



Screening of organic and metal contaminants in Australian humpback dolphins (*Sousa sahulensis*) inhabiting an urbanised embayment



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HIGHLIGHTS

- Australian humpback dolphins are exposed to a variety of pollutants.
- Levels of PCBs and DDXs in blubber were near or above toxicity thresholds.
- PBDEs, PAHs, HCB, organotins, other POPs and 'drins' were not detected or at low levels.
- Element profiles were similar in epidermis compared to other tissues.
- Results useful for prioritizing pollutants for biopsy samples from this population.

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ABSTRACT

As a marine mammal species that inhabits shallow nearshore waters, humpback dolphins are likely exposed to a wide range of pollutants from adjacent land-based activities. Increased mortality rates of Australian humpback dolphins (*Sousa sahulensis*) in waters off a major urbanised centre triggered investigations into the threats to these species, including their contaminant exposure. The present study utilised archived tissues from 6 stranded animals to screen for a range of pollutants (PCDD/Fs, PBDEs, PCBs, organochlorine pesticides, PAHs, organotins, essential and non-essential elements) to inform future biopsy based biomonitoring strategies. Concentrations of PCBs and DDXs in blubber of some of these animals were remarkably high, at levels near or above toxicological thresholds associated with immune- and reproductive toxicity or population declines in other marine mammals. PBDEs, PAHs, HCB, organotins, 'drins' as well as other organic pesticides were not detected, or present at relatively low concentrations. Profiles of elements were similar in epidermis compared to other tissues, and apart from some exceptions (e.g. Fe, Cr, Co, Cu) their concentrations fell within 25th–75th percentiles of cetacean baselines in four of the five animals. Non-essential elements (Al, V, Pb, Ba, Ni, Cd) were notably elevated in one specimen which may have experienced poor health or nutritional status. These data provide a first insight into the contaminant status of a rare and poorly studied population inhabiting an urbanised area. The results highlight a need for future biomonitoring of live populations, and inform on priorities in the typically limited blubber and skin sample volumes obtained through biopsies.

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1. Introduction

Estuarine and nearshore environments are highly productive

and ecologically diverse areas which support a number of threatened or endangered marine mammal species. Apart from being subject to fluctuating environmental conditions, such habitats pose significant challenges to marine mammals due to anthropogenic influences associated with urbanisation, agriculture, industry and shipping activities (Parra et al., 2004). This includes pollutant exposure, which can be considerably higher for species frequenting inshore compared to offshore waters.

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Over the last decades, there have been numerous studies demonstrating elevated exposure of marine mammals to persistent organic pollutants. As long-lived top predators in many aquatic food chains, these animals are prone to accumulate contaminants in their tissues to levels that may affect biological functions. Common pollutants and trace elements such as PCBs (polychlorinated biphenyls), DDXs (total of 6 DDEs, DDDs and DDTs) and other pesticides, PAHs (polycyclic aromatic hydrocarbons) or Hg (mercury) have been suggested to be involved in increased mortality, incidence of diseases and impaired reproduction in beluga whales (Martineau et al., 2002), harbour seals (de Swart et al., 1996; Frouin et al., 2010), striped dolphins (Aguilar and Borrell, 1994), California sea lions (DeLong et al., 1973), ringed seals (Helle et al., 1976), harbour porpoises (Jepson et al., 2005) and bottlenose dolphins (Venn-Watson et al., 2015).

Australian humpback dolphins (AHDs) (*Sousa sahulensis*) are a rare tropical dolphin species that occurs as discrete localised sub-populations in northern Australia. Low population growth rates, high site fidelity and geographic isolation make this species vulnerable to decline (Parra et al., 2004). They are mostly found within 20 km off the coast, but have a preference for shallow, inshore waters and estuaries (Parra et al., 2004). AHDs have only recently been recognised as a species in their own right (Jefferson and Rosenbaum, 2014). To date, however, only limited information exists for robust evaluations of the status and threats to Australian humpback dolphins.

During 2011 to 2014, elevated mortality rates of AHDs were recorded in the densely urbanised region of South East Queensland (Moreton Bay region). During that time, approximately 9–12% ($n = 15$) of the estimated AHD population were reported dead, causing heightened concern regarding the sustainability of the resident population. This sparked a range of investigations, including an ongoing population dynamics study and the current exposure assessment study.

The present study utilised archived tissues (blubber, and where available, liver, kidney and skin) to undertake a comprehensive screening of pollutants in AHDs, covering persistent organic pollutants (PCDD/Fs, PCBs, PBDEs, organochlorine pesticides) as well as other persistent contaminants (PAHs, organotin, essential and non-essential elements). These compounds are known to be highly relevant for marine mammals, both in terms of exposure as well as potential toxicity. The goal was to get an insight into the contaminant status of AHD populations residing in habitats impacted by urbanisation, and to inform prioritisation of chemicals for future biopsy sampling campaigns and biomonitoring efforts.

2. Materials & methods

2.1. Sample information

Blubber ($n = 6$), skin ($n = 5$), liver ($n = 1$), kidney ($n = 1$) and muscle ($n = 4$) tissue samples were collected opportunistically between 2002 and 2014 from 6 Australian humpback dolphins (*S. sahulensis*; 4 males, 2 females) that stranded in the Moreton Bay region of South East Queensland. Information about location, morphometrics and health condition of the individuals is provided in the Supporting Information (Table S1). Tissues were wrapped in aluminium foil which was sealed in plastic zip lock bags and stored frozen at -20°C . Where possible, subsamples for analysis were taken below the top ~ 0.5 – 1 cm surface. Due to limited archived tissue availability, analyses across all chemical groups could only be carried out for three individuals. Based on the initial results, PCBs and PCDD/Fs were analysed also for the remaining specimens, and metals and metalloids were analysed for a total of 5 individuals.

2.2. Analysis

2.2.1. Polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) and polychlorinated biphenyls (PCBs)

All six blubber samples were analysed for the 2,3,7,8-substituted PCDD/Fs and tetra- to heptaCDD/F homologue groups ($\Sigma\text{D/Fs}$), the 12 dioxin-like PCBs (dl-PCBs; coplanar and mono-ortho PCBs to which WHO-TEFs have been assigned) and the 7 non-planar indicator PCBs (Tables S2 and S3). Sample clean-up, quantification and QA/QC are detailed in the SI. Briefly, blubber was extracted using an acid digestion method and lipid content was determined gravimetrically. Sample clean-up was based on isotope dilution US EPA methods 1613 (PCDD/Fs) and 1668A (PCBs). Separation was performed on an Agilent 6890 GC coupled to a Waters Autospec HRMS at mass resolution $R \geq 10,000$ for PCDD/Fs and $R \geq 9,000$ for PCBs. Quantification was carried out over two recorded masses by isotope dilution for all analytes against the added $^{13}\text{C}_{12}$ -labelled quantification standards.

Toxic equivalent concentrations for PCDD/Fs ($\text{TEQ}_{\text{PCDD/F}}$) and DL-PCBs (TEQ_{PCB}) were calculated using 2005 WHO TEFs (mammalian) (Van den Berg et al., 2006) and are reported using lower bound concentrations (i.e. congeners < limit of detection (LOD) were regarded as zero) unless otherwise stated.

2.2.2. Polybrominated diphenylethers (PBDEs)

Muscle ($n = 3$), liver ($n = 1$) and kidney ($n = 1$) were analysed for 24 tri- to nonabrominated DE congeners (Table S4) based on isotope dilution methods modified from a method previously described (Päpke et al., 2004), which is further detailed in the SI together with QA/QC measures. In brief, tissues were cold extracted and lipid content determined gravimetrically. After sample cleanup and purification quantification was performed by GC/MS on a HP 6890 GC coupled with an Agilent 5973 mass selective detector (MSD) using labelled quantification standards.

2.2.3. Organochlorine pesticides (OCPs)

Thirty OCPs, including those listed as POPs under the Stockholm Convention were analysed using isotope dilution methods in 3 blubber samples, as detailed in the SI (text and Table S5). In brief, blubber was cold-extracted and subjected to a multi-step clean-up. A main OCP fraction was eluted separately from endosulfans, which underwent additional clean-up. Quantification of OCPs was performed by HRGC-HRMS on a Thermo Trace GC Ultra coupled to a Thermo DFS high resolution mass spectrometer at mass resolution $R \geq 8000$. Endosulfans were measured on an Agilent 7000C quadrupole HRGC-PCI-MS/MS. Quantification of both groups was carried out by isotope dilution and internal standard method, according to the internal standards added.

2.2.4. Polycyclic aromatic hydrocarbons (PAHs)

The 16 EPA priority polycyclic aromatic hydrocarbons (PAHs) (Table S6) were analysed in 3 blubber samples using isotope dilution methods described in full in the SI. In brief, blubber was soxhlet extracted and cleaned by column chromatography on deactivated silica. Instrumental analysis was performed using HRGC-HRMS on a Thermo Trace GC Ultra coupled to a Thermo DFS high resolution mass spectrometer (HRMS) operated at mass resolution $R \geq 10,000$. Quantification was carried out mainly by isotope dilution.

2.2.5. Organotin compounds (OTCs)

Three blubber samples were analysed for a total of eight OTC compounds (Table S7), following isotope dilution methods further detailed in the SI. In brief, blubber was homogenised and treated with trimethyl ammoniumhydroxide followed by methanol.

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