

## Risk assessment of trace elements in the stomach contents of Indo-Pacific Humpback Dolphins and Finless Porpoises in Hong Kong waters

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### Abstract

The potential health risks due to inorganic substances, mainly metals, was evaluated for the two resident marine mammals in Hong Kong, the Indo-Pacific Humpback Dolphin (*Sousa chinensis*) and the Finless Porpoise (*Neophocaena phocaenoides*). The stomachs from the carcasses of twelve stranded dolphins and fifteen stranded porpoises were collected and the contents examined. Concentrations of thirteen trace elements (Ag, As, Cd, Co, Cr, Cs, Cu, Hg, Mn, Ni, Se, V and Zn) were determined by inductively coupled plasma mass spectrometer (ICP-MS). An assessment of risks of adverse effects was undertaken using two toxicity guideline values, namely the Reference Dose (RfD), commonly used in human health risk assessment, and the Toxicity Reference Value (TRV), based on terrestrial mammal data. The levels of trace metals in stomach contents of dolphins and porpoises were found to be similar. Risk quotients (RQ) calculated for the trace elements showed that risks to the dolphins and porpoises were generally low and within safe limits using the values based on the TRV, which are less conservative than those based on the RfD values. Using the RfD-based values the risks associated with arsenic, cadmium, chromium, copper, nickel and mercury were comparatively higher. The highest RQ was associated with arsenic, however, most of the arsenic in marine organisms should be in the non-toxic organic form, and thus the calculated risk is likely to be overestimated.

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### 1. Introduction

Hong Kong is located on the southern coast of China, where it is the centre of a rapidly expanding area and is host to the second largest port in the world. Marine ecosystems in Hong Kong and the adjacent Guangdong Province have been the subject of considerable research during the

last 10–20 years. A number of studies have been conducted on the dolphin and porpoise populations, but only a small number were related to pollution (Parsons, 1999a; Minh et al., 1999, 2000a,b; Leung et al., 2005; Ramu et al., 2005; Hung et al., 2006).

There are 16 species of dolphins, porpoises and whales that have been recorded in Hong Kong waters. Only the Indo-Pacific Humpback Dolphin, or locally known as Chinese White Dolphin (*Sousa chinensis*), and the Finless Porpoise (*Neophocaena phocaenoides*) are resident marine mammals (Parsons et al., 1995). The two species tend to

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have different distributions in Hong Kong waters. Indo-Pacific Humpback Dolphins are estuarine animals, which can be found in western waters, situated at the mouth of Pearl River, while the porpoises are found in the eastern and southern waters of Hong Kong.

To date, the size of the humpback dolphin population in the Pearl River Estuary is estimated to be 1300 (Jefferson, 2005) and the abundance estimation of finless porpoises is about 217 in Hong Kong waters (<http://www.afcd.gov.hk/>). Both of them are protected species included in Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES).

Many studies showed that humpback dolphins and finless porpoises in Hong Kong are threatened by habitat loss, over-fishing, fast-moving boats, infrastructural projects and pollution. Post-mortem investigations on stranded cetacean carcasses found in Hong Kong waters demonstrated that many accumulated a range of contaminants that may cause adverse health effects (Parsons, 1999a; Minh et al., 1999, 2000a,b; Leung et al., 2005; Ramu et al., 2005; Hung et al., 2006). For example tissue analyses showed that some specimens had high levels of trace metals with the highest mercury levels in dolphins and porpoises at 910 and 390  $\mu\text{g g}^{-1}$  dw, respectively. Arsenic levels were also very high in dolphins (12.9  $\mu\text{g g}^{-1}$  dw) and porpoises (40.3  $\mu\text{g g}^{-1}$  dw) (Parsons, 1999a).

Based on the stomach content of stranded cetaceans, some studies have found that humpback dolphins mainly feed on estuarine fish, including the families Engraulidae, Sciaenidae and Clupeidae (Jefferson, 2000; Barros et al., 2004). The feeding habits of finless porpoises are somewhat different from the dolphins. Their diet consists of fish, crustaceans and cephalopods (Barros et al., 2002). The trace element concentrations in fish collected in western waters of Hong Kong have been measured (Parsons, 1999b; Hung et al., 2004) and the risk to humpback dolphins, due to consuming these species of fish was assessed (Hung et al., 2004). However, there are only a very small number of studies on the trace metal concentrations in the actual stomach content of the cetaceans. In fact, the stomach acts as a very specific sampler collecting the numbers of species, sizes and other characteristics of the biota consumed, and thus the trace metal content of this is particularly relevant in risk assessment.

The main objective of the present study was to measure the trace element concentrations in stomach contents of stranded cetacean carcasses in Hong Kong waters and use these data to evaluate the risk to the humpback dolphins and finless porpoises.

## 2. Materials and methods

### 2.1. Sample preparation

The stranded dolphins and porpoise carcasses were collected and dissected by personnel of the HK Cetacean Research Project (HKCRP) funded by the Agricultural,

Fisheries and Conservation Department (AFCD) of Hong Kong SAR. The whole stomachs, with adequate contents, were stored at  $-20\text{ }^{\circ}\text{C}$ . Prior to analysis the stomach contents were removed, stored in pre-washed PVC tubes and freeze-dried (Dura-Dry™, US).

### 2.2. Analytical method

The dried stomach contents were digested following the methods of Connell et al. (2002) and Hung et al. (2004). The weighed samples were digested in a mixture of 3-D water (2 ml, double-distilled deionized water) and 70% nitric acid (5 ml, trace metal grade, Tedia, USA). The pressure in the digestion tubes was 65 psi and this was continued for 15 min in a microwave oven (CEM, model MDS-2000). After cooling,  $\text{H}_2\text{O}_2$  (2 ml, 35%, Riedel-de Haën, Germany) was added and the procedure, 65 psi for 15 min, was repeated. The cooled digests were filtered through a disposable syringe filter disc (Macherey-Nagel, pore size 0.45  $\mu\text{m}$  and diameter 25 mm, with cellulose mixed esters as filtering material) equipped with 50 ml plastic syringes and the filtrates were diluted to 25 ml with 3-D water in volumetric flasks. The samples were kept in PVC tubes at  $4\text{ }^{\circ}\text{C}$  for subsequent trace element analysis.

Concentrations of trace elements (Ag, As, Cd, Co, Cr, Cs, Cu, Hg, Mn, Ni, Se, V and Zn) were determined using an Inductively Coupled Plasma Mass Spectrometer (ICP-MS) (Perkin–Elmer, Elan DRC Plus). The detection limits of Ag, As, Cd, Co, Cr, Cs, Cu, Hg, Mn, Ni, Se, V and Zn were 0.22, 0.68, 0.04, 0.26, 0.21, 0.26, 0.69, 0.21, 0.44, 0.37, 0.49, 0.52 and 0.57  $\text{ng g}^{-1}$ , respectively. All specimens were analyzed in batches which included a procedural blank. Standard reference material (SRM2977, freeze-dried mussel tissue, National Institute of Standards and Technology, USA) were also analyzed using the same method. A good agreement was found between the data generated and those certified by the SRM. The percentage recoveries ranged from 85% to 118%. All the concentrations were expressed in  $\text{ng g}^{-1}$  wet weight (ww) for easier comparison with other studies and calculation in risk assessment, and were not corrected for recovery.

### 2.3. Risk assessment

Exposure was assessed from plots of the percent cumulative probability against the concentration. The dose-response assessment was carried out using two guideline values namely the Reference Dose (RfD,  $\text{mg kg}^{-1}$  wet weight  $\text{day}^{-1}$ ) commonly used in relation to human health and Toxicity Reference Value (TRV,  $\text{mg kg}^{-1}$  wet weight  $\text{day}^{-1}$ ), used in relation to mammal health. These were used to derive the Maximum Allowable Concentration (MAC) which represents the maximum concentration of each toxicant that can occur in prey items (food taken by dolphin or porpoise) without causing adverse health effects. The MAC based on the RfD ( $\text{MAC}_{\text{RfD}}$ ) is derived using the equation for intake of a chemical

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