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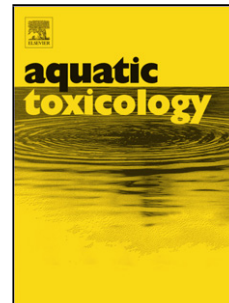
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Supporting evidence for PCB pollution threatening global killer whale population

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In a recent Science report, Desforges et al. (2018) predicted the global killer whale population to collapse due to pollution with polychlorinated biphenyls. Using an individual-based model framework and globally available data on PCB concentrations in killer whale tissues, they showed that PCB-mediated effects on reproduction and immune function threaten the long-term viability of >50% of the world's killer whale populations. Here, we present empirical evidence, which supports and extends the reports' statement.

On 8 February 2016, a neonate male killer whale stranded on the beach of Rantum, German island of Sylt (Figure 1). Killer whale strandings are rare and post-mortem examinations on fresh individuals are scarce. This stranding probably marks the ninth recorded killer whale stranding along German coasts since the mid-1800s (Reckendorf et al., 2018). The carcass was completely necropsied approximately 10 h after collection and was examined according to standard protocol (Siebert et al., 2001). Total length, body weight, different body measurements, girths and blubber thickness (at nine locations along the left flank: dorsal, medial and ventral behind the flipper, in front of as well as behind the dorsal fin) were recorded. The stomach contained ~20 mL milk residue and no pathologies explaining the cause of death could be detected (Reckendorf et al., 2018). Blubber samples (n=3) presenting low lipid concentrations [16-22%] were analysed for a suite of persistent organic pollutants, such as

PCBs (28 congeners), polybrominated diphenyl ethers (PBDEs) and Dichlorodiphenyltrichloroethane and metabolites (DDTs). The method used for sample extraction and clean-up has been previously described by Das et al., 2017. Liver samples were analysed for mercury following the method described by Cransveld et al., 2017. Skin samples were collected caudal to the dorsal fin for genotyping of the mitochondrial control region using established standard odontocete primers ProL and DLH (Tiedemann et al., 1996; Wiemann et al., 2010). For the analysed part of the mtDNA, many sequences from killer whales around the world are available in genetic databases. Hence, such genetic analyses can provide insight into the putative origin of the individuals, enabling them to be assigned to a certain population/ecotype (Foote et al., 2009; Morin et al., 2010).



Figure 1. Picture of the neonate male killer whale stranded on 8 February 2016 on the beach of Rantum, on the German island of Sylt.

Neonatal attributes including colouration, freshly healing umbilicus, distinct neonatal papillae on the tongues and partly erupted teeth were found and indicated an age of at least 3 days (Reckendorf et al., 2018). The blubber PCB concentrations were very high [Σ PCBs, average 225 mg/kg lipid weight (lw)], largely exceeding the PCB toxicity thresholds reported for the onset of immunosuppression [9 mg/kg lw Σ PCB] (Kannan et al., 2000) and for severe reproductive impairment [41 mg/kg lw Σ PCB] (Helle et al., 1976) in marine mammals (Jepson et al., 2016) (Figure 2).

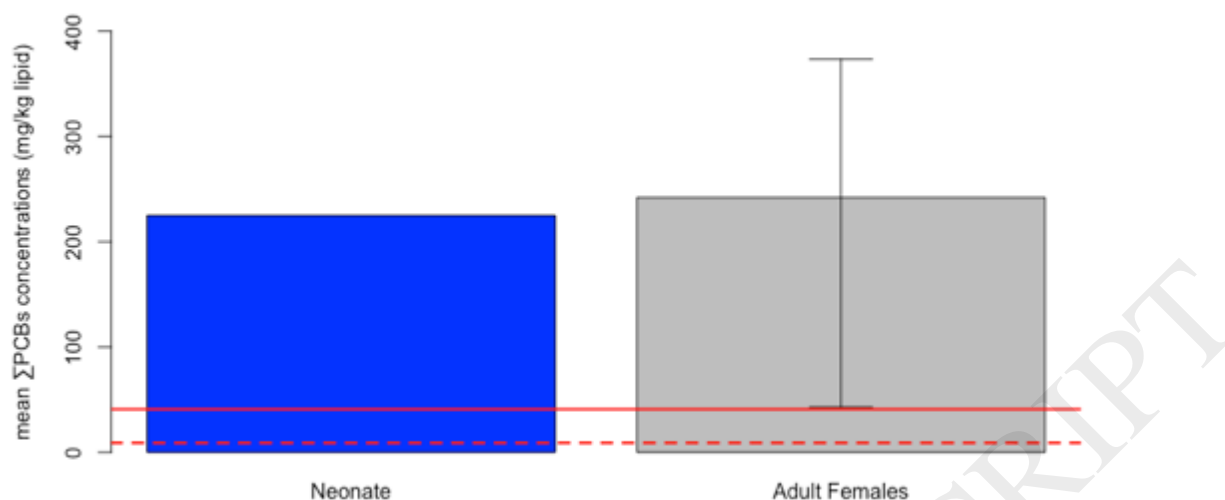


Figure 2. Mean Σ PCBs concentrations in neonate and adult female killer whales (from Jepson *et al.* 2016). The blue bar represents the neonate and the grey bar are adult females. The red dashed line is the equivalent Σ PCBs concentrations threshold (9.0 mg/kg lipid) for onset of physiological effects in experimental marine mammal studies (Kannan *et al.*, 2000). The red solid line is the equivalent Σ PCBs concentrations threshold (41.0 mg/kg lipid) for the highest PCB toxicity threshold published for marine mammals based on marked reproductive impairment in ringed seals in the Baltic Sea (Helle *et al.*, 1976). Error bars = LowerCI and UpperCI.

The study of Desforbes *et al.* focuses on a single chemical class (PCBs), but a large number of additional contaminants are present in killer whale tissues. This individual showed equally high concentrations in p,p' -DDE [average 226 mg/kg lw]. In a previous study, adult killer whales from British and Irish coastal waters showed lower p,p' -DDE concentrations [ranging from 7 to 179 mg/kg lw], except one individual that presented a higher p,p' -DDE concentration of 567 mg/kg lw (McHugh *et al.*, 2007). A number of contaminant studies on marine mammals have determined the p,p' -DDE/ Σ DDT ratio in order to assess the chronology of DDT inputs and state that a ratio > 0.6 is indicative of a stable system with no new DDT inputs (Aguilar, 1984; Tanabe *et al.*, 1997). In the current study, our neonate presented a p,p' -DDE/ Σ DDT ratio of 0.98, suggesting that DDT residues are derived from historic contamination. However, several studies in delphinid species have shown higher transfer rates of p,p' -DDE from mother to calf during lactation than for p,p' -DDT or Σ DDT, which might potentially alter the observed ratio (Aguilar, 1984; Borrell *et al.*, 1995; McKenzie *et al.*, 1997).

Polybrominated diphenyl ethers (PBDEs) are a group of flame-retardant compounds which have been widely used in recent years. Compared to other orcas from UK waters, elevated levels of Σ PBDEs [average 5 mg/kg lw] were found in this individual. In a previous study,

four adult killer whales showed lower Σ PBDE concentrations, [ranging from 0.69 to 4.37 mg/kg lw], while one individual presented a higher Σ PBDE concentration [of 23.8 mg/kg lw] (Law et al., 2005).

Environmental mercury (Hg) concentrations have increased over the past 150 years, resulting in over 92% of the mercury body burden in higher trophic level species being of man-made origin (Dietz et al., 2013). Total mercury (THg) analysed in liver samples of the neonate [1.1 μ g/g dry weight dw] revealed concentrations below the threshold [20 μ g/g dw] defined for liver lesion in adult marine mammals (Dietz et al., 2013; Rawson et al., 1993), but close to the mercury-associated neurochemical effect threshold in the 3 to 5 μ g/g dw range (Basu et al., 2007, 2006; Dietz et al., 2013). More subtle changes were observed at lower Hg concentrations in the brain of toothed whales with associated signs of neurochemical effects. The meaning of such a high Hg concentration in the tissue of a neonate is difficult to interpret, but methyl-Hg is immunotoxic and neurotoxic even at low doses (Basu et al., 2007, 2006; Dietz et al., 2013).

All these compounds may contribute to reproductive and immune failure or other health endpoints not included here, and raise concerns about the potential for other persistent contaminants to generate additional toxicological effects in long-lived, high-trophic level aquatic species. These results suggest a high placental transfer of pollutants from mother to foetus. Consequently, blubber and plasma PCB concentrations and calf mortality rates are both high in primiparous females (Schwacke et al., 2002; Wells et al., 2005). With such high pollutant levels, this neonate had poor prerequisites for survival. The neonate belonged to Ecotype I (generalist feeder) and carried the mitochondrial haplotype 35 present in about 16% of the North Atlantic killer whales from or close to the North Sea (*i.e.*, the UK, the Netherlands, Denmark) (Foote et al., 2009; Reckendorf et al., 2018). The relevance of this data becomes apparent in the UK West Coast Community, the UK's only resident orca population, which is currently composed of only eight individuals (each four males and females) and no calves have been reported over the last 19 years (Beck et al., 2014).

Despite worldwide regulations, marine pollutants, including mercury, organochlorine pesticides and PCBs, persist in the environment and remain a severe concern for killer whale populations, placing calves at high risk due to the vertical mother-offspring pollutant-transfer resulting in a high toxicological burden of the neonates.

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