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Chemosphere 63 (2006) 845–852

CHEMOSPHERE

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An assessment of the risks associated with polychlorinated biphenyls found in the stomach contents of stranded Indo-Pacific Humpback Dolphins (*Sousa chinensis*) and Finless Porpoises (*Neophocaena phocaenoides*) from Hong Kong waters

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Received 3 June 2005; received in revised form 8 July 2005; accepted 20 July 2005
Available online 16 September 2005

Abstract

The risks to Indo-Pacific Humpback Dolphins and Finless Porpoises associated with polychlorinated biphenyls (PCBs) were assessed. Stomach contents from twelve stranded Humpback Dolphins and sixteen stranded Finless Porpoises were collected. Concentrations of total and isomer-specific PCBs in the stomach contents were determined using dual-column gas chromatography equipped with electron capture detectors (GC-ECD). Risks due to the PCBs were assessed in three scenarios, based on total PCBs (summation of 41 PCB congeners), total toxicity equivalency (TEQs) and PCB 118, using the toxicity reference values (TRVs) as the threshold effects benchmarks. The calculated risk quotients (RQs) showed that risks due to PCBs were generally low or negligible. Specifically, RQs from total TEQs and total PCBs for Finless Porpoises are below one, suggesting that PCBs should be a low risk for the Finless Porpoise in Hong Kong waters. However, the Humpback Dolphin has RQs larger than 1 for total TEQs and total PCBs when the 95th percentile data were used in the evaluation. This indicates that further investigation may be needed to examine more closely the potential impact of toxic contaminants in the habitat of the Humpback Dolphin.

Keywords: PCB118; Risk quotient; Toxicity equivalency

1. Introduction

The polychlorinated biphenyls (PCBs) are synthetic chemicals which had been used in huge quantities

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0045-6535/$ - see front matter
doi:10.1016/j.chemosphere.2005.07.059

throughout the world. They are toxic, persistent and bioaccumulative contaminants that are detectable in many environmental compartments. They consist of 209 congeners that exhibit different physicochemical properties and biological effects. Twelve of these congeners are grouped as the dioxin-like PCBs because their coplanar structure and toxic effects are similar to dioxins. Thus the toxicities of these 12 PCB congeners have been estimated with respect to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) to obtain the toxicity equivalency factors (TEFs) from which toxicity equivalencies (TEQ) can be estimated.

Due to the PCBs being highly bioaccumulative, the top predator species in the food chain, including humans and marine mammals, may be vulnerable to the toxic effects of PCBs. In Hong Kong, sixteen species of marine mammals have been recorded, but only the Indo-Pacific Humpback Dolphin (*Sousa chinensis*) and Finless Porpoise (*Neophocaena phocaenoides*) are considered as local residents (Parsons et al., 1995). The geographical distributions of these two species are different. The Humpback Dolphin mainly inhabits the estuarine western waters of Hong Kong (Jefferson, 2000), while the Finless Porpoise can be found in the eastern and southern waters (Jefferson et al., 2002a). Both of them are listed in Appendix 1 of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES).

Some studies have shown that pollution may have an impact on the health of Humpback Dolphins and Finless Porpoises in Hong Kong because of the high level of environmental contaminants, including trace elements (Parsons, 1999) and organochlorines (Minh et al., 1999, 2000a,b; Jefferson et al., 2002b), found in the body tissues of these two species. Especially high concentrations of total PCBs were found in the blubber of Humpback Dolphins and Finless Porpoises with the highest concentrations being 50 and 48 $\mu g\ g^{-1}$ (wet weight: ww) in dolphins and porpoises, respectively. Total TEQ levels based on the dioxin-like PCBs in dolphins and porpoises were 510 and 400 $pg\ g^{-1}$ ww, respectively (Minh et al., 2000a). In the previous studies contaminant levels in the tissue, namely in liver, kidney and blubber, were the targets of interest in the Humpback Dolphins and Finless Porpoises.

In fact, the persistent organic pollutants, such as the PCBs and several chlorinated pesticides, reach the marine mammals through the food chain (via biomagnification). The relatively high contaminant levels found in the tissue of the animals may indicate that cetaceans in Hong Kong are ingesting elevated levels of PCBs in their diets. It is, therefore, instructive to examine the levels of contaminants in prey items and food consumed by these cetaceans to see how significant a source of these contaminants they are for the animals. Stomach contents of dead animals should provide a good source of their actual diets. Consequently the stomachs of the stranded animals provide a ready source of such material that can be analyzed for contaminants. Based on previous analyses of stomach contents of these animals the Humpback Dolphin mainly feeds on estuarine fish, including the most important families of Engraulidae, Sciaenidae and Clupeidae (Jefferson, 2000; Barros et al., 2004), while the diet of Finless Porpoises consists of fish, crustaceans and cephalopods (Barros et al., 2002). The aims of the present study are to determine the concentrations of PCBs in the stomach contents of Indo-Pacific Humpback Dolphins and Finless Porpoises, and to assess the risk to the dolphin and porpoise due to the PCBs.

2. Materials and methods

2.1. Sample preparation

The stranded Indo-Pacific Humpback Dolphin and Finless Porpoises were collected and sampled by researchers from the Hong Kong Cetacean Research Project (HKCRP), which is supported by the Agriculture, Fisheries and Conservation Department (AFCD) of Hong Kong SAR. Stomachs of the dead stranded animals were assessed and those with sufficient content, about 0.5 kg, were sliced open and examined. The contents were diced and about 15 g placed in separate pre-cleaned PVC tubes and stored at −20 °C until analyzed. At the time of the analysis, the samples were removed from the pre-cleaned PVC tubes, freeze-dried (Dura-Dry^{TM}) and ground into powder in order to enhance extraction efficiency.

2.2. Analytical method

About 2 g of the freeze-dried sample was weighed into a pre-cleaned cellulose thimble and then extracted with 200 ml of a hexane and dichloromethane (DCM) mixture (3:1 v/v) for 16 h in a Soxhlet apparatus. The cooled extract was concentrated to about 1 ml with a rotary evaporator.

The extract was re-dissolved in hexane and then passed through two chromatography columns. The alumina (aluminum oxide, neutral, Sigma), silica gel (silica gel 60, Merck) and anhydrous sodium sulphate (Na_2SO_4) (Riedel-de Haën) used to prepare the columns were pre-heated to 450 °C, while all the organic solvents used were of analytical grade and were distilled before use. The first glass column (1 cm i.d.) was packed with a mixture of 10 g of alumina and 1.5 g of Na_2SO_4 and washed with 50 ml hexane. The second column was packed with 4 g alumina, 4.5 g silica gel and 1.5 g of Na_2SO_4 and washed three times with two aliquots of different hexane and DCM solvent mixtures (1:3 and 1:1 v/v) and pure hexane. The extract (1 ml) was placed on the top of the first column and eluted with 150 ml hexane. The concentrated eluant from the first column (rotary-evaporated to 1 ml) was then added to the second column and two fractions were eluted, one with 130 ml hexane and one with 200 ml of a mixture of hexane and DCM (1:1 v/v).

Concentrations of 41 PCB congeners were determined by gas chromatography with an electron capture detector (GC-ECD, Hewlett–Packard 6890). A sequential dual column system, two capillary columns (DB-5 and DB-XLB, 60 m length × 0.25 mm i.d. and 0.25 μm film thickness, J&W Scientific Inc., USA), was used to enhance identification and confirmation of PCB congeners in the GC chromatograms (Fig. 1). The oven temperature for the columns was programmed from 100 to 130 °C at a rate of 10 °C/min, then increased to 255 °C at 1 °C/min, finally it was increased to 285 °C at 2 °C/min and held for 5 min. Helium was used as the carrier gas. The injector and detector were kept at 230 °C and 300 °C, respectively. All specimens were analyzed in batches which included procedural blank and Standard Reference Material (SRM 2977, freeze-dried mussel tissue, National Institute of Standards and Technology, USA). The percentage recoveries of individual PCB congeners ranged from 75% to 120%, while the detection limit of individual PCB congeners was 0.5 ng ml^{-1}. The concentrations of organochlorine pesticides (OCPs) were not determined in the present study because a low recovery was found when OCP standards were spiked into stomach content samples. This may be due to the complex matrices of the stomach contents and/or their acidity not favoring extraction.

2.3. Risk assessment

The method used to assess the risk due to the PCBs was similar to that in Hung et al. (2004). For exposure assessment, probabilistic plots, based on data from all stomach content samples from Humpback Dolphins or Finless Porpoises, were constructed by plotting percent cumulative probability on a probabilistic scale against concentrations on a logarithmic scale. An equation describing the relationship was obtained by least-squares linear regression, and the corresponding 5th, 50th and 95th percentile data were calculated.

Dose–response assessment aims to derive a maximum allowable concentration (MAC) for a specific chemical in the stomach content (assumed to represent ingested prey items), which represents the highest level of toxicant that can occur in the prey items without causing harm to a dolphin or a porpoise. The MAC in terms of adverse biological effects on dolphins and porpoises were estimated based on the toxicity reference value (TRV, mg kg^{-1} ww day^{-1}). The NOAEL (no observable adverse effect level) values for the contaminants of interest are available for several mammals. Due to the absence of dolphin- or porpoise-specific toxicity data, these values were used as surrogates to derive the MAC. A body weight scaling factor (Sample et al., 1996) was applied to these NOAEL values to determine TRVs which were used in the present risk assessment (Hung et al., 2004).

$$\text{Scaling factor: } TRV_r = NOAEL_t\ (BW_t/BW_r)^{1/4}$$

where TRV_r is the toxicity reference value for receptor species (mg kg^{-1} ww day^{-1}); $NOAEL_t$, the no observable adverse effect level for the test species (mg kg^{-1} ww day^{-1}); BW_r, the body weight of the receptor species (kg ww) and BW_t is the body weight of the test species (kg ww).

The maximum allowable concentration based on TRV (MAC_{TRV}) is calculated using the following equation for intake of a contaminant:

$$\text{Intake (mg kg}^{-1}\text{ day}^{-1}) = \frac{CF \times IR \times EF \times ED}{BW \times AT}$$

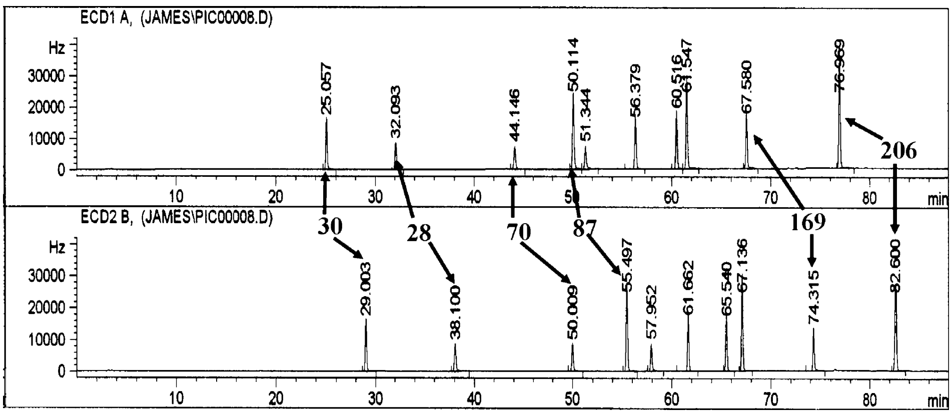

Fig. 1. Typical chromatograms of the dual column system showing different retention times of various PCB congeners (e.g. PCB IUPAC Nos. 28, 30, 70, 87, 169 and 206). The two capillary columns used are DB-5 (upper) and DB-XLB (lower).

where CF is the contaminant concentration in fish (mg kg^{-1} ww); IR, the ingestion rate (kg day^{-1}); EF, the exposure frequency (day $year^{-1}$); ED, the exposure duration (years); BW, the body weight (kg) and AT is the averaging time (period over which exposure is averaged in days).

For the highest rate of intake of a specific contaminant at which health risk is still at an acceptable low level,

$$\text{Intake} = \text{TRV} \quad \text{and} \quad \text{CF} = \text{MAC}_{\text{TRV}}$$

Thus,

$$\text{TRV} = \frac{\text{MAC}_{\text{TRV}} \times \text{IR} \times \text{EF} \times \text{ED}}{\text{BW} \times \text{AT}}$$

$$\text{MAC}_{\text{TRV}} = \frac{\text{TRV} \times \text{BW} \times \text{AT}}{\text{IR} \times \text{EF} \times \text{ED}}$$

The default values used in the above derivation are summarized in Table 1 and the calculated MAC_{TRV} values are summarized in Table 2.

For risk characterization, results of exposure and dose–response assessments were integrated to estimate the risk. The risk quotient (RQ), which indicates the level of risk, can be calculated as follows:

$$\text{RQ} = \text{Concentration of PCBs in stomach content (expressed as ng g}^{-1}\text{ ww)}/\text{MAC}_{\text{TRV}}$$

3. Results and discussion

3.1. Total PCB residue level

The total PCB levels (sum of 41 PCB congeners) in stomach content samples are summarized in Table 3. PCB concentrations from Indo-Pacific Humpback Dolphins ranged from 15.9 to 2105 ng g^{-1} ww (mean: 313.41) while those from Finless Porpoises ranged from 1.2 to 287.1 ng g^{-1} ww (mean: 78.56). The minimum, maximum and mean concentrations of total PCBs in stomach contents from Finless Porpoises are lower than those from Humpback Dolphins but there is no significant difference, probably due to the similar ranges of PCB concentrations in the stomach content samples, except for specimen SC01-03/06.

The tissue concentrations of organochlorines in stranded samples of Humpback Dolphins and Finless Porpoises have been previously reported (Parsons and Chan, 1998; Minh et al., 1999, 2000a,b). In Humpback Dolphins the mean PCB tissue concentrations ranged from 24 to 31 μg g^{-1} ww and in Finless Porpoises the mean PCB concentrations ranged from 12 to 20 μg g^{-1} ww. These values are generally 1–2 orders of magnitude greater than those found in the present study which might be expected as PCBs accumulate up the food chain into the final predator and stomach contents were analyzed in this study while blubber samples were ana-

Table 1
Values of the variables used in the risk assessment for the dolphins and porpoises

Variables	Values (Indo-Pacific Humpback Dolphin)	Values (Finless Porpoise)
Contaminant concentration in stomach content (CF)	5th, 50th and 95th percentile data	5th, 50th and 95th percentile data
Ingestion rate (IR)	9 kg day^{-1} (about 5% of body weight)	3 kg day^{-1} (about 5% of body weight)
Exposure frequency (EF)	365 day $year^{-1}$[a]	365 day $year^{-1}$[a]
Exposure duration (ED)	35 years (assuming life-time exposure)[a]	20 years (assuming life-time exposure)[b]
Body weight (BW)	185 kg[a]	60 kg[b]
Average time (AT)	12,775 days (35 × 365 days)	7300 days (20 × 365 days)

[a] Data from ERM (2000).
[b] Data from www.afcd.gov.hk.

Table 2
TRV and MAC_{TRV} values used in the risk assessment for dolphins and porpoises (TRV and MAC_{TRV} of TEQ are expressed in pg kg^{-1} ww day^{-1} and pg kg^{-1}, respectively)

	Indo-Pacific Humpback Dolphin		Finless Porpoise	
	TRV (μg kg^{-1} ww day^{-1})	MAC_{TRV} (μg kg^{-1})	TRV (μg kg^{-1} ww day^{-1})	MAC_{TRV} (μg kg^{-1})
TEQs	1[a]	22.56	1[a]	20.0
PCB 118	3.55[b]	72.87	4.7[b]	93.96
Total PCBs	37.96[c]	780.3	50.3[c]	1006

[a] Data from WHO (1998).
[b] Data from Chu et al. (1995).
[c] Data from ERM (2000) and the references therein.

Table 3
The total PCB concentrations and TEQs (wet weight) in the stomach contents of Humpback Dolphins and Finless Porpoises

Sample no.	Sex	Total PCBs ($ng\ g^{-1}$)	Total TEQs ($pg\ g^{-1}$)
Indo-Pacific Humpback Dolphin			
SC00-01/08	F	452.3	22.9
SC01-11/02	F	36.4	1.3
SC00-26/08	M	81.1	4.9
SC00-06/07	F	182.8	0.4
SC01-06/02	M	153.8	7.7
SC00-23/07	M	335.5	0.7
SC01-30/07	M	227.0	0.3
SC01-03/06	M	2105.0	27.9
SC01-28/06	M	126.7	5.7
SC02-08/02	M	15.9	0.7
SC03-08/01	?	21.5	0.8
SC02-22/03	M	22.8	0.8
Mean		313.41	6.18
Range		15.9–2105.0	0.3–27.9
Finless Porpoise			
NP01-09/12	M	83.2	0.5
NP01-20/03	M	124.5	4.6
NP00-26/12	M	244.8	8.4
NP02-21/04	M	87.0	0.3
NP01-12/04	M	287.1	2.3
NP00-25/12	F	46.2	1.9
NP01-15/06	?	8.7	0
NP03-08/05	?	140.2	0.7
NP00-23/06	?	70.8	0.4
NP00-27/09	F	1.2	0
NP00-28/12	M	7.5	0.03
NP00-05/09	M	23.9	0.05
NP00-09/11	M	1.2	0.01
NP01-24/05	M	115.0	3.3
NP01-13/10A	?	3.9	0.2
NP00-02/11	M	11.8	0.08
Mean		78.56	1.43
Range		1.2–287.1	0–8.4

lyzed previously (Minh et al., 1999, 2000a,b). Moreover, the total PCBs were also calculated using a different method. In this study, 41 PCB congeners were determined to represent the total PCBs. However, Minh et al. (1999, 2000a,b) did not specify clearly the composition of total PCBs in their study.

3.2. Isomer-specific accumulation

The percentage composition of PCB congeners is shown in Fig. 2. Hexachlorobiphenyls were the predominant congeners which comprised 40% and 37.7% of total PCBs found in the stomach contents of Humpback Dolphins and Finless Porpoises, respectively. As seen from Fig. 2, penta- to heptachlorobiphenyls were the major congeners as they accounted for nearly 90% of total PCBs in both species.

Isomer-specific analysis showed that hexachlorobiphenyl IUPAC No. 153 was the dominant congener which accounted for 32.47% (Humpback Dolphin) and 41.33% (Finless Porpoises) of the total PCBs. This is similar to the results of Minh et al. (2000a,b). PCB IUPAC Nos. 99, 101, 180 and 182 were also the dominant congeners in the present study.

3.3. Coplanar PCBs

To assess the risk of dioxin-like PCBs in the stomach contents, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) toxicity equivalencies (TEQs) were estimated based on the mammal-specific toxicity equivalency factors (TEFs) that have been proposed by the World Health Organization (WHO) and Van den Berg et al. (1998). The levels of total TEQs are summarized in Table 3. The TEQs of Humpback Dolphins ranged from 0.3 to 27.9 $pg\ g^{-1}$ ww (mean: 6.18), and of Finless Porpoises from 0 to 8.4 $pg\ g^{-1}$ ww (mean: 1.43).

The non-*ortho* coplanar congeners, IUPAC Nos. 77, 81, 126 and 169, were generally in lower concentration than the mono-*ortho* congeners IUPAC Nos. 105, 114, 118, 123, 156, 157, 167 and 189, in the stomachs of Humpback Dolphin. Although the concentrations of non-*ortho* congeners were lower, they still accounted for 26% of the total TEQ as they are comparatively more toxic than the mono-*ortho* ones. On the other hand, no non-*ortho* congeners were found in the stomachs of Finless Porpoises. Thus, only the mono-*ortho* congeners contributed to the total TEQ. This finding, which may be related to the different feeding habits and habitats of the two species, deserves further investigation.

The total TEQs previously reported in the blubber of Humpback Dolphins and Finless Porpoises were 20–50 times higher than the TEQs found in this study (Minh et al., 2000a), probably due to the bioaccumulation of the lipophilic PCBs in the blubber lipid over a long period which will inevitably occur even if low levels of TEQs occur in the food.

3.4. Assessment of the risk of adverse effects

The risks due to PCBs, based on three aspects that included total TEQs, PCB 118 and total PCBs, have been calculated as a risk quotient (RQ) and the calculated risks are summarized in Table 4. The plots of percent cumulative probability (probabilistic scale) against concentrations of PCB 118, total TEQs and total PCBs in stomach contents (logarithmic scale) with corresponding MAC_{TRV} are presented in Fig. 3.

The risks to Finless Porpoises appear to be lower than those for Humpback Dolphins. This may be

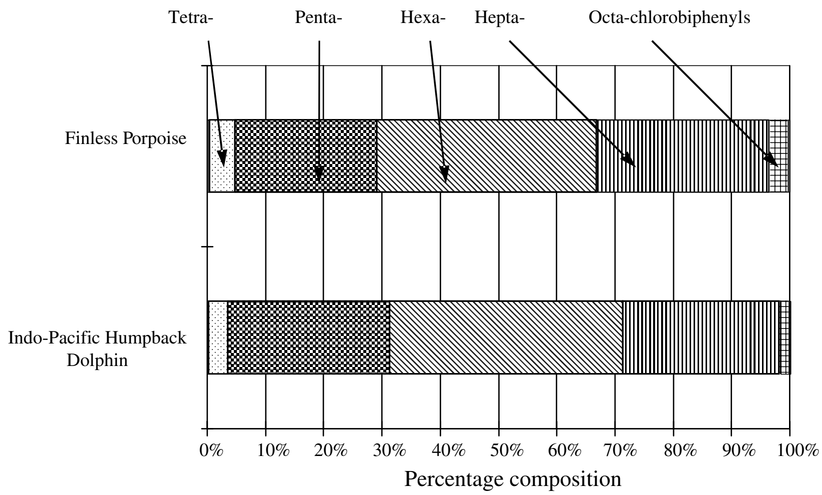

Fig. 2. Percentage composition of PCB congeners in stomach contents of Humpback Dolphins and Finless Porpoises.

Table 4
Risk quotient (RQ) calculated for total TEQs, PCB IUPAC No. 118 and total PCBs using 5th, 50th and 95th percentile concentration data

		RQ (using 5th percentile data)	RQ (using 50th percentile data)	RQ (using 95th percentile data)
Indo-Pacific Humpback Dolphin	Total TEQs	<0.01	0.1	1.4
	PCB IUPAC No. 118	<0.01	<0.01	0.5
	Total PCBs	0.01	0.1	1.7
Finless Porpoise	Total TEQs	<0.01	0.01	0.6
	PCB IUPAC No. 118	<0.01	<0.01	0.2
	Total PCBs	<0.01	0.03	0.6

because Humpback Dolphins inhabit the western waters of Hong Kong, into which the Pearl River empties, which are generally believed to be more polluted than the eastern waters. The RQs from PCB 118 are smaller than unity for porpoises and dolphins even in the case of the 95th percentile data. This suggests that the risks from PCB 118 should be low and negligible. RQs from total TEQs and total PCBs for Finless Porpoises are below one, suggesting that PCBs should be a low risk for the Finless Porpoise in Hong Kong. However, the Humpback Dolphin has RQs larger than 1 for total TEQs and total PCBs when the 95th percentile data were used in the evaluation. This indicates that further investigation may be needed to examine more closely the potential impact of toxic contaminants in the habitat of the Humpback Dolphin.

In the present study, the risk from PCBs was analyzed in three directions. The total PCBs are commonly used in order to assess the impact of mixtures of PCBs in the environment, and for easier comparison between different studies. For the total PCBs, the TRV was derived from the NOAEL of mink (*Mustela* sp.) (ERM, 2000 and the reference therein). Mink are species sensitive to the toxic effect of PCBs (Kannan et al., 2000). In addition, with the body weight scaling factor, the toxic threshold concentrations derived from mink may be a conservative estimate for the protection of marine mammal populations.

The risks from PCB IUPAC No. 118 were also assessed in this study. The NOEAL on rats (*Rattus* sp.) was used to derive the TRV for dolphins and porpoises. The end points used to determine the NOAEL of rat were hepatic EROD activity and vitamin A content (Chu et al., 1995). However, the reproductive effects usually occur at greater exposure concentrations than the physiological effects (Kannan et al., 2000).

The risks from dioxin-like PCBs were assessed by calculating the total TEQs. The TRVs of total TEQs were obtained from the WHO. The reported values were a range of tolerable daily intakes (TDI) which are the thresholds derived for humans with the application of a safety factor (Table 2). The lowest value in the range of TDIs was used and this threshold should be a stringent value so no body weight scaling factor was applied

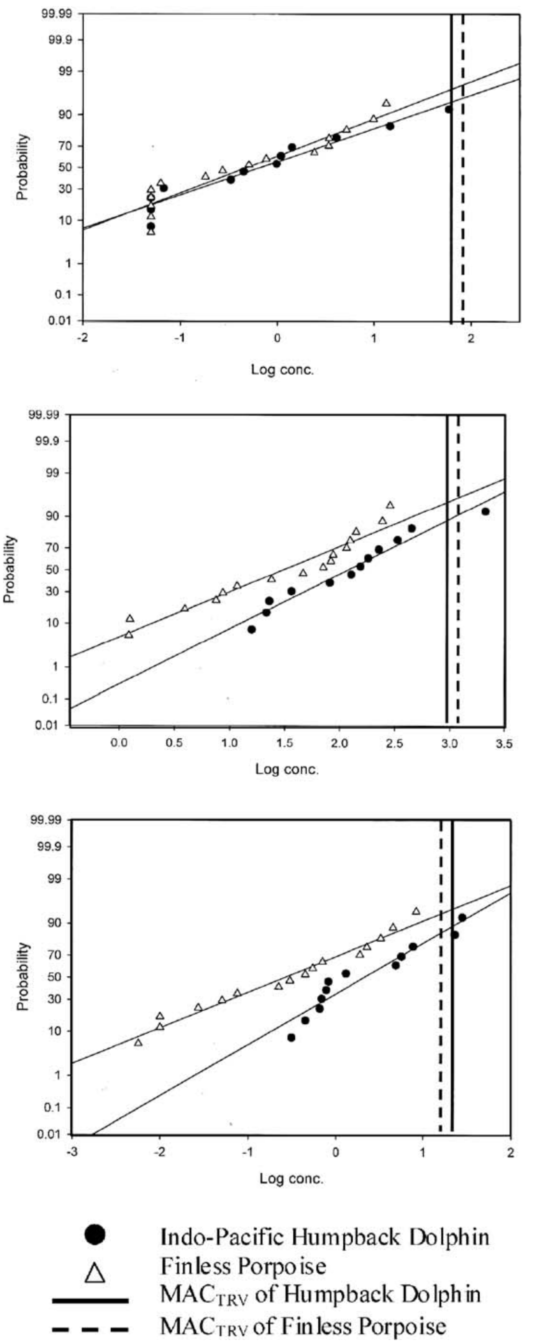

Fig. 3. Plot of percent cumulative probability (probabilistic scale) against concentrations of PCB IUPAC No. 118 (upper), total TEQ (middle), and total PCBs (lower) in stomach contents of Humpback Dolphins and Finless Porpoises (logarithmic scale), with corresponding MAC_{TRV} values.

and the value from WHO (1998) was directly used as the TRV for total TEQs.

The results of this investigation suggest that risks to Humpback Dolphin and Finless Porpoise in Hong Kong due to PCBs are generally low based on their food consumed. However, PCBs are known to bioaccumulate and thus higher tissue levels of PCBs may be found in marine mammals. Further studies are required to investigate the tissue levels of trace organic pollutants in cetaceans in Hong Kong waters. Moreover, a number of other contaminants may need closer study, including DDTs, PBDEs, perfluorinated compounds, butyltins and certain trace contaminants such as mercury and arsenic (Hung et al., 2004).

Acknowledgments

This project was funded by the Hong Kong Research Grants Council (CityU1103/04M). The grant also provided a postgraduate studentship to CLHH. The stranding program is conducted by the HKCRP and supported by AFCD. The authors thank the many research assistants, interns, and volunteers who have assisted with the collection of the stranding data and samples.

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