PERSISTENT ORGANIC POLLUTANTS IN HUMPBACK DOLPHINS FROM NEARSHORE AND ESTUARINE ENVIRONMENTS IN QUEENSLAND, AUSTRALIA

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Introduction

Australian humpback dolphins (*Sousa sahulensis*) are a small delphinid species that occur in nearshore and estuarine habitats in the northern half of Australia. This species has long been classified as the Indo-Pacific humpback dolphin (*Sousa chinensis*), but was in 2014 formally identified as a separate species[1]. Current estimates for discrete populations in Queensland range from approximately 60 (Townsville) to 250 (Capricorn Curtis Coast and Shoalwater Bay) individuals[2], suggesting they are rare and likely threatened. In recent years, elevated mortality rates have been observed in southeast Queensland (Moreton Bay region) where 15 dead individuals (out of a population of ~120) were reported from 2011 to 2014. This has highlighted the urgent need for studies that improve understanding on the status and threats to this poorly studied species.

Even though nearshore and estuarine environments are rich in nutrients, they pose great challenges in terms of pollution. It has been shown already that levels of persistent organic pollutants (POPs) in marine mammals are often higher in species from estuarine and coastal environments compared to species from open waters[3]. Furthermore, studies have shown toxic impacts of several pollutant classes, such as PCBs (polychlorinated biphenyls), pesticides (DDT and isomers/metabolites) and PCDD/Fs (polychlorinated dibenzo-*p*-dioxins/furans), on the immune, endocrine and reproductive systems of various marine mammal species[4-8]. These adverse effects, combined with the nearshore habitat of the humpback dolphins, the elevated mortality rate for this species in recent years and the lack of toxicological information for these animals, have triggered an investigation on the humpback dolphins in southeast Queensland. As part of this investigation, it was intended to also get an understanding on contaminant exposure and potential risks. For the latter, archived samples from stranded specimens were used to analyse a range of persistent organic pollutants (POPs), metals and other bioaccumulative compounds. Here, the results of PCBs, DDXs, PCDD/Fs in blubber tissue are discussed.

Materials and methods

Samples. Archived blubber samples of six humpback dolphins were available (4 males, 2 females). PCBs, PCDDs and PCDFs were investigated in all 6 samples while DDXs were investigated only in 3 samples due to limited sample volume.

Lipid extraction. Lipid was extracted from blubber tissue using a previously described method[9]. In brief, ~8 g of blubber and 40 mL of 4 M HCl was heated at 70-80˚C for 3-4 hours and liquid-liquid extracted with 100 mL hexane and 150 mL warm water, followed by double extractions with a mix of 50 mL of hexane and 100 mL water. The hexane fractions were filtered through sodium sulphate, concentrated until stable weight was achieved and the percent lipid was determined gravimetrically.

PCDD/F and PCB analysis. The samples were analysed for all 2,3,7,8- substituted PCDD/Fs as well as sum homologue concentrations, all 12 dioxin-like PCBs and the 7 indicator PCBs (PCB 28, 52, 101, 118, 138, 153, 180) based on improved US EPA methods 1613B and 1668C, respectively. An aliquot of the lipid extract (1.4-1.5 g) was taken from each sample and spiked with $^{13}C_{12}$-labelled PCDD/Fs and PCBs. The clean-up consisted of a mixed silica column with silica gel/44 % H_2SO_4 conc. and pure silica followed by fractionation on an alumina column (basic, activity super I) with elution of PCB using toluene/hexane and elution of PCDD/Fs taking place with hexane:DCM (1:1, v:v)). PCDD/F fractions were further cleaned with florisil (3% water deactivation). The fractions were evaporated and a set of $^{13}C_{12}$-labelled PCDD/Fs and PCBs, respectively, were added as injection standards. Analytical measurement of PCDD/Fs (with totals) and PCBs was performed by HRGC/HRMS on a Waters Autospec Premier HRMS at mass resolution R≥10,000 equipped with a VFXms/SLB5ms column (60 m × 0.25 mm i.d. × 0.25 µm dF). Quantification was carried out by isotope dilution against daily calibration points together with a multipoint calibration. For quality control, method blanks were run with each sample batch to monitor for background contamination. Reference materials (pooled samples) are regularly monitored.

DDX analysis. Blubber samples were spiked with quantification standards (^{13}C-labeled β-HCH, γ-HCH, *p,p´*-DDT, *p,p´*-DDE, pentachlorobenzene, hexachlorobenzene, endosulfan sulfate, β-endosulfan and dieldrin). Clean up was performed by column chromatography including basic alumina and Florosil. Hexane was used for elution of the main fraction, evaporated and spiked with ^{13}C-PCB 105 as an injection standard. Analyses was performed by HRGC-HRMS on a Thermo DFS at mass resolution R≥8000 on a DB5-type fused silica column (60 m × 0.32 mm i.d. × 0.25 µm dF). Quantification was carried out by isotope dilution and internal standard methods against daily calibration points, together with a multipoint calibration.
TEQs. Toxic equivalencies (TEQs) for 2,3,7,8-substituted PCDD/Fs and dioxin-like PCBs were calculated using mammalian toxic equivalency factors (TEFs) adopted by the World Health Organisation[10].

Results and discussion:
Levels. The concentrations of ΣPCBs (i.e. sum concentration of dioxin-like and indicator congeners) in the six analysed blubber samples were highly variable, ranging from 1,600 to 370,000 ng g^{-1} lipid weight (lw). These levels are generally higher compared to ΣPCB concentrations reported in previous studies on humpback dolphins, which include specimens from Queensland[11], Hong Kong and China[12-15] (Table 1). It is, however, important to note that these comparisons should be interpreted with caution because of the relatively small sample numbers as well as the different number and type of PCB congeners included in the ∑PCBs across all studies. To our knowledge, the maximum ΣPCB concentrations quantified here were among the highest recorded for any marine mammal worldwide. For example, Ross et al[16] found the highest levels of sum PCBs in male transient killer whales (*Orcinus orca*; 250,000 ± 55,000 ng g^{-1} lw) and reported that PCB 153 was on average 26% of the sum, giving a value of 64,000 ng g^{-1} lw for PCB 153. In the present study, the concentration of PCB 153 in #0756 was 46% of the sum of all PCBs which is equivalent to 170,000 µg g^{-1} lw. This is more than 2.5 times higher than the levels found by Ross et al[16] which were measured in one of the most contaminated species in the world.

In contrast to PCBs, concentrations of ΣPCDD/Fs (i.e. sum concentration of all congeners; 0.2 – 2.5 ng g^{-1} lw for PCDDs and <0.01 – 0.52 ng g^{-1} lw for PCDFs; Table 1) were within the range expected for marine mammals in Queensland (for TEQ values, see below). While there is only one previous study that investigated PCDD/Fs in *Sousa* species (PCDD levels of 0.13 ng g^{-1} lw and PCDF levels of 0.013 ng g^{-1} lw; n=1[17]), the ΣPCDD/Fs concentrations are also within the range of those observed in other nearshore marine mammals, such as for example dugongs[9].

Table 1. Minimum-maximum ranges (ng g^{-1} lw) of POPs in Australian (grey shaded) and Indo-pacific humpback dolphins from different regions. Levels in wet weight were re-calculated to lipid weight by using lipid percentages from the respective study or by using an average lipid percentage of 31% (based on data from Minh et al[12], Ramu et al[13], Wu et al[18]). na = not analysed.

Reference	n	Location	∑ PCBs	∑ DDXs	∑ PCDDs	∑ PCDFs
This study	6	Australia	1,600-370,000	1,800-17,000	0.2-2.5	<0.01-0.52
11	18	Australia	800-94,000	310-6,200	na	na
17	1	Australia	na	na	0.13	<0.02
18	15	China	1,000-86,000	7,200-670,000	na	na
12	11	China	6,100-160,000	9,400-200,000	na	na
19	45	China	3,800 ± 6,200	130,000 ± 120,000	na	na
14	10	China	160-130,000	29,000-190,000	na	na
15	7	China	9,400-83,000	51,000-470,000	na	na
15	2	India	1,400-2,600	66,000-84,000	na	na
13	15	China	2,800-83,000	19,000-470,000	na	na

The maximum DDXs levels in the present study were more than twice as high as in humpback dolphins from central Queensland[11] (Table 1), but an order of magnitude lower than the concentrations measured in tissues of humpback dolphins from China[12-15,18,19] (Table 1). The higher DDX levels in animals from southeast Queensland compared to central Queensland can be due to the residence adjacent to a major urban centre in southeast Queensland. Since humpback dolphins feed mostly in estuarine and inshore waters, they are likely to have higher exposure to contaminants compared to dolphins from a similar trophic level but with much wider foraging grounds such as common dolphins (*Delphinus delphis*) or Indo-Pacific bottlenose dolphins (*Tursiops aduncus*).

Profiles. In all humpback dolphins analysed for the present study, PCB 153 contributed the most to the ΣPCBs, with percentages ranging from 36 to 48%, followed by either PCB 138 or PCB 180. Although only few samples were available for robust temporal or other comparisons, there was no apparent difference in PCB profiles among samples from male or female specimens, nor across the 12 years they were collected. Among PCDD/Fs, PCDDs dominated the profiles while most PCDFs were near or below the limit of detection. This is typical for Australian samples across environmental matrices including soil, sediment and marine biota as well as humans [20]. Similar to PCDD profiles in other biota from Australia, OCDD contributed the highest proportions (68 to 86%) to the ΣPCDDs with decreasing contributions towards TCDDs (< 1 – 5 %). The origin of this contamination has been debated by various studies, and most recent investigations suggest a significant contribution from historical, as well as to some degree currently used, pesticides[21], in combination with photolytic or other fate processes[21,22]. Interestingly, with the exception of one individual, the proportions of TCDDs in humpback dolphins seemed to decrease from 2002 to 2014 in favor of OCDD. More samples would need to be available, however, to confirm or evaluate such a trend.

Among DDXs, *p,p'*-DDE was the most dominant compound in two out of three samples analysed for DDX. This is a common pattern in several marine mammal species worldwide that are foraging distant to any DDT point sources[23]. In contrast, *p,p'*-DDD had the highest levels of all DDXs in the remaining sample. Again, the limited sample number and unknown feeding grounds of the stranded animals analysed here, makes it difficult to interpret these results. Overall, these results do, however, suggest that exposure to POPs, and possibly point-source exposure, may be of concern to some individuals (or pods) in southeast Queensland waters. Analysis of biopsies from different pods (with different feeding grounds) would be warranted to evaluate any spatial trends and potential threats in more detail.

Impact? Despite the low sample numbers, we can tentatively compare the toxic equivalencies (TEQs) for 2,3,7,8-substituted PCDD/Fs and dioxin-like PCBs as well as compare the concentrations found in the present study with various effect levels for marine mammals reported in the literature. Sum TEQ levels ranged from 32 to 1,300 pg g^{-1} lw and dioxin-like PCBs contributed the greatest proportion (53 – 98 %) to the total TEQ in all individuals, which is consistent with results from a previous study across a range of Australian marine mammal species[17].

Immunotoxicity in harbour seals fed herring from the Baltic Sea was observed for animals with blubber TEQs of 286 ± 17 pg g^{-1} lw[24]. Using this value as a threshold, the results of the present study suggest that the normal functioning of the immune system was compromised in at least 2 out of 6 humpback dolphins (Fig 1A). The same conclusion can be drawn when comparing PCB and DDX levels to toxic endpoints observed in marine mammal species worldwide (Fig 1B), indicating that some individuals from the humpback dolphin population in southeastern Queensland may experience adverse effects on their reproductive and immune systems, thereby compromising their survival and overall wellbeing. This is of particular concern in species that are already rare and, due to their habitat, exposed to numerous anthropogenic stressors.

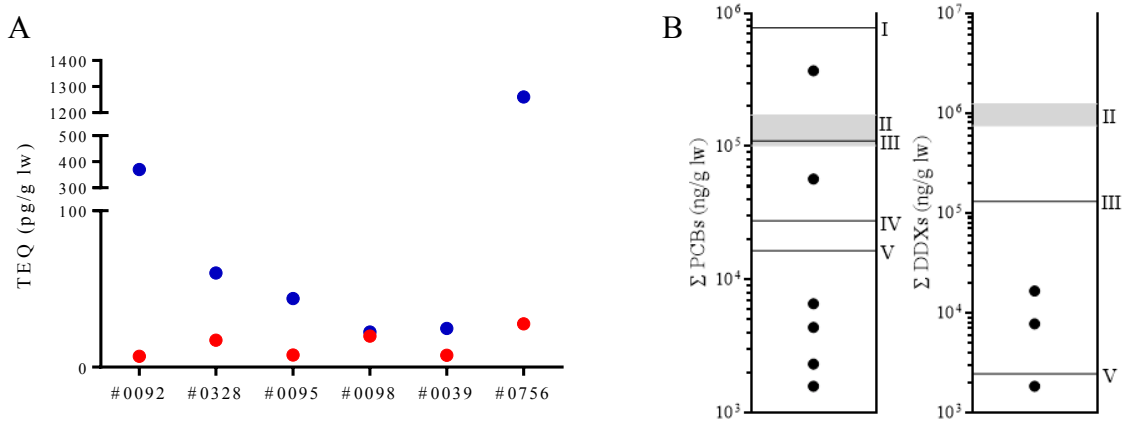

Fig 1. Assessment of toxicity in Australian humpback dolphins (n = 6) by using TEQ values (● for dioxin-like PCBs, ● for 2,3,7,8-PCDD/Fs) (A) and toxicity endpoints (B). All endpoints are based on concentrations in blubber. I) Epizootic, diseased striped dolphins[4], II) Premature pupping in California sea lions[5], III) Impaired reproduction in ringed seals[6], IV) Infectious diseases in harbour porpoises[7], V) Immunotoxicity in harbour seals[8]. ● = humpback dolphin, present study.

Despite being recognised as a priority for conservation, very little is known of the impact of pollution on the health of Australian humpback dolphins. In part, this has been because of the logistics involved in salvaging carcasses and the fact that they are often too decomposed by the time the carcass can be reached. Humpback dolphins are also often misidentified in the first instance as another dolphin species. With sufficient sample numbers and reasonable carcass conditions, stranded animals could, however, provide valuable information to inform species conservation efforts.

Acknowledgements:
The authors wish to acknowledge funding from the Australian Marine Mammal Centre (AMMC) used for sample analysis.

References:

1. Jefferson, T.A., Rosenbaum, H.C. (2014) *Marine Mammal Science* 30, 1494-1541.
2. Cagnazzi, D. (2013) Report, Gladstone Port Corporation. 53pp.
3. Dorneles, P.R., Lailson-Brito, J., Dirtu, A.C., Weijs, L., Azevedo, A.F., Torres, J.P.M., Malm, O., Neels, H., Blust, R., Das, K., Covaci, A. (2010) *Environment International* 36, 60-67.
4. Aguilar, A., Borrell, A. (1994) *The Science of the Total Environment* 154, 237-247.
5. DeLong, R.L., Gilmartin, W.G., Simpson, J.G. (1973) *Science* 181, 1169-1170.
6. Helle, E., Olsson, M., Jensen, S. (1976) *Ambio* 5, 261-262.
7. Jepson, P.D., Bennett, P.M., Deaville, R., Allchin, C.R., Baker, J.R., Law, R.J. (2005) *Environmental Toxicology and Chemistry* 24, 238-248.
8. De Swart, R.L., Ross, P.S., Vos, J.G., Osterhaus, A.D.M.E. (1996) *Environmental Health Perspectives* 104, 823-828.
9. Jin, L., Gaus, C., Escher, B.I. (2015) *Environmental Science & Technology*. DOI: 10.1021/acs.est.5b00947
10. Van den Berg, M., Birnbaum, L., Denison, M., De Vito, M., Farland, W., Feeley, M., Fiedler, H., Hakansson, H., Hanberg, A., Haws, L., Rose, M., Safe, S., Schrenk, D., Tohyama, C., Tritscher, A., Tuomisto, J., Tysklind, M., Walker, N., Peterson, R.E. (2006) *Toxicological Sciences* 93, 223-241.
11. Cagnazzi, D., Fossi, M.C., Parra, G.J., Harrison, P.L., Maltese, S., Coppola, D., Soccodato, A., Bent, M., Marsili, L. (2013) *Environmental Pollution* 182, 490-494.
12. Minh, T.B., Watanabe, M., Nakata, H., Tanabe, S., Jefferson, T.A. (1999) *Marine Pollution Bulletin* 39, 383-392.
13. Ramu, K., Kajiwara, N., Tanabe, S., Lam, P.K.S., Jefferson, T.A. (2005) *Marine Pollution Bulletin* 51, 669-676.
14. Leung, C.C.M., Jefferson, T.A., Hung, S.K., Zheng, G.J., Yeung, L.W.Y., Richardson, B.J., Lam, P.K.S. (2005) *Marine Pollution Bulletin* 50, 1713-1744.
15. Kajiwara, N., Kamikawa, S., Ramu, K., Ueno, D., Yamada, T.K., Subramanian, A., Lam, P.K.S., Jefferson, T.A., Prudente, M., Chung, K.H., Tanabe, S. (2006) *Chemosphere* 64, 287-295.
16. Ross, P.S., Ellis, G.M., Ikonomou, M.G., Barrett-Lennard, L.G., Addison, R.F. (2000) *Marine Pollution Bulletin* 40, 504-515.
17. Gaus, C., Correll, R., Mueller, J., Holt, E., Ellis, D., Prange, J., Shaw, M., Bauer, U., Symons, R., Burniston, D. (2005) *Organohalogen Compounds* 67, 1271-1275.
18. Wu, Y., Shi, J., Zheng, G.J., Li, P., Liang, B., Chen, T., Wu, Y., Liu, W. (2013) *Science of the Total Environment* 444, 423-429.
19. Gui, D., Yu, R., He, X., Tu, Q., Chen, L., Wu, Y. (2014) *Chemosphere* 114, 106-113.
20. Haynes, D., Muller, J.F., McLachlan, M.S. (1999) *Chemosphere* 38, 255-262.
21. Holt, E., Weber, R., Stevenson, G., Gaus, C. (2012) *Chemosphere* 88, 364-370.
22. Camenzuli, L., Scheringer, M., Gaus, C., Ng, C.A., Hungerbuhler, K. (2012) *Organohalogen Compounds* 74, 363-366.
23. Hellou, J., Lebeuf, M., Rudi, M. (2013) *Environmental Reviews* 21, 53-69.
24. Kannan, K., Blankenship, A.L., Jones, P.D., Giesy, J.P. (2000) Human and Ecological Risk Assessment 6, 181-201.