IS THERE A CONNECTION BETWEEN POLLUTION, MASS-STRANDINGS AND PILOT WHALES FROM AUSTRALIA?

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Introduction

As already shown in several studies, pollution can have an impact on the health as well as on the survival of marine mammals[1,2]. Because of their long life spans and high position in marine food webs, these animals are exposed to elevated pollutant concentrations and their toxic effects. In Tasmania, Australia, pilot whale (*Globicephala melas*) mass stranding events are more frequent than in other places (DPIPWE Unpublished) and several plausible explanations have been explored so far. In such events, animals appear to be disorientated or ill which can have both natural (e.g. escaping from predators, anomalous magnetic fields) or anthropogenic (e.g. military sonar exercises, pollution) causes. Previous studies have suggested that pollution can lead to impaired immune systems in marine mammals, thereby leading to illnesses[2]. However, little information is available regarding the levels of various pollutant classes in any tissue of Australian pilot whales or the impact of these levels on the incidence of mass strandings. The objective of the present study is therefore to investigate the bioaccumulation of persistent organic pollutants (POPs) in Australian pilot whales and its potential relationship with the occurrence of mass stranding events.

Materials and methods

Samples, chemicals and target compounds. Blubber samples were collected from 55 pilot whales (*Globicephala melas;* one fetus, 9 lactating females, 24 non-lactating females, 21 males). Body sizes were recorded for all animals, except for the fetus and its mother. The age of the animals could not be assessed through counting dentine layers, but was estimated via the recorded body size of each animal and the growth equations for long-finned pilot whales from Bloch et al.[3]. The animals were divided in groups according to their estimated age, gender and lactation status (for females). All animals were victims of the mass-stranding at Sandy Cape, Tasmania on December 14, 2008. In all samples, 37 PCB congeners, 6 PBDEs, 6 DDXs, HCB, chlordanes (CHLs) and 5 MeO-PBDEs were investigated.

Sample preparation and analysis. The method used for the sample extraction and clean-up has been previously described[4] and is briefly presented below. Approximately 0.2 g of blubber was spiked with internal standards BDE 77, BDE 128 and CB 143 and extracted by hot Soxhlet for 2h with hexane/acetone (3/1; *v/v*). After lipid determination (performed on an aliquot of the extract), the extract was cleaned on 8 g of acidified silica and analytes eluted with 20 ml hexane and 15 ml dichloromethane. The cleaned extract was evaporated to dryness and reconstituted in 150 µl iso-octane. PBDEs, MeO-PBDEs and CHLs were measured by GC-ECNI/MS (gas chromatography-electron capture negative ion/mass spectrometry) on a 30m x 0.25mm x 0.25µm DB-5 column by monitoring ions *m/z* = 79 and 81 (for PBDEs and MeO-PBDEs) and 2 specific ions for each CHL. PCBs and DDXs were measured by GC-EI/MS (gas chromatography-electron ionization/mass spectrometry) on a 25m x 0.22mm x 0.25µm HT-8 column by monitoring 2 ions for each homologue group. This system was also used to confirm MeO-PBDEs.

Quality assurance/quality control (QA/QC). Recoveries for individual PCB and PBDE congeners ranged between 75 and 104 % (RSD < 12 %). For each analyte, the mean procedural blank value was used for subtraction. After blank subtraction, the limit of quantification (LOQ) was set at 3 times the standard deviation of the procedural blank. For analytes that were not detected in procedural blanks, LOQs were calculated for a ratio S/N equal to 10. LOQs depended on the sample intake and on the analyte and ranged between 1 and 4 ng/g lipid weight (lw). QC was performed by regular analyses of procedural blanks, by random injection of standards and solvent blanks. A standard reference material SRM 1945 (PCBs, OCPs and PBDEs in whale blubber) was used to test the method accuracy. Obtained values did not deviate more than 10 % from the certified values.

Statistical analysis. Statistical analyses were conducted using the SPSS 18.0 statistical package (PASW Statistics 18). The level of statistical significance was defined at $p < 0.05$. For compounds that were detected in more than 50% of the samples, concentrations < LOQ were replaced by a value of f (detection of frequency) * LOQ. Groups with samples sizes of 1 (1 fetus, 1 lactating sexually immature female) were not included in the statistical analysis. Results were log-transformed in order to fit a normal distribution. The parametric ANOVA test was used to test the differences in lipid percentages and pollutant concentrations between the groups.

Results and discussion:

PCB congener 18 was not detected in any sample, whereas PCB 28, PCB 47, PCB 44, PCB 209, TC (trans-chlordane) and BDE 28 were detected in less than 50% of the samples. These compounds were therefore not discussed or used in any further calculation. The lipid percentage of the blubber in the present study averaged 84.2 ± 4.0 % and ranged from 73.7 % for the fetus to 96.9 % for a non-lactating adult female. The results for the fetus and the lactating sexually immature female could not be tested statistically due to low sample size (n = 1), but there were no statistically significant differences in lipid percentage of the blubber between all other groups.

Overall contamination profile

Fig 1 shows the overall contamination profile in all age-gender groups of the pilot whales from the present study. In all groups, DDXs represented the greatest proportion of the overall contamination profile, whereas the proportions of PCBs (sum of tetra to nona) and of MeO-PBDEs were often comparable (Fig 1). Contributions of PBDEs to the overall profile were < 1% while the percentages of CHL remained similar (5.7- 6.3%) (Fig 1).

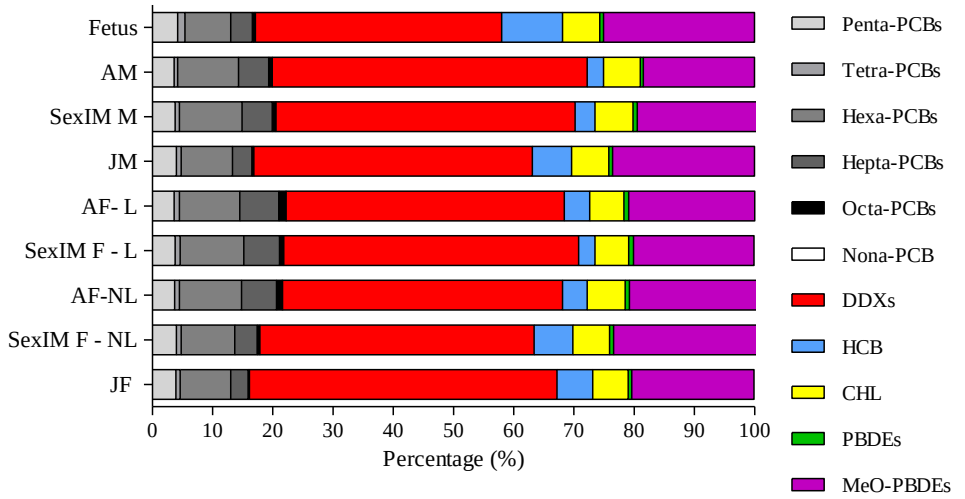

Figure 1. Percentages of PCBs, PBDEs, MeO-PBDEs, DDXs, HCB and CHLs in blubber of 55 pilot whales from Sandy Cape, Australia. SexIM-sexually immature, A-adult, F-female, M-male, J-juvenile, NL-non-lactating, L-lactating.

Adult females (including the lactating sexually immature female), had similar profiles regardless of whether they were lactating or not. The estimated ages for the adult females ranged from 7.5 to > 30 years, with the non-lactating adult females being the oldest. Considering that age was only estimated, the similarity in contamination profiles is quite remarkable and may point towards a stable bioaccumulation profile coupled to a consistent offloading of pollutants to offspring throughout their lives. Indeed, long-finned pilot whales seem to reproduce more than short-finned pilot whales[5] and as for several Odontocetes, such as short-finned pilot whales and killer whales, it is not uncommon to find that non-reproductive females are still producing milk for their offspring's offspring[6] suggesting that a gradual or even a slow build up of concentrations throughout life might never occur in adult females. Compared to the sexually immature female group, the adult females had higher percentages of PCBs and of DDXs, but lower percentages of HCB and MeO-PBDEs. Regarding their continuous reproductive cycle, this probably means that there is a selective retention and transfer of compounds to their offspring.

The profile of the juvenile males showed considerable resemblance to the profile of the fetus and to profiles of the juvenile and sexually immature non-lactating females which was expected. The profiles of adult males, sexually immature males and adult females (lactating or not) were also similar. This provides evidence to show

that, although elimination through gestation/lactation can be beneficial for reducing the levels of contaminants, it does not change the basic contents of the contaminant cocktail the animals are exposed to.

Concentrations of PCBs, PBDEs and MeO-PBDEs

Among the PCBs, PCB 153 was the dominant congener in all groups, except for some lactating adult females where PCB 180 was more important. Statistical tests were performed for PCB 153, PCB 180, for each PCB-class (tetra- to nona-CBs) and for the sum PCBs. Although the levels of PCB 153, PCB 180, all PCB-classes and the sum PCBs were lowest in the lactating adult females showing that the reproductive cycle (gestation and subsequent lactation) is an efficient way to eliminate pollutants in marine mammals, the differences with the other groups were not always statistically significant. There were no statistically significant differences for individual PCBs, PCB-classes or the sum PCBs, between juvenile males and females and between sexually immature males and females indicating that there is an equal bioaccumulation process in all animals as long as they are not reproducing. Compared to other marine mammal species worldwide[4,7], the concentrations of sum PCBs in these pilot whales were more than 10 to 25 times lower (Table 1). In contrast, they were higher than the concentrations of the sum PCBs in several marine mammals from Australia[8].

For PBDEs, PBDE 47 was predominant in all animals from the present study. Similar to the PCBs, all differences in PBDE 47 or sum PBDEs involved the group of the lactating adult females which had the lowest concentrations compared to all other groups. Similar to the sum of PCBs, the sum PBDEs in the pilot whales (Table 1) was much lower than those in marine mammal species worldwide[4,7,9]. Considering the naturally produced MeO-PBDEs, levels of 6-MeO-BDE 47 was the most dominant, followed by 2-MeO-BDE 68 which is in contrast with what was reported previously for animals from the southern hemisphere[10,11]. Most differences among the groups were caused by the lower concentrations found in the group of the lactating adult females (Table 1). The levels of sum MeO-PBDEs in the pilot whales from Tasmania (Table 1) were higher than those in harbour porpoises and harbour seals[4] from the Northern Hemisphere, similar to the levels in several marine mammal species from Australia[11] and lower than the levels in marine mammal species inhabiting the continental shelf and open ocean in Brazil[10].

Levels of DDXs, CHLs and HCB

p,p'-DDE had the highest concentration of the DDXs and all statistically significant differences involved the lactating adult female group. The lactating female group was significantly different for *p,p'*-DDE and the sum DDXs from all other female groups and from all male groups. With DDXs being detected in the fetus (Table 1) and in the milk of marine mammals (e.g. harbour porpoises[15]), this finding was not a surprise. Compared to other marine mammal species and other areas around the globe, the pilot whales from the present study have lower concentrations of sum DDXs than harbour porpoises[4] and pilot whales from the Faroe Islands[12], but similar concentrations to harbour porpoises from the North Sea[7] and other parts of Europe (based on DDE[1]) and several marine mammal species from Australia (based on *p,p'*-DDE[11]). Trans-nonachlor (TN) was the most dominant compound among the CHLs and for TN as well as for CHLs and HCB, the largest differences were found between the lactating adult females and the other groups. Information about CHLs and HCB in pilot whales is scarce. Levels of HCB in the pilot whales were lower than in harbour porpoises from the Black Sea[4], but comparable to levels in harbour porpoises from the North Sea[7] and in pilot whales from the Faroe Islands[12]. Levels of CHLs were lower in the Tasmanian pilot whales than in the pilot whales from the Faroe Islands[12], whereas they were comparable to levels in harbour porpoises from the North Sea[7].

Implications for health and survival

In terms of bioaccumulation, concentrations of PCBs, PBDEs, MeO-PBDEs, CHLs, DDXs and HCB decreased with age. For all compounds, this could be explained by the elimination pathway through gestation/lactation for females as they were all present in the fetus (Table 1) and in milk of other marine mammal species (e.g. harbour porpoises[15]). Yet, such pathway does not exist for other groups. For these animals, there are a number of explanations as to why the concentrations of these chemicals decreased with age. For sexually immature animals, the growth dilution effect plays an important role as the animals experience a rather steep growth curve until the age of 10-15 years. After that age, a possible explanation is the change in ability to metabolise and eliminate these chemicals that occurs across a lifespan. This would mean that juveniles have a limited capacity to eliminate the chemicals while adults have maybe much more capacity. Another explanation

would be the worldwide controls on several of these chemicals that have been introduced over the last 20 years which may have lowered exposure of these animals. For DDE, the levels found in these animals are similar to those found in harbour porpoises from various European waters which were shown to have adverse effects on the thyroids of the porpoises[1]. In laboratory animals, PCBs and other POPs also have an endocrine-disrupting potential even at low concentrations[13,14]. Although the toxicological understanding of dose response is limited in marine mammals and average concentrations of chemicals in the present study seem lower than concentrations reported worldwide, it is of concern that the highest levels of POPs were found in the youngest animals. It is unknown whether these levels are toxic for this particular group or whether these levels induce changes that are compromising the well-being of pilot whales on the longer term. However, within the limits of the present study, it is not possible to determine if pollutants or any specific pollutant class could explain the mass-stranding in this particular pilot whale population.

Table 1. Concentrations, expressed in ng/g lw (lipid weight) in blubber of long-finned pilot whales from Tasmania. Values are mean ± SD and minimum – maximum.

	Fetus	JF	SexIM F-NL	AF-NL	SexIM F-L	AF-L	JM	SexIM M	AM
n	1	6	9	9	1	8	6	10	5
∑ PCBs	80	676 ± 562 67 – 1382	418 ± 208 242 – 933	244 ± 150 77 – 472	306	167 ± 114 59 – 399	446 ± 223 97 – 709	404 ± 53 320 – 504	380 ± 88 287 – 512
∑ PBDEs	3	25 ± 19 3 - 54	16 ± 7 10 – 31	8 ± 4 3 – 14	11	6 ± 4 2 – 15	17 ± 8 4 – 26	13 ± 3 10 – 20	10 ± 3 7 – 13
∑ MeO-PBDEs	117	843 ± 561 105 – 1635	553 ± 262 292 - 1100	237 ± 142 82 – 431	280	157 ± 110 59 – 410	628 ± 297 157 – 1007	389 ± 72 267 – 492	353 ± 73 278 – 434
∑ DDXs	191	2123 ± 1965 174 – 4748	1072 ± 623 543 – 2613	528 ± 408 133 – 1255	687	347 ± 311 92 – 1036	1229 ± 635 246 – 2071	983 ± 141 769 – 1177	997 ± 223 810 – 1366
∑ CHLs	29	246 ± 205 27 – 530	144 ± 81 83 – 344	71 ± 51 20 – 152	78	43 ± 33 12 – 116	164 ± 88 38 – 287	125 ± 19 98 – 153	116 ± 19 92 – 139
HCB	47	244 ± 110 45 - 375	149 ± 69 78 - 254	47 ± 26 18 - 90	38	32 ± 24 15 - 86	171 ± 66 75 - 258	64 ± 24 23 - 113	52 ± 13 40 - 72

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