



Potential association between exposure to legacy persistent organic pollutants and parasitic body burdens in Indo-Pacific finless porpoises from the Pearl River Estuary, China

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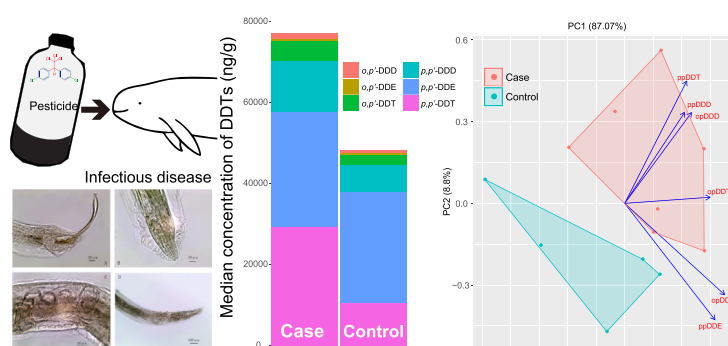
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HIGHLIGHTS

- Σ DDTs concentrations in blubber of *N. phocaenoides* from the Pearl River Estuary are among the highest in cetaceans globally.
- A high prevalence of nematode parasites (mostly lungworms) were found in our samples.
- Only the concentrations of *p,p'*-DDT and DDDs were significantly higher in diseased than in healthy ones.
- Contrasted accumulation pattern of DDTs was found between animals with different health status.
- First time to describe a significant positive correlation between parasitic infections and DDT exposure in cetaceans.

GRAPHICAL ABSTRACT



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ABSTRACT

A high prevalence of infectious diseases (mostly lungworms) is found in finless porpoises (genus *Neophocaena*) in the coastal waters of China, which is one of the most dichlorodiphenyltrichloroethane (DDT)-polluted areas worldwide, while its association with contaminant exposure remains undetermined. To address this gap, we investigated blubber levels of polychlorinated diphenyls (PCBs), organochlorine pesticides (OCPs) and polycyclic aromatic hydrocarbons in Indo-Pacific finless porpoises (*Neophocaena phocaenoides*) stranded in the Pearl River Estuary (PRE) of China. In the post-mortem examinations, lungworms (*Halocercus* species) were found to be the most common parasites, with a high density observed in lungs and bronchi. Severe infections by nematode parasites were also found in the uterus (*Cystidicola* species), intestine (*Anisakis typica*) and muscle (*A. typica*). For all the pollutant compounds analyzed, only the concentrations of *p,p'*-DDT, *p,p'*-dichlorodiphenyldichloroethane (DDD) and *o,p'*-DDD were significantly higher in porpoises died of infectious diseases than in the “healthy” individuals (died from physical trauma). Contrasted accumulation pattern of DDTs and their metabolites was found between animals with different health status. The proportion of *p,p'*-DDT in Σ DDTs was higher than that of *p,p'*-dichlorodiphenyldichloroethylene (DDE) in diseased animals,

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whereas an opposite pattern was shown for “healthy” ones. While this study is the first to describe a significant positive correlation between parasitic diseases and high levels of DDTs in cetaceans, the direction of causality cannot be determined in our data: either a parasitic infection affected the porpoises' ability to metabolize DDTs, resulting in high levels of *p,p'*-DDT in their blubber, or the pollutant burden rendered them more susceptible to parasitic infection.

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1. Introduction

Persistent organic pollutants (POPs) are resistant to environmental degradation, bioaccumulative as they move through food webs and have detrimental effects on biota (United Nations Environment Programme, 2001). Odontocetes (toothed whales, dolphins and porpoises) are prone to accumulate high levels of POPs due to their high trophic-level feeding, large deposits of fat, relatively long life-span and a relatively low capacity to metabolize and excrete POPs (Law, 2014; Tanabe et al., 1994; Aguilar et al., 2002). Currently, alarmingly high levels of legacy POPs are still found in populations inhabiting coastal regions with significant industrial and agricultural development (Jepson and Law, 2016). For example, legacy industrial-use polychlorinated biphenyls (PCBs) showed a static or even increasing trend in European odontocetes in recent years (Jepson et al., 2016). Numerous experimental and epidemiological studies have indicated that PCBs are associated with increases in the risk of cancer and infectious diseases, population declines and low or zero reproduction rates in populations of cetaceans and pinnipeds (Helle et al., 1976; Hall et al., 2006; Hickie et al., 2007; Buckman et al., 2011; Bull et al., 2006; Jepson et al., 2005; Murphy et al., 2015). However, no such relationship has yet been established for organochlorine pesticides (OCPs), e.g., dichlorodiphenyltrichloroethanes (DDTs), even though DDTs have higher reproductive transfer rates than PCBs, (Borrell et al., 1995; Mckenzie et al., 1996) causing serious toxic effects to the newborns. DDTs are still in use in certain regions of developing countries, such as South Africa and China, mainly for malaria control and dicofol production, respectively (Van den Berg, 2009). Our previous studies have shown that DDT levels in cetaceans from these regions are high enough to cause concern (Gui et al., 2014, 2016).

Indo-Pacific finless porpoise (*Neophocaena phocaenoides*) is one of the two residential cetacean species in the Pearl River Estuary (PRE) (Jefferson et al., 2002a). The PRE is reported to be one of the most DDT-polluted areas in the world (Fu et al., 2003; Ying et al., 2009). Sources of DDTs in the PRE include the river discharge of technical DDTs and dicofol from agricultural areas, as well as emissions from anti-fouling paints on ships in PRE coastal waters (Guo et al., 2008; Qiu et al., 2005). *Neophocaena phocaenoides* is recently included in Appendix I of the Convention on International Trade in Endangered Species (CITES) (<https://www.cites.org/eng/app/appendices.php>) and is identified as “vulnerable” by IUCN (Wang and Reeves, 2017). The highest number of finless porpoises sighted per year in the PRE was 260 individuals during the period 2013–2014, which is consequently the minimum estimate for the size of this population (Jefferson et al., 2002a). However, there were on average approximately 30 cases of dead finless porpoise strandings that occurred in HK waters each year during the period 2004–2013, (http://www.afcd.gov.hk/english/conservation/con_mar/con_mar_fin/con_mar_fin_fin/con_mar_fin_fin_rep_from.html) which raises serious concern about their survival and health. It has long been suspected that the apparent high ratio of mortality and diseases seen among the newborn finless porpoises and Indo-Pacific humpback dolphins (*Sousa chinensis*) from the PRE are linked to the high levels of pollutants found in their tissues, particularly DDTs (Gui et al., 2014, 2017; Ramu et al., 2005; Yeung et al., 2009; Chan, 1998; Minh et al., 1999; Lam et al., 2009; Jefferson et al., 2002b; Jefferson et al., 2006; Parsons, 2004).

Our objective was to investigate the relationship between exposures to legacy POPs, including PCBs (21 congeners), 10 groups of OCPs and 16

United States Environmental Protection Agency (USEPA) priority polycyclic aromatic hydrocarbons (PAHs) and postmortem findings of infectious diseases in the Indo-Pacific finless porpoises that were stranded and necropsied from the PRE between 2007 and 2016. Significant and positive correlation between percentages of DDT metabolites and total DDT burden in whales suggests intensification of the dehydrochlorinative and differential excretive functions at higher pollutant levels (Borrell and Aguilar, 1987). Thus, we also aim to examine variations of dichlorodiphenyldichloroethylene (DDE) proportion between the diseased and “healthy” finless porpoises to provide insights into the influence of diseases on pollutant metabolism.

2. Materials and methods

2.1. Sample collection and post-mortem examination

Of the nearly 30 *N. phocaenoides* strand along the shore of the PRE from Zhuhai to Jiangmen between 2007 and 2016, 19 were necropsied and sampled for blubber tissues (Fig. 1) within the collaborative cetacean stranding network program run by the Pearl River Estuary Chinese White Dolphin Reserve, Jiangmen Guangdong Chinese White Dolphin Provincial Nature Reserve and Sun Yat-Sen University. In addition, three *N. phocaenoides* and one narrow-ridged finless porpoise (*Neophocaena asiaorientalis*) were collected from the adjacent regions of the PRE along the shore of China (Table 1). Gross post-mortem examinations were carried out on all samples, using the protocols described in Law et al. (2006). When nematodes were found, we counted their number, measured their size, and identified to species using both morphological and genetic methods (detailed in the Supporting Information). Histopathological examination was carried out on representative individuals which are freshly dead (code 2) or early moderately decomposed (code 3) ($n = 14$) to assist in cause of death analysis. Infected lungs were examined visually for worms and histologically for lesions consistent with verminous pneumonia. Tissues were fixed in 10% neutral buffered formalin and submitted to the Sun Yat-sen University Histopathology Center for evaluation. Sex was determined by observing the reproductive organs, and if sex could not be determined in the field, it was determined by DNA analysis. Age was estimated from the growth layer groups in the dentine of teeth (Jefferson, 2000; Myrick et al., 1983). The sexual maturity of the animals was determined by examining the reproductive organs. If it could not be determined in the field, body length was used to estimate the age class. The adult porpoises were categorized as either females with body lengths 137 cm and longer or males with body lengths 138 cm and longer (Jefferson et al., 2002c). The maturity and sex composition were Juvenile Females (JF = 7, 30.4%), Juvenile Males (JM = 5, 21.7%), Adult Females (AF = 4, 17.4%), and Adult Males (AM = 7, 30.4%). Organs were packed in clean plastic storage bags and frozen at $-20\text{ }^{\circ}\text{C}$ for further analysis.

2.2. Chemical analysis

The analyzed compounds were 21 PCB congeners (CB28, CB37, CB52, CB77, CB81, CB101, CB105, CB114, CB118, CB123, CB126, CB153, CB138, CB128, CB156, CB157, CB167, CB169, CB180, CB188 and CB209); Σ DDTs (dichlorodiphenyltrichloroethanes, *o,p'*-DDT, *p,p'*-DDT,

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