an increased concentration of both lipids and xenobiotic compounds is expected. The

Table 2
Concentrations of organohalogens (μg/g lipid wt.) in blubber, liver and kidney of cetaceans collected from Hong Kong coastal waters

ID	Year	Sex	BL (cm)	Tissue	Lipid (%)	PBDEs	PCBs	DDTs	CHLs	HCHs	HCB	TCPMe	TCPMOH
Finless porpoise													
NP00-25/12	2000	F	145	Blubber	28	0.47	18	110	0.43	0.10	0.28	0.099	0.044
				Liver	7.1	0.38	14	95	1.4	0.12	0.57	0.27	0.16
				Kidney	3.7	0.22	6.7	43	0.21	0.077	0.19	0.078	0.052
NP01-12/4	2001	M	163	Blubber	32	0.84	22	260	1.9	0.31	0.25	0.082	0.066
				Liver	4.3	0.29	9.3	73	0.44	0.13	0.36	0.061	0.22
				Kidney	2.1	0.29	7.8	61	0.41	0.11	0.19	0.061	0.089
NP01-20/3	2001	M	121	Blubber	63	0.23	1.4	26	0.14	0.034	0.087	0.024	0.034
				Liver	11	0.25	2.7	60	0.32	0.066	0.17	0.096	0.10
				Kidney	3.2	0.089	1.4	25	0.15	0.036	0.11	0.048	0.048
NP01-15/6	2001	UK	123	Blubber	27	0.28	1.9	10	0.15	0.032	0.075	0.019	0.019
				Liver	12	0.071	2.1	19	0.16	0.067	0.13	0.55	0.033
				Kidney	10	na	1.1	9.4	0.29	0.041	0.057	0.35	0.027
Humpback dolphin													
SC00-30/11	2000	M	231	Blubber	34	2.6	78	470	3.8	1.0	0.43	0.26	0.25
				Liver	6.3	0.79	27	230	2.3	1.3	0.85	0.19	0.33
				Kidney	3.6	1.0	18	83	0.88	0.46	0.24	0.12	0.0046
SC01-11/2	2001	F	234	Blubber	25	1.6	17	76	1.4	0.36	0.24	0.086	0.042
				Liver	11	0.34	0.61	67	1.1	0.60	0.55	0.058	0.14
				Kidney	5.3	0.33	4.6	28	0.73	0.32	0.16	0.062	0.055
SC01-6/5	2001	M	106	Blubber	29	0.28	72	160	1.7	2.2	0.35	0.074	0.058
				Liver	3.4	0.25	30	150	1.6	2.4	0.52	0.056	0.16
				Kidney	5.6	0.26	18	91	0.98	1.5	0.27	0.053	0.051

UK: unknown; M: Male; F: Female; na: no data available; BL: body length.

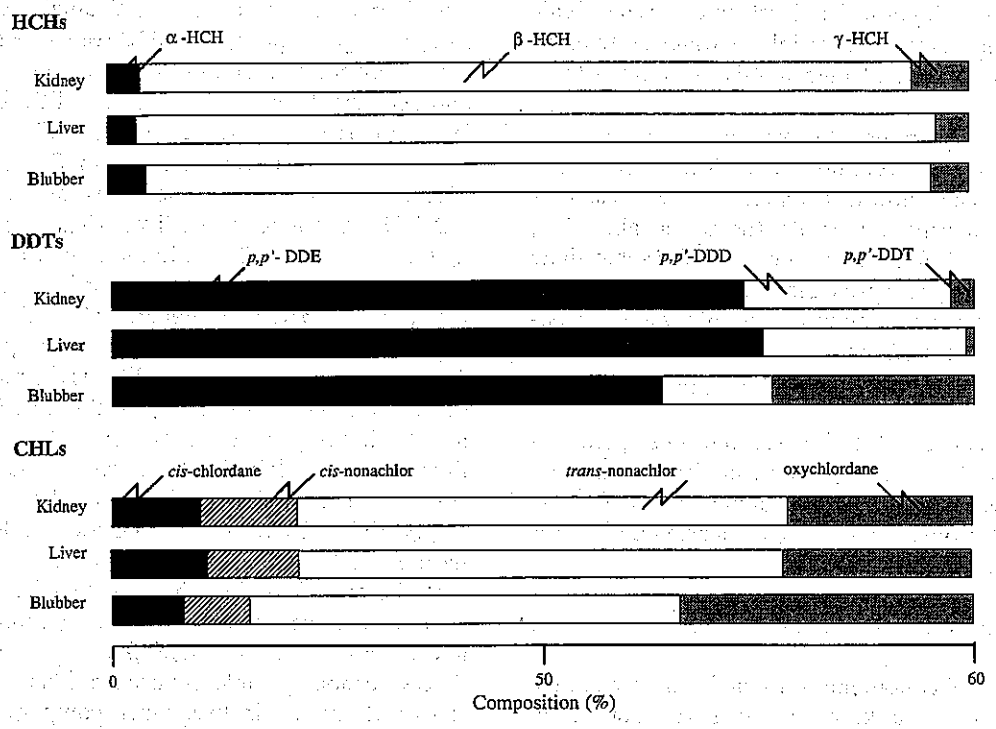

Fig. 3. Percentage compositions of organochlorine pesticides in the blubber, liver and kidney of finless porpoises from Hong Kong coastal waters.

differences in the accumulation pattern among the tissues can be attributed to metabolism of the compounds, seasonal changes in the stored fat or recent exposure to the contaminants.

3.5. Toxic assessment

Reproductive impairments and declining populations in marine mammals have been associated with high burdens of organic pollutants (Delong et al., 1973; Helle et al., 1976). The concentrations of DDTs and PCBs in Hong Kong cetaceans were compared with the concentrations of DDTs and PCBs in other marine mammals, which showed adverse effects (Fig. 4). From the figure, it is evident that for both the cetaceans, concentrations of DDTs and PCBs were in the range where there may be impairment of reproduction as well as suppression of the immune system. Histopathological studies of

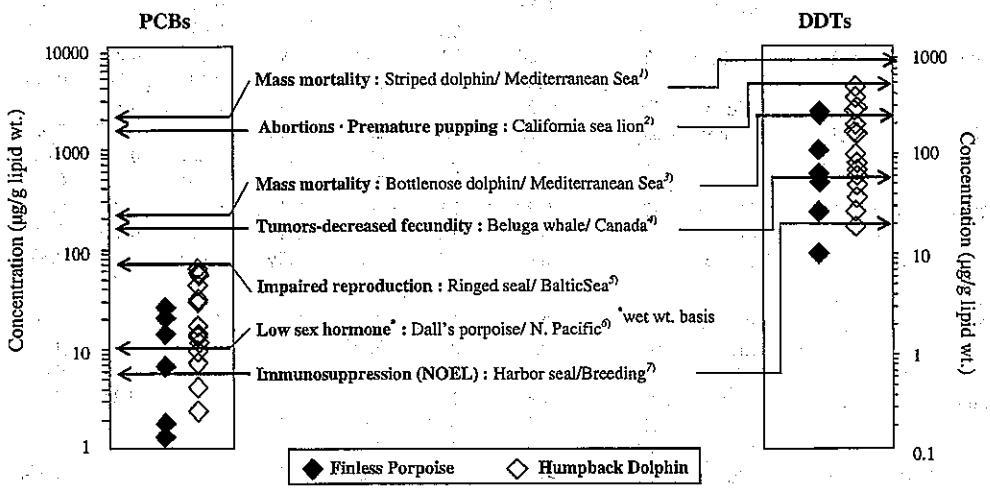

Fig. 4. Toxic assessment of PCBs and DDTs in cetaceans from Hong Kong coastal waters. Data were cited from (1) Kannan et al. (1993); (2) Delong et al. (1973); (3) Corsolini et al. (1995); (4) Martineau et al. (1987); (5) Helle et al. (1976); (6) Subramanian et al. (1987); (7) de Swart et al. (1996). *: wet weight basis.

finless porpoises from Hong Kong showed that the lymphocytes had toxic granules, which could be suggestive of an immune system problem (Parsons et al., 1999). Also, there was some possible indications of finless porpoise specimens that were in poor health having somewhat higher levels of some organochlorines in their blubber (Jefferson et al., 2002b). Thus, the high level of contaminants found in the cetaceans from Hong Kong are of serious concern. Continuous monitoring and ecotoxicological studies are needed to understand the status of contamination as well as actual health effects of man made chemicals on this population of cetaceans.

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