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CONCENTRATIONS OF SOME LEGACY POLLUTANTS HAVE INCREASED IN SOUTH AUSTRALIAN BOTTLENOSE DOLPHINS FROM 1989 TO 2014

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Abstract

Information about pollution and its potential impact in Australian marine wildlife is scarce. To fill this knowledge gap, our study investigated concentrations of legacy pollutants as well as naturally produced methoxylated polybrominated diphenyl ethers (MeO-PBDEs) in blubber, liver, kidney and muscle of Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) from a large inverse estuary in South Australia from 1989-1995 and 2009-2014. Our results show that concentrations of most pollutant classes are relatively low compared to the literature but at the higher end of the ranges reported for marine mammals in Australia. Results of some individuals

exceed toxicity thresholds indicative for immunotoxicity in marine mammals. It is important to note that concentrations of some compound classes, particularly PBDEs and polychlorinated biphenyls (PCBs), increased over a time interval of 20 years thereby placing more individuals at risk in recent years. Some of the highest concentrations of persistent organic pollutants (POPs) were measured in juveniles, which may jeopardize their development and the success of future generations. These results indicate that legacy pollutants may play a role in the wellbeing of these animals and therefore should be included in biomonitoring efforts.

Keywords

Bottlenose dolphins, Australia, tissue distribution, temporal trends, POPs, MeO-PBDEs

1. Introduction

Despite the bans and restricted uses that were enacted years or even decades ago, legacy contaminants such as PCBs (polychlorinated biphenyls), PBDEs (polybrominated diphenyl ethers) and pesticides continue to be a threat to marine wildlife (McKinney et al., 2011; Desforges et al., 2018; Williams et al., 2020). Although numerous studies have reported the bioaccumulation of these legacy contaminants as well as associated adverse effects in a wide range of marine mammal species worldwide over the last couple of decades (e.g. De Swart et al., 1996; Jepson et al., 2005; Frouin et al., 2010), the long-term health implications of the persistent organic pollutant (POP) body burdens are underestimated and poorly understood. In this regard, the term 'legacy contaminants' is somewhat misleading as it implies that exposure occurred in the past and chemicals are (slowly) phasing out. However, in reality, large quantities are still stockpiled in consumer goods (Shaw and Kannan, 2009), new sources have recently been identified (Bartlett et al., 2019), and restricted uses of selected legacy POPs are still currently permitted (Van den Berg et al., 2017). Some compounds such as polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) even lack a 'phasing out' stage as they can be produced either intentionally (i.e. small quantities for research purposes) or unintentionally through anthropogenic activities (i.e. smelting, manufacturing of other chlorinated pollutants such as pesticides) and natural processes (i.e. wildfires, volcanoes). These factors justify the need for a sustained focus on legacy pollutants in toxicological research at a time when a lot of attention has been focused on detection of new and emerging pollutants in a variety of environmental matrices.

Bottlenose dolphins (*Tursiops* spp.) have a worldwide distribution and are probably among the most studied marine mammals from a physiological, biological, ecological, genetic as well as toxicological perspective. For Australia, however, information about the presence of toxicants in dolphins is rarely published. Organochlorines (PCBs and DDT) were analysed in only 6 bottlenose dolphins (*Tursiops* spp.) to 1994 as reviewed in Kemper et al. (1994). In the following decade, these compounds were reported in only 17 specimens (n = 12 in EPA (2000); n = 4 in Vetter et al. (2001); n = 1 in Gaus et al. (2005)). Since 2006, only perfluorinated compounds have been monitored (n = 44; EPA, 2017).

In the few publications that have included PCB results, detected levels ranged from 760 - 39,000 ng/g lw (lipid weight) (EPA, 2000; Vetter et al., 2001; Gaus et al., 2005) with 4 specimens exceeding thresholds for immunotoxicity (*Phoca vitulina* - harbour seals; De Swart

et al., 1996) and 3 specimens also exceeding PCB levels that were associated with infectious diseases (*Phocoena phocoena* - harbour porpoises; Jepson et al., 2005). In 2013, an unusual mortality event occurred in South Australia during which the number of deceased dolphins (*Tursiops aduncus* - Indo-Pacific bottlenose dolphins) more than doubled compared to previous and subsequent years (Kemper et al., 2016). Most of the animals were young (neonates, calves and juveniles) and infectious disease was found to be a major contributor to the death of 23 out of 31 Indo-Pacific bottlenose dolphins (Kemper et al., 2016). Correlations between diseases in cetaceans and pollution in marine mammals have been suggested in previous research (Jepson et al., 2015). However, toxicity tests have never been performed in any Australian cetacean and legacy compounds such as PCBs and DDT have not been analysed in bottlenose dolphins in more than a decade.

A major objective of the present study was to investigate the levels of a range of legacy pollutants such as PCBs, DDT (and isomers/metabolites), PCDD/Fs, PBDEs, chlordanes, HCB, and HCHs in coastal bottlenose dolphins (*Tursiops aduncus*) from South Australia. In addition, naturally produced methoxylated PBDEs (MeO-PBDEs) were also measured. These compounds are produced naturally by algae and sponges, which makes them different from the other compounds analysed in the study. Nevertheless, they have also been linked to adverse effects in wildlife and have already been detected at considerable levels in Australian marine mammals (Vetter et al., 2001; Weijs et al., 2019). The specific aims of this study were to 1) investigate the different POPs and MeO-PBDEs in several tissues of *Tursiops aduncus* from all ages and both genders, 2) assess how the pollutants are distributed across different tissue types (liver, muscle, kidney and blubber), 3) explore potential temporal trends between dolphins collected during 1989-1995 and dolphins from 2009-2014, 4) examine whether there are differences in pollutant levels between animals with different health status (e.g., cetacean morbillivirus), and 5) compare the results to levels and toxicity thresholds reported for marine mammals in the literature.

2. Materials & Methods

2.1 Samples. Blubber, liver, kidney, and muscle samples from 43 Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) from South Australia were included in this study (Fig S1, Table S1). All animals were from Spencer Gulf, Gulf St Vincent (both are poorly flushed inverse estuaries) and Investigator Strait. All samples were obtained from dead animals (e.g. stranded, victims of fisheries by-catch, illegally killed) from two time periods, namely 1989-1995 (referred to as the YI group) and 2009-2014 (referred to as the YII group), with the latter group including selected specimens (n = 12) from the unusual mortality event in 2013 (Kemper et al., 2016). In the case of blubber, tissue samples were collected from non-specific parts of the body. Tissues were stored at -20°C either in aluminium foil and a plastic bag or simply a plastic bag. To subsample for this study, they were cored and stored in aluminium foil and plastic bag. For all individuals, biological information (e.g. age, gender) as well as pathological findings were recorded and can be found in Table S1 in the Supporting Information. Animals were divided into relative age categories based on observations recorded at necropsy: a neonate has features consistent with being less than two months old (e.g. neonatal folds, rostral hairs); a juvenile has lost the neonatal features, has signs of suckling (tip of tongue with papillae), and/or is clearly not fully grown; subadults are close to but not fully grown; adults are fully grown and appear to be sexually mature on gross evidence. Future studies will adopt a refined set of categories that incorporate physical and sexual maturity, and estimated time of weaning.

In all samples, 29 polychlorinated biphenyl congeners (PCBs; IUPAC numbers 28, 52, 47, 49, 66, 74, 95, 99, 101, 105, 110, 118, 146, 149, 128, 138, 153, 156, 170, 171, 177, 180, 183, 187, 194, 196/203, 199, 206, 209), 5 chlordanes (CHLs; oxychlordane-OxC, *trans*-nonachlor-TN, *cis*-nonachlor-CN, *trans*-chlordane-TC, *cis*-chlordane-CC), 3 hexachlorocyclohexanes (HCHs; α, β, γ), hexachlorobenzene (HCB), DDT and metabolites (DDXs; *p,p'*-DDE, *p,p'*-DDT, *p,p'*-DDD), 3 polybrominated diphenyl ethers (PBDEs; IUPAC numbers 47, 99, 100) and 2 naturally occurring methoxylated PBDEs (MeO-PBDEs; 6-MeO-BDE47 and 2'-MeO-BDE68) were measured.

In 10 blubber and 5 liver samples of adult males, PCDDs (Congeners and homologue groups: 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 1,2,3,4,7,8-HxCDD, 1,2,3,6,7,8-HxCDD, 1,2,3,7,8,9-HxCDD, 1,2,3,4,6,7,8-HpCDD, OCDD, total TCDD isomers, total PeCDD isomers, total HxCDD isomers, total HpCDD isomers) and PCDFs (Congeners and homologue groups: 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF, 2,3,4,7,8-PeCDF, 1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDF,

2,3,4,6,7,8-HxCDF, 1,2,3,7,8,9-HxCDF, 1,2,3,4,6,7,8-HpCDF, 1,2,3,4,7,8,9-HpCDF, OCDF, total TCDF isomers, total PeCDF isomers, total HxCDF isomers, total HpCDF isomers) were also measured.

2.2 Sample preparation.

PCBs, CHLs, DDXs, HCB, HCHs, PBDEs, and MeO-PBDEs: Approximately 0.150 g (wet weight) of blubber, 1.50 g of liver, or 1.75 g of kidney and muscle was homogenized with anhydrous Na_2SO_4 and spiked with internal standards which included PCB 143, ε-HCH, ^{13}C-HCB and BDE 77. Homogenized samples were added to a glass column and extracted 3 times: homogenized samples were left covered by the solvent mixture (acetone/*n*-hexane (1/3, v/v)) for a minimum of 2 h (twice) and overnight (once). The extracts were combined per sample and evaporated to approximately 10 mL. About 1/8th of each of the resulting extracts was used for gravimetric lipid determination by evaporation on aluminium dishes in the oven (100°C, 1 h). The remaining 7/8th of each extract was cleaned up on approximately 8 g of acid silica (H_2SO_4, 44%) with 20 mL *n*-hexane and 15 mL dichloromethane (DCM). Extracts were evaporated to near dryness, re-dissolved in 100 µL recovery standard (PCB 207 in iso-octane) and kept chilled until GC-MS analysis.

PCDD/Fs: Approximately 10 g (wet weight) of blubber, or 2 g of liver was spiked with a range of isotopically labelled surrogate PCDD/Fs standards, and exhaustively extracted with a concentrated hydrochloric acid/organic solvent mixture (hexane/dichloromethane (3/1, v/v)). The extracts were solvent exchanged into hexane and partitioned with sulphuric acid then distilled water. The extracts were exchanged into DCM and further purification was performed using gel permeation chromatography followed by column chromatography on acid and base modified silica gels, basic alumina and carbon dispersed on Celite. Extracts were evaporated to near dryness then redissolved in 10 µL recovery standard (isotopically labelled PCDD/Fs) and kept at -20°C until GC-HRMS analysis.

2.3 Sample analysis.

PCBs, CHLs, DDXs, HCB, HCHs, PBDEs, and MeO-PBDEs: All analytes, except the lower chlorinated PCBs (tri and tetra-CBs) were measured with an Agilent 6890 gas chromatograph coupled with a 5973 mass spectrometer system (GC-MS). The GC was equipped with a 30 m

× 0.25 mm × 0.25 µm DB-5 capillary column. The MS operated in electron capture negative ionisation (ECNI) mode and was used in selected ion-monitoring (SIM) mode with 2 ions monitored for each analyte of homologue group. For the measurement of lower chlorinated PCBs (tri and tetra-CBs), an Agilent 6890 gas chromatograph coupled with a 5973 mass spectrometer system (GC-MS) was equipped with a 25 m × 0.22 mm × 0.25 µm HT-8 capillary column. The MS operated in electron ionisation (EI) mode and was used in SIM mode with 2 ions monitored for each analyte of homologue group.

PCDD/Fs: Samples were analysed with Thermo Trace high resolution gas chromatography coupled with a Thermo DFS high resolution mass spectrometry (HRGC-HRMS). A 1 µL aliquot of the extract was injected into the GC, the analytes were separated by high-resolution GC (60 m × 0.25 mm × 0.25 µm ZB-5ms, 60 m × 0.25 mm × 0.15 µm DB-Dioxin capillary columns) and detected by a high-resolution mass spectrometer.

2.4 Quality Assurance/Quality Control (QA/QC).

Mean recoveries ± RSD (both expressed in %) for surrogate PCDD/F standards were 62 ± 8, 58 ± 10, and 61 ± 9 for 2,3,7,8-TCDD, 1,2,3,7,8-PeCDF and OCDD, respectively. PCDD/F data was not blank subtracted. Mean recoveries ± SD (both expressed in %) for internal standards were 94 ± 15, 71 ± 9, 71 ± 15 and 78 ± 21 for PCB 143, ε-HCH, ^{13}C-HCB and BDE 77, respectively. 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, which ensures > 99 % certainty that the reported value is originating from the sample. For analytes that were not detected in procedural blanks, LOQs were calculated for a signal to noise (S/N) ratio equal to 10. For POPs other than PCDD/Fs and for MeO-PBDEs, LOQs depended on the sample intake and on the analyte and ranged between 2 and 20 pg/mL extract. QC was performed by regular analyses of procedural blanks, by random injection of standards and solvent blanks. Blubber of a humpback dolphin (*Sousa sahulensis,* ID BIO-328) was included as an uncertified reference sample. This matrix was analysed in three different institutions with different procedures for sample preparation and analysis thereby providing the opportunity to assess the performance of the current method (Table S2).

2.5 Statistics.

The influence of gender (female, male), age class (neonate, juvenile, subadult, adult) and 'year' group (1989 - 1995 = cohort YI, 2009 - 2014 = cohort YII) was tested on the lipid percentages and on the concentrations of pollutant groups (PCBs, CHLs, DDXs, HCB, HCHs, PBDEs, MeO-PBDEs) in blubber (F), muscle (M), liver (L) and kidney (K) with three-way ANOVA statistical tests. For compounds detected in more than 50% of the samples, concentrations below LOQ were replaced by a value of f (frequency of detection) × LOQ. Compounds detected in less than 50% of the samples were removed from statistical analysis and any calculations. For every compound, outliers were detected through boxplots and excluded from statistical analysis.

PCDD/Fs were only analysed in 10 blubber samples and 5 liver samples of adult males. For these compounds as well as for the WHO-TEQ results, only the influence of 'year' on PCDD/F levels in blubber was tested with one-way ANOVA. Temporal differences were not tested statistically in the liver samples due to the low sample sizes (n = 2 in YI; n = 3 in YII). Although the results of some pollutants were normally distributed, levels of all compound classes and of WHO-TEQs were log-transformed to achieve normality (with log(x+1) to avoid negative values) for consistency. Principal Component Analysis (PCA) was done to assess any potential correlations between pollutant levels and the animals' health status (morbillivirus positive (tested with immunohistochemistry or IHC) and negative). ANOVA tests were performed using SPSS (IBM SPSS Statistics version 25) and the level of statistical significance was set at $p = 0.05$. The PCA analyses were done with MetaboAnalyst (version 4.0). All graphs, except for the PCA plots, were made with GraphPad Prism (version 8).

3. Results

3.1 Lipid percentages and non-detects

Blubber. The lipid percentages in blubber ranged from 5 to 86% (Table S3) and were not significantly different between animals regardless of gender, age and time period (Table S7). PCB 49, PCB 209, α-HCH, γ-HCH, BDE 100, 1,2,3,7,8-PeCDF, 2,3,4,7,8-PeCDF, 2,3,7,8-TCDD, total TCDD isomers, 1,2,3,4,7,8-HxCDD, and 1,2,3,7,8,9-HxCDD were detected in less than 50% of all samples.

Liver. The lipid percentages in liver ranged from 0.2 to 31% (Table S4) and did not differ between dolphins regardless of gender, age and year (for p-values, see Table S7). Five compounds were detected in less than 50% of all samples: PCB 49, *p,p'*-DDT, α-HCH, γ-HCH, and BDE 100. OCDD was detected at levels above LOD (limit of detection) in only 1 out of the 5 liver samples. All other PCDDs and PCDFs were below LOD.

Kidney. The lipid percentages in kidney varied from 2.0 to 14% (Table S5). Similar to the lipid percentages in the liver and blubber, lipid percentages in the kidney did not differ significantly between animals regardless of age, gender and year (Table S7). All compounds targeted were detected, however, PCB 28, PCB 49, PCB 74, PCB 110, PCB 209, *p,p'*-DDT, α-HCH, BDE 100 and BDE 99 were detected in less than 50% of all kidney samples.

Muscle. The lipid percentages in muscle ranged from 0.2 to 4.3% (Table S6) and statistically significantly higher lipid percentages in juvenile males compared to adult males in YII (2009-2014) were found (Table S7). PCB 66, PCB 52, PCB 49, PCB 74, PCB 110, *p,p'*-DDT, BDE 100 and BDE 99 were detected in less than 50% of all samples.

3.2 Influence of gender, age class and year for MeO-PBDEs and for POPs other than PCDD/Fs

Blubber. Levels of hexa-PCBs, especially PCB 153, were consistently high among PCBs in all groups regardless of age, gender and year. PCB 153 was followed by PCBs 99, 118 (both penta-PCBs), 138 (hexa-PCB), 187 or 180 (hepta-PCBs) in a random order. Total PCB concentrations differed significantly between age/gender groups in YI as well as YII (3-way interaction: $p = 0.015$; Table S7). These differences were driven by the differences in hexa- to nona-PCBs (3-way interaction: $0.018 < p < 0.036$; Table S7) as age/gender groups did not differ significantly

in YI or YII for tri- to penta-PCBs (3-way interaction: $0.063 < p < 0.133$; Table S7). For CHLs, DDT, HCB, β-HCH and PBDEs, concentrations in blubber were different between animals from YI and animals from YII (main effect of year: $0.001 < p < 0.031$; Table S7). However, these differences did not follow any age or gender pattern as none of the 3-way interactions were statistically significant. MeO-PBDE concentrations were age-dependent ($p = 0.014$) but no influences of gender or year were found. *trans*-Nonachlor was predominant among CHLs in all samples in the present study, followed by either oxychlordane or *cis*-nonachlor. For DDXs, *p,p'*-DDE was dominant in all samples. This compound was followed by *p,p'*-DDT in 13 out of 16 samples (81%) in the YI group and in 14 out of 26 samples (54%) in the YII group. The blubber samples were also similar for PBDEs and MeO-PBDEs across all age/gender groups in YI and YII with BDE 47 and 2'-MeO-BDE 68 dominating PBDEs and MeO-PBDEs, respectively. Comparing all samples within YI to all samples within YII, general patterns were fairly similar: the order for YI was DDXs > MeO-PBDEs > PCBs > CHLs > HCB > PBDEs > HCHs, while the order for YII was PCBs > MeO-PBDEs > DDXs > CHLs > PBDEs > HCB > HCHs. Average concentrations of HCB and DDXs in blubber of YII animals (0.024 ± 0.013 μg/g lw for HCB, 5.6 ± 10 μg/g lw for DDXs) were approximately half the levels found in YI animals (0.059 ± 0.037 μg/g lw for HCB, 11 ± 12 μg/g lw for DDXs). In contrast, PBDEs were about 7 times higher in blubber of YII animals (0.29 ± 0.54 μg/g lw) compared to YI animals (0.041 ± 0.028 μg/g lw) while PCB concentrations almost doubled over the same time period (5.5 ± 7.4 μg/g lw in YI, 8.6 ± 15 μg/g lw in YII).

Liver. Two out of 14 liver samples of animals from the YI group had PCB 66 as dominant PCB while PCB 153 dominated in the liver samples of all other animals in YI and YII. In all liver samples, except for one of those two, hexa-PCBs had the highest levels followed by either penta- or hepta-PCBs (PCBs 99 and 118 for penta, PCBs 180 and 187 for hepta). There was a high degree of variability between the different ages and genders in groups YI and YII for octa- to deca-PCBs (3-way interaction: $0.018 < p < 0.036$; Table S7). However, this seemed to be significant for females only (two-way interaction between age and year: $0.001 < p < 0.004$ for females, $0.152 < p < 0.159$ for males). In contrast to blubber, deca-PCB was detected in 29 out of 39 liver samples and more differences were found between age/gender/year groups for the higher chlorinated PCBs (hepta- to deca-PCBs) than for the lower chlorinated ones (tri- to hexa-PCBs). No statistically significant differences were found for total PCBs, tri- to hexa-PCBs, HCB and PBDEs indicating that these are either distributed uniformly or randomly across all groups. For CHLs, there were age and gender-related differences in concentrations

with the highest concentrations in juveniles (compared to adults) and in females (compared to males). Juvenile females from YI had significantly higher DDT levels than juvenile females from YII while there was a different effect of gender on MeO-PBDE levels for adults in YI compared to YII (i.e. higher MeO-PBDE levels for adult males in YII compared to YI but lower levels for adult females in YII compared to YI) . The profiles of the respective classes in liver were very similar to the profiles in blubber, with TN, BDE 47 and 2'-MeO-BDE 68 being prevalent among CHLs, PBDEs and MeO-PBDEs, respectively. The greatest difference in profiles was found for DDXs: while *p,p'*-DDE dominated the DDX profiles in blubber and liver, DDT was present in all blubber samples but only in < 50% of the liver samples. In the liver samples where *p,p'*-DDT was detected, concentrations were up to 90 times lower than *p,p'*-DDD. Comparing overall profiles between YI and YII shows an almost ten-fold increase in PBDEs (0.025 ± 0.017 µg/g lw in YI, 0.23 ± 0.059 µg/g lw in YII), modest increases for MeO-PBDEs (3.5 ± 2.3 µg/g lw in YI to 5.0 ± 5.4 µg/g lw in YII) and PCBs (5.1 ± 9.7 µg/g lw in YI to 7.7 ± 14 µg/g lw in YII), and decreases for CHLs (1.1 ± 2.6 µg/g lw in YI, 0.74 ± 1.7 µg/g lw in YII), DDXs (6.5 ± 8.5 µg/g lw in YI to 4.3 ± 7.9 µg/g lw in YII), HCB (0.13 ± 0.26 µg/g lw in YI to 0.041 ± 0.037 µg/g lw in YII) and HCH (0.058 ± 0.067 µg/g lw in YI to 0.008 ± 0.009 µg/g lw in YII).

Kidney. Tri- and deca-PCBs were detected sporadically in the kidney, while hexa-PCBs dominated in all kidney samples except for 3 out of 34 samples. For these three exceptions, PCB 66 (tetra-PCB) had the highest levels. For all other samples, the hexa-PCBs 138 (one sample) or 153 (30 samples) were dominant. No statistically significant influences of either age, gender or year could be found on the levels of tetra-PCBs, penta-PCBs, HCB and MeO-PBDEs in kidney (Table S7) indicating that differences and variation could not be attributed to either of these factors. For all other compound groups, the year of death (YI or YII) was critical on the effect that age or gender had. For total PCB and hexa-PCBs, there was an effect of gender and year ($p = 0.037$ and 0.030, respectively; Table S7), but only for juveniles ($p = 0.039$ (juveniles) and $p = 0.398$ (adults) for total PCBs; $p = 0.042$ (juveniles) and $p = 0.298$ (adults) for hexa-PCBs). For hepta- and octa-PCBs, effects could mostly be attributed to the gender with age and gender influences apparent in the males ($p = 0.044$ and 0.048, respectively) but not females. Adult males from YI had significantly lower BDE 47 concentrations compared to adult males from YII. Despite statistically significant two-way interactions for CHLs and DDT, further statistical analyses did not reveal any significant influence of age, gender or year. Compound class profiles are comparable to the profiles in blubber and liver, with TN, *p,p'*-

DDE, β-HCH, and 2'-MeO-PBDE 68 dominating among CHLs, DDXs, HCHs, and MeO-PBDEs, respectively. Over a time span of 20 years, the concentrations of BDE 47 increased ten-fold in the kidney (0.012 ± 0.010 μg/g lw in YI, 0.13 ± 0.30 μg/g lw in YII), while concentrations of total PCBs doubled (2.1 ± 4.2 μg/g lw in YI, 4.6 ± 8.4 μg/g lw in YII) and levels of MeO-PBDEs and DDXs increased slightly (2.1 ± 1.2 μg/g lw in YI, 3.2 ± 3.0 μg/g lw in YII for MeO-PBDEs; 2.0 ± 3.4 μg/g lw in YI, 2.6 ± 4.6 μg/g lw in YII for DDXs). In contrast, levels of HCHs were three times lower in YII (0.004 ± 0.005 μg/g lw) compared to YI (0.013 ± 0.011 μg/g lw) while levels of CHLs remained roughly the same (0.48 ± 1.1 μg/g lw in YI, 0.47 ± 1.0 μg/g lw in YII) and HCB decreased slightly (0.039 ± 0.019 μg/g lw in YI, 0.025 ± 0.017 μg/g lw in YII).

Muscle. In contrast to kidney, blubber and liver, there were very few statistically significant influences of age, gender or year on the levels of POPs and of MeO-PBDEs in the muscle of dolphins (Table S7). The year and age played a role for bioaccumulation of nona- and deca-PCBs ($p = 0.016$ and 0.031, respectively; Table S7). For nona-PCBs, this interaction was weakly significant for females ($p = 0.045$) but not males ($p = 0.087$), however, no significant difference was found for either gender for deca-PCB ($p = 0.093$ and 0.089 for females and males, respectively). The muscle and liver were the only tissues in which PCB 209 was detected in more than 50% of the samples. In all muscle samples, PCB 153 was the most dominant PCB congener followed by PCBs 180, 187 and 118 in no specific order. Despite being present in more than 50% of all blubber samples and even dominating the PCB profiles in some kidney and liver samples, PCB 66 was only found in 15 out of 36 muscle samples with a higher occurrence in YII (3 out of 11 muscle samples from YI, 12 out of 25 muscle samples from YII) compared with YI. Muscle concentrations were also different from all other tissue types in that all three HCH isomers (α, β, γ) could be detected in more than half of the samples. TN, *p,p'*-DDE and 2'-MeO-PBDE 68 were dominant among CHLs, DDXs and MeO-PBDEs, respectively, which is similar as the results for other tissues. BDE 47 was the only PBDE congener detected in more than 50% of the muscle samples. Although BDE 47 had relatively low levels in the muscle, its concentrations increased by an order of magnitude over 20 years (0.027 ± 0.036 μg/g lw in YI, 0.32 ± 0.87 μg/g lw in YII). Concentrations of CHLs almost doubled (0.54 ± 1.1 μg/g lw in YI, 0.95 ± 2.4 μg/g lw in YII) while PCB concentrations more than doubled over the same time period (3.8 ± 5.6 μg/g lw in YI, 9.5 ± 20 μg/g lw in YII). Levels of DDXs, HCB and HCHs decreased (6.4 ± 9.1 μg/g lw in YI, 5.1 ± 11 μg/g lw in YII

for DDXs; 0.056 ± 0.027 µg/g lw in YI, 0.043 ± 0.044 µg/g lw in YII for HCB; 0.014 ± 0.010 µg/g lw in YI, 0.007 ± 0.006 µg/g lw in YII for HCHs).

3.3 Influence of year on the bioaccumulation of PCDD/Fs

PCDD/Fs were only analysed in blubber (Table S8) and liver (Table S9) of a limited number of adult males thereby eliminating the potential influence of age or gender. No significant temporal trends were found for the total sum of PCDDs or PCDFs in blubber ($p = 0.283$ and 0.163, respectively) or for any PCDD/F congener or isomer group ($0.101 < p < 0.342$ for PCDD congeners, $0.122 < p < 0.674$ for PCDF congeners). With one exception, OCDD was dominant among PCDDs in all blubber and liver samples with levels up to 22 pg/g lw. For PCDFs, TCDFs dominated in 5 blubber samples with levels up to 2.5 pg/g lw while PeCDFs were prevalent in the other 5 blubber samples with levels up to 2.9 pg/g lw.

3.4 Distribution between tissues

Overall, the number of compounds found in the blubber and liver was higher than in muscle and kidney: 41 out of 46 analytes were found in more than 50% of the liver and blubber samples whereas 37 and 38 analytes were found in kidney and muscle samples, respectively. This total number of 46 analytes does not include the PCDD/Fs which were mostly below LOD and sporadically detected in only a limited number of samples. Per age/gender group, the overall profiles were comparable across the different tissue types (Fig S2). However, there were differences in concentrations (Table 1; Fig S3). The highest levels of PCBs, CHLs, DDXs, PBDEs and MeO-PBDEs were found in the blubber for most animals (18, 31, 30, 30 and 30 out of 43 animals, respectively) while the lowest levels of these compounds were usually in the kidney. In contrast, the liver had the highest levels of HCHs and HCB while the lowest concentrations were often detected in the blubber. For adult males and females, concentrations were highest in the blubber followed by the muscle, liver and kidney. For younger animals (neonates, juveniles, subadults), the liver and muscle often bioaccumulated higher levels of pollutants compared to the blubber (Fig S3).

3.5 POPs and MeO-PBDEs versus health

379 The potential impact of pollution on the health of the dolphins was investigated in two ways.
380 Firstly, potential differences in POP and MeO-PBDE profiles were assessed between
381 morbillivirus victims and morbillivirus carriers (Fig 1 for blubber and Fig S4 for liver, kidney
382 and muscle). Some of the animals included in this study (n = 12) were part of the unusual
383 mortality event in South Australia in 2013 during which 45 dolphins were found dead. All 12
384 animals tested morbillivirus positive using a PCR test, which indicates that RNA of the virus
385 was detected regardless of disease stage or activeness. However, depending on whether the
386 virus acted as a pathogen or not, which is detected via immunohistochemistry test (IHC),
387 animals are classified as 'morbillivirus victims' (morbillivirus positive via IHC test) or
388 'morbillivirus carriers' (morbillivirus negative via IHC test) in the present study. Of the 12
389 animals, 9 were morbillivirus victims and 3 were morbillivirus carriers. Although this is a
390 relatively small sample size, especially for the negative/carrier group, a PCA analysis was
391 conducted (Fig 1 for blubber and Fig S4 for liver, kidney and muscle). Results show a
392 separation of carriers and victims in all tissues along the PC1 and PC2 axis with the
393 anthropogenic POPs influencing the distribution horizontally and the naturally occurring
394 compounds affecting the distribution vertically. Although the victims and carriers did not
395 separate completely and only 3 carriers were included, the clusters of the victims and carriers
396 are perpendicular indicating that both clusters are influenced by the naturally occurring and
397 anthropogenic compounds differently. Secondly, World Health Organization Toxic EQuivalent
398 (WHO-TEQ) values were calculated for PCDD/Fs and dioxin-like PCBs in the blubber and
399 liver of 10 and 5 dolphins, respectively (Fig 2C; Table S10). WHO-TEQs ranged from 0.89 to
400 57 pg/g lw in blubber with dioxin-like PCBs contributing 53 to 98% to the total TEQ. In the
401 liver, WHO-TEQs ranged from 9.1 to 27 pg/g lw with dioxin-like PCBs contributing 7 to 57%
402 to the total TEQ (Fig 2). For the latter, it is important to note that PCDD/F concentrations in
403 the liver were all below LOD so calculations were made with half the LOD value.
404

4. Discussion

The taxonomic relationships of bottlenose dolphins have been widely debated. Two species are recognised, including for Australia: *Tursiops truncatus* is found worldwide in both pelagic and coastal environments; *T. aduncus* generally occurs in shallow water close to continents and islands (Reeves et al., 2002). A third species, *T. australis*, has been described from inshore Victoria, Australia but it is not currently recognised (Committee on Taxonomy 2020, Jedensjo et al. in press). Some authors (Möller et al., 2008; Moura et al., 2013), use the common name southern Australian bottlenose dolphin, which they assumed to be *T. australis,* but without published evidence. In the present paper, we use the species name *T. aduncus* i.e. the inshore bottlenose dolphin in South Australia (Kemper, 2004).

4.1 POPs in Australian bottlenose dolphins

This study is the first to report on the presence of PCBs, DDXs, CHLs, HCB, HCHs, PBDEs, PCDD/Fs and MeO-PBDEs in different tissues of all age/gender groups for an Australian marine mammal species (Table 1). Organohalogens have received little attention in Australian marine mammals. The focus of most toxicological research in these animals has been on inorganics while POPs were investigated only in a limited number of studies. Kemper et al. (1994) reviewed pollution in blubber of Australian marine mammals and documented organochlorine concentrations (PCBs, DDXs, oxychlordane, HCB) from a handful of sources (3 published and 2 unpublished) in 39 specimens from 9 species. Assuming an average lipid percentage in blubber of 69%, which was calculated using the lipid percentages in blubber of dolphins from the present study, the highest DDX, PCB, OxC and HCB comparative concentrations were up to 54,000 ng/g lw (bottlenose dolphin), 5,600 ng/g lw (*Arctocephalus pusillus doriferus* - Australian fur seal), 68 ng/g lw (*Hydrurga leptonyx* - leopard seal) and 590 ng/g lw in killer whales (*Orcinus orca*), respectively (Table 2).

From the Kemper et al. (1994) review until 2019, less than 10 studies were published reporting on POPs in Australian odontocetes with the following levels: up to 53,000 ng/g lw (bottlenose dolphin; Vetter et al., 2001), 370,000 ng/g lw (humpback dolphin; Weijs et al., 2016), 1,000 ng/g lw (humpback dolphin; Weijs et al., 2016), 380 ng/g lw (*Globicephala melas* - long-finned pilot whale; Weijs et al., 2013), 440 ng/g lw (humpback dolphin; Weijs et al., 2016), and 3.0 ng/g lw (humpback dolphin; Weijs et al., 2016) for DDXs, PCBs, CHLs, HCB, PBDEs, and

437 PCDD/Fs, respectively (Table 2). In general, our results exceed or are at the higher end of the
438 ranges of the concentrations reported for Australian marine mammals (Table 2). This can be a
439 result of the inshore lifestyle of the species which brings the animals close to urban impacts
440 and anthropogenic activities. However, with data from only a limited number of studies
441 encompassing animals from different species, different states across Australia, different ages
442 and genders, comparisons are likely skewed. Despite being at the higher end of the
443 concentrations reported for Australian marine mammals, our results are low compared to
444 results reported for odontocetes elsewhere (e.g. striped dolphins (*Stenella coeruleoalba*) in
445 Aguilar and Borrell, 1994; several species in Dorneles et al., 2010; killer whales (*Orcinus orca*)
446 in Desforges et al., 2018; harbour porpoises (*Phocoena phocoena*) in Williams et al, 2020).
447
448 The blubber of marine mammals is undoubtedly one of the most investigated tissues with
449 regards to lipophilic pollutants such as PCBs and DDXs. Influences of factors such as age and
450 gender on POP levels in this matrix have been studied and described numerous times (e.g.
451 Hickie et al., 1999; Ross et al., 2000; Weijs et al., 2012). Generally, POP concentrations are
452 different in adult males and adult females but comparable for marine mammals of both genders
453 that have not yet reached a reproductive age. Distinct and sometimes statistically significant
454 differences in POP concentrations between the blubber of adult females and adult males (with
455 lower concentrations in the females as a result of maternal offloading) can be seen for example
456 for CHLs, DDXs and PBDEs in both YI and YII cohorts (Table 1, Table S7, Fig S3).
457 Comparable levels in the blubber of juvenile males and females were also found for PBDEs,
458 HCB and HCHs in the YII cohort.
459 However, not all results follow these common principles. A few exceptions are the PCB and
460 CHL levels in juveniles of the YI cohort, which are more than 75 times higher in juvenile
461 females compared to juvenile males, or the higher PCB levels found in blubber of adult females
462 of the YII cohort compared to adult males. These exceptions are not sustained in both cohorts
463 and may therefore be anomalies rather than general trends for this species. Possible
464 explanations range from POP hotspots in specific locations to statistical errors due to low
465 sample sizes for specific age/gender groups. Age/gender influences on POP concentrations in
466 blubber are also not necessarily reflected in other tissues. PCBs in the YI cohort are a good
467 example to illustrate this: levels of PCBs are higher in adult males compared to adult females
468 in the blubber, liver and muscle, but not in the kidney. These discrepancies highlight a need for
469 a better understanding of POP distribution in marine mammals and emphasize the importance
470 of consistency across studies.

This study confirms the importance of blubber for biomonitoring lipophilic pollutants in marine mammals. Concentrations of most POPs investigated in our study are higher in the blubber compared to liver, kidney and muscle (Table 1, Fig S3). However, exceptions exist for HCB and HCHs as well as for selected age/gender groups such as young animals (i.e. neonates, juveniles) and adult females. HCB and HCHs have recently been shown to preferentially accumulate in tissues other than blubber in dugongs because of their lower log K_{ow} values compared to PCBs, DDXs and PBDEs (Weijs et al., 2019). Gestation and lactation are responsible for developing a blubber layer in young animals and cause a re-mobilisation of POPs that are present in the blubber of mothers (Debier et al., 2003; Brown et al., 2016). Furthermore, there are several studies reporting selective maternal offloading of POPs with higher proportions of lower chlorinated and brominated compounds transferred to offspring than higher chlorinated and brominated compounds (Debier et al., 2003; Brown et al., 2016). Consequently, POP levels in adult females and in the younger members of the population are in a state of flux, which may explain the lower concentrations of selected POPs in the blubber compared to some of the other tissues. In adult females and young (neonate, juvenile) animals, the highest levels of HCB and HCHs can be found in the muscle which is probably an artefact of the very low lipid levels in the muscle thereby giving higher concentrations when calculated on a lipid weight basis.

POP profiles in the blubber were similar to those in the kidney, muscle and liver of the dolphins in this study: PCB 153, TN, *p,p'*-DDE and BDE 47 were the most dominant compounds among PCBs, CHLs, DDXs and PBDEs, respectively. These compounds are known to be highly persistent in the environment and dominate PCB, CHL, DDX and PBDE profiles in most marine mammal species worldwide. As commercial mixtures often have larger proportions of, for example, DDT and higher brominated diphenyl ethers, our findings suggest the absence of new POP inputs into the South Australian marine environment.

4.2 MeO-PBDEs in Australian bottlenose dolphins

MeO-PBDEs are naturally produced by algae and sponges and are therefore different than all other compounds analysed in this study. Two MeO-PBDEs were targeted in our study, namely 2'-MeO-BDE 68 and 6-MeO-BDE 47. According to a hypothesis raised by Vetter et al. (2001),

both MeO-PBDEs have different sources with 6-MeO-BDE 47 produced by (red) algae and 2'-MeO-BDE 68 produced by sponges. Without exception, both compounds were detected in all blubber, liver, kidney and muscle samples indicating that MeO-PBDE producing algae and/or sponges are present in South Australian waters. In all samples, except for the kidney of one adult female from the YII cohort, 2'-MeO-BDE 68 levels were higher compared to those of 6-MeO-BDE 47. This pattern is considered common for marine mammals from the southern hemisphere (Melcher et al., 2005; Dorneles et al. 2010; Weijs et al., 2019) although a predominance of 6-MeO-BDE 47 has been reported previously in long-finned pilot whales from Tasmania (Weijs et al., 2013). Our results show that age, gender and year do not influence MeO-PBDE levels significantly in blubber, muscle, kidney or liver, which is probably due to the high degree of variability within each group, the lack of true mother/offspring pairs in either cohort and the knowledge that maternal offloading of MeO-PBDEs is limited as suggested previously (Weijs et al., 2019) (Table 1). As MeO-PBDEs are naturally produced compounds, this variability is most likely attributable to the different locations of the dolphins (Fig S1). Within most groups, however, there is a tendency to find the highest concentrations (expressed in lipid weight) in blubber, followed by muscle, liver and kidney which is also the case for most anthropogenic POPs.

4.4 What has changed over 20 years?

Because of the general scarcity of toxicological data from Australian marine mammals, a major objective of this study was to assess temporal trends. The two groups (YI and YII) included in our study were from 1989-1995 and 2009-2014 thereby providing the opportunity to assess potential changes in toxicological status over a 20-year interval. It is important to note that the dolphins investigated in the present study were primarily from inverse estuaries (Spencer Gulf, Gulf St Vincent). Inverse estuaries are characterized by higher salinity compared to the adjacent ocean because of an excess of evaporation over freshwater input. These areas are poorly flushed and water circulation occurs more laterally than vertically thereby creating ideal conditions for a build-up of contaminants (Kampf, 2014). Although differences depend on the age/gender group and may or may not be statistically significant, concentrations of all compound classes generally increased from 1989 until 2014 except for DDXs, HCB and HCHs (Fig S4). A portion of the increases can probably be attributed to the inverse estuaries where the dolphins live. However, as increasing temporal trends were not observed for all compounds

536 investigated, there are likely other factors playing a role. The most dramatic increase was seen
537 for PBDEs, with a difference of at least an order of magnitude between levels in the YI and
538 YII cohorts. This contrasts with recent studies which analysed human blood samples from
539 Australia: both Drage et al. (2018) and Gyalpo et al. (2015) reported declining PBDE levels in
540 children over a time period of more than 10 years. While the decreasing levels in human blood
541 samples correspond with the ban on importation and manufacture of octa- and penta-BDE
542 mixtures in Australia, the increasing concentrations detected in the South Australian bottlenose
543 dolphins indicate that these compounds are still present and bioavailable in the marine
544 environment. This may be a sign of slow movement of PBDEs from the land to the marine
545 environment and/or a higher persistence of these compounds in the the latter.

546 Like PBDEs, PCB concentrations increased over the 20-year interval. These compounds were
547 banned globally in the 1970s and were never used extensively in Australia. Consequently,
548 concentrations of PCBs in Australian wildlife were usually relatively low, which probably
549 explains the scarcity of PCB data in Australian marine apex predators. Despite this, it is
550 concerning that PCB levels have almost doubled in South Australian marine top predators
551 while they have been decreasing or stabilising in other parts of the world (Ross et al., 2013;
552 Jepson et al., 2016; Robinson et al., 2019). For both compound classes, explanations are
553 probably a combination of local inputs such as effluent discharge, poor (electronic) waste
554 management, industrial and agricultural runoff, and global sources such as oceanic currents
555 and atmospheric deposition fluxes.

556 CHLs were used in pesticide formulations in Australia but have been replaced by other
557 pesticides since the 2000s, which is probably too recent to see any decreases in marine wildlife.
558 Like all POP classes mentioned here, increases in PCDD/F concentrations were also observed
559 in dolphins over the 20-year interval. However, unlike other POP classes mentioned so far,
560 PCDD/Fs are produced unintentionally via anthropogenic activities (e.g. smelting, barbeque)
561 as well as natural processes (e.g. bushfires, volcano eruptions). PCDD/Fs have also been found
562 as impurities in organochlorine pesticide formulations (Holt et al., 2010) and can be formed by
563 exposing pesticides to natural sunlight (Holt et al., 2012). As a result, it is difficult to manage
564 or interpret PCDD/F releases into the marine environment and impossible to pinpoint the exact
565 source(s).

566 Like PCDD/Fs, MeO-PBDEs are hard to manage as they are produced via natural processes.
567 Theoretically, MeO-PBDEs can be formed through methylation of hydroxylated PBDE

568 metabolites (HO-PBDEs). They can also be formed by condensation of bromophenols and
569 methylation in algae, sponges and cyanobacteria. Higher water temperatures and diatom
570 blooms (and the biotoxins they produced) were investigated and dismissed as causative agents
571 by Kemper et al (2016) in the morbillivirus-associated unusual mortality event in South
572 Australian dolphins in 2013. Those higher water temperatures could be responsible for an
573 increased activity of MeO-PBDE producing algae or sponges, thereby providing an explanation
574 for the higher MeO-PBDE concentrations in the YII cohort compared to YI.

575

576 *4.5 Animal health implications*

577 Despite the relatively low concentrations found in this study, our PCA results indicate that
578 morbillivirus victims (morbillivirus positive according to the IHC test) and morbillivirus
579 carriers (morbillivirus negative according to the IHC test but positive from PCR test) animals
580 have a different distribution in the PC1 and PC2 planes, which correspond with different POP
581 and MeO-PBDE signatures (Fig 1 for blubber and Fig S4 for liver, kidney and muscle). MeO-
582 PBDEs have been investigated in several marine mammal species worldwide but have never
583 been associated with adverse effects in these species to our knowledge. The toxicity of naturally
584 produced compounds, however, should not be underestimated. Biotoxins, such as domoic acid,
585 which are also produced by algae or cyanobacteria, have been linked to marine mammal mass
586 mortalities in the US (Bossart et al., 1998). In a recent study, Davis et al. (2019) also detected
587 high levels of the cyanobacterial neurotoxin β-methylamino-L-alanine (BMAA) and
588 potentially associated neuropathological changes in the brains of 13 out of 14 stranded dolphins
589 (7 bottlenose dolphins and 7 common dolphins (*Delphinus delphis*)). POPs, on the other hand,
590 have been associated with impacts on the reproductive, immune and endocrine systems of
591 marine mammals (DeLong et al. 1973; Helle et al., 1979; Aguilar and Borrell, 1994; De Swart
592 et al., 1996; Kannan et al., 2000; Jepson et al., 2005). In the present study, PCB concentrations
593 of two out of 16 animals from the YI cohort and 5 out of 26 animals from the YII cohort
594 exceeded PCB levels associated with immunotoxicity in harbour seals (De Swart et al., 1996).
595 Of the latter 5 animals, 4 also exceeded PCB levels found in harbour porpoises with infectious
596 diseases (Jepson et al., 2005) (Fig 2A). With regards to PCBs, potential health impacts were
597 more severe in the YII cohort compared to the YI cohort. The opposite is true for *p,p'*-DDT
598 which exceeded thresholds indicative for immunotoxicity in 4 out of 16 animals from the YI
599 cohort but none from the YII cohort (Fig 2B). It should be noted, however, that decreasing

600 *p,p'*-DDT levels imply increasing *p,p'*-DDE concentrations as DDE is a metabolite of DDT.
601 DDE has been shown to have a longer half-life and may be more toxic than DDT. Toxicity
602 thresholds based on *p,p'*-DDT only may therefore be misleading with regards to the toxicity
603 resulting from exposure to DDXs. None of the PCB or *p,p'*-DDT results in the bottlenose
604 dolphins from the present study were close to toxicity thresholds associated with reproductive
605 effects in marine mammals or diseased dolphins from the Mediterranean (> 100,000 ng/g lw
606 for both *p,p'*-DDT and PCBs; DeLong et al. 1973; Helle et al., 1979; Aguilar and Borrell,
607 1994). In addition, WHO-TEQ values of PCDD/Fs and dioxin-like PCBs were much lower
608 than toxicity thresholds reported in seal blubber (LOAEL: 286 pg/g lw; Kannan et al., 2000;
609 Fig 2C). This study has not conducted an in-depth assessment of the health of the dolphins in
610 South Australia over time, which makes it hard to draw conclusions about the current health
611 status of the animals. Nevertheless, our findings indicate that some compound classes have
612 increased in internal concentration and reached toxic levels in several individuals and suggest
613 that the health of the dolphins may have deteriorated over a 20-year interval.
614

5. Conclusions

This investigation is part of a larger study that aims to characterize pollution and its possible effects on Australian marine mammals. Pollution has been implicated as a stressor, potentially impacting on the health of Australian marine mammals, however, it has been the focus of few investigations. Our study is the first to analyse naturally produced compounds as well as a variety of legacy pollutants in several tissues of more than 40 individuals of an Australian cetacean species. This study is also the first to assess pollution in the same population of dolphins over a time frame of 20 years. Our results generally confirm several well-known principles in marine mammal toxicology such as higher levels of pollutants in adult males due to bioaccumulation of persistent compounds with age, and low concentrations of pollutants in adult females because of maternal offloading of pollutants to offspring. There are some concerns regarding the very high levels of pollutants in some (female) juvenile animals as this may influence their development as well as exposure in future generations. Our results also support the use of blubber as a biomonitoring tool in marine mammals but stress the importance of consistency in subsampling considering the variability in pollutant levels and profiles in different tissues across several age/gender groups. A large portion of this variability can possibly be addressed and better understood with more detailed approaches such as toxicokinetic modelling in future studies. Although the impact of naturally produced compounds on the health of the animals is unknown, the relatively high concentrations of naturally produced compounds found in all tissues of all dolphins in our study suggest that toxicological research in the future should go beyond the scope of only targeting anthropogenic compounds. Overall, the levels of POPs in the South Australian dolphins were low compared to marine mammals from severely polluted areas such as the Mediterranean but at the higher end of the ranges reported for marine mammal species in Australia. Our data show that levels of several pollutant classes have increased over a time interval of two decades, from 1989 to 2014, resulting in more animals that surpass available toxicity thresholds for other marine mammals in the literature. Finally, this study emphasizes the need to biomonitor legacy and emerging pollutants in Australian marine wildlife on a more regular basis as low levels of even legacy pollutants can increase over time.

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Table 1A. Summary of all compounds analysed in South Australian bottlenose dolphin (*Tursiops aduncus*) tissues from 1989 to 1995 (group YI). Values represent average ± SD (expressed in ng/g lw). Sample sizes and min-max ranges are presented in Tables S3-S6. F = blubber, L = liver, K = kidney, M = muscle.

		PCBs	CHLs	DDXs	HCB	HCHs	PBDEs	MeO-PBDEs
Adult male	F	5,000 ± 5,700	1,300 ± 2,300	15,000 ± 12,000	53 ± 39	3 ± 1	41 ± 27	7,100 ± 6,100
	L	2,000 ± 1,400	290 ± 370	6,100 ± 6,900	55 ± 21	54 ± 43	21 ± 12	2,600 ± 1,900
	K	480 ± 400	40 ± 12	780 ± 920	29 ± 8	17 ± 3	0 ± 0	2,000 ± 1,800
	M	3,100 ± 3,200	260 ± 190	12,000 ± 14,000	56 ± 12	16 ± 4	25 ± 33	6,500 ± 4,400
Subadult male	F	1,800 ± 540	200 ± 73	3,900 ± 1,800	60 ± 29	5 ± 4	52 ± 36	12,000 ± 12,000
	L	880 ± 160	78 ± 14	1,700 ± 990	380 ± 560	130 ± 120	26 ± 15	4,300 ± 3,000
	K	490 ± 130	48 ± 13	770 ± 480	43 ± 19	21 ± 15	14 ± 5	2,400 ± 1,900
	M	1,300 ± 810	140 ± 95	1,900 ± 1,000	75 ± 35	20 ± 13	37 ± 64	9,700 ± 10,000
Juvenile male	F	330 ± 260	120 ± 62	1,900 ± 2,200	74 ± 11	1 ± 1	19 ± 11	3,400 ± 3,200
	K	140	60	290	72	1	20	2,200
	M	120	44	220	78	4	22	2,100
Neonate male	F	2,000	800	2,200	36	0	36	1,500
	L	1,600	320	1,000	31	6	18	470
	K	2,100	560	1,400	32	5	24	1,100
	M	2,700	500	1,300	44	2	24	830
Adult female	F	2,000 ± 2,700	180 ± 160	4,900 ± 6,100	34 ± 16	3 ± 1	22 ± 30	15,000 ± 9,100
	L	960 ± 840	95 ± 77	2,900 ± 2,600	51 ± 28	32 ± 21	15 ± 13	4,700 ± 2,600
	K	1,100	66	1,700	14	12	0	2,000
	M	3,000	150	4,200	8	23	0	4,100
Juvenile female	F	22,000 ± 5,000	9,200 ± 1,400	31,000 ± 2,100	100 ± 73	5 ± 2	75 ± 20	5,200 ± 900
	L	27,000 ± 7,700	7,100 ± 2,800	23,000 ± 4,700	120 ± 31	24 ± 10	51 ± 19	4,500 ± 1,200
	K	13,000	3,400	11,000	48	4	22	2,100
Neonate female	F	7,400	2,900	9,900	38	3	29	580
	M	19,000	3,800	12,000	42	6	38	890

Table 1B. Summary of all compounds analysed in South Australian bottlenose dolphin tissues from 2009 to 2014 (group YII). Values represent average ± SD (expressed in ng/g lw). Sample sizes and min-max ranges can be found in Tables S3-S6. F=blubber, L=liver, K=kidney, M=muscle.

		PCBs	CHLs	DDXs	HCB	HCHs	PBDEs	MeO-PBDEs
Adult male	F	8,000 ± 6,400	1,100 ± 1,200	10,000 ± 5,300	21 ± 9	3 ± 3	610 ± 700	7,500 ± 4,100
	L	5,300 ± 4,300	550 ± 590	6,200 ± 3,500	30 ± 8	15 ± 11	300 ± 350	3,800 ± 1,800
	K	4,400 ± 4,200	450 ± 550	3,800 ± 2,400	20 ± 7	10 ± 12	210 ± 230	2,700 ± 1,500
	M	8,500 ± 7,000	810 ± 850	8,100 ± 6,200	35 ± 27	13 ± 6	470 ± 570	5,200 ± 2,900
Juvenile male	F	8,400 ± 14,000	1,400 ± 2,500	5,200 ± 9,000	22 ± 12	1 ± 1	500 ± 900	6,200 ± 3,600
	L	15,000 ± 27,000	2,000 ± 3,800	8,300 ± 15,000	63 ± 82	9 ± 8	750 ± 1,400	7,600 ± 6,200
	K	8,300 ± 14,000	1,200 ± 2,200	5,000 ± 8,700	37 ± 37	4 ± 3	380 ± 690	5,300 ± 3,900
	M	24,000 ± 46,000	3,100 ± 6,000	13,000 ± 25,000	63 ± 95	8 ± 10	1,100 ± 2,100	6,700 ± 8,000
Neonate male	F	9,000 ± 19,000	730 ± 1,500	3,900 ± 8,200	30 ± 20	1 ± 1	110 ± 170	3,500 ± 4,100
	L	7,200 ± 15,000	440 ± 860	2,400 ± 4,800	31 ± 17	6 ± 5	69 ± 100	5,400 ± 9,600
	K	5,900 ± 13,000	370 ± 760	1,900 ± 3,900	23 ± 15	2 ± 1	58 ± 92	2,500 ± 3,400
	M	7,800 ± 16,000	520 ± 1,100	2,900 ± 6,000	29 ± 22	5 ± 3	77 ± 100	5,700 ± 10,000
Adult female	F	11,000 ± 18,000	700 ± 1,000	2,300 ± 2,900	22 ± 14	0 ± 0	100 ± 98	3,400 ± 4,500
	L	8,000 ± 14,000	400 ± 600	1,500 ± 1,900	47 ± 45	9 ± 14	62 ± 65	2,900 ± 4,200
	K	2,600 ± 3,500	150 ± 170	500 ± 490	18 ± 10	3 ± 1	21 ± 17	1,400 ± 2,400
	M	5,800 ± 8,000	310 ± 320	1,100 ± 960	45 ± 50	7 ± 6	62 ± 46	2,900 ± 4,700
Subadult female	F	120	28	160	27	0	49	19,000
	L	130	18	100	46	5	20	12,000
	K	124	17	80	31	3	28	8,900
	M	230	41	220	57	3	79	27,000
Juvenile female	F	11,000 ± 22,000	1,800 ± 3,400	10,000 ± 20,000	29 ± 10	1 ± 0	370 ± 700	12,000 ± 17,000
	L	8,000 ± 14,000	960 ± 1,700	6,800 ± 12,000	38 ± 15	4 ± 6	180 ± 300	4,400 ± 3,000
	K	4,900 ± 8,700	570 ± 990	3,700 ± 6,700	25 ± 8	2 ± 1	100 ± 160	3,400 ± 2,600
	M	9,700 ± 14,000	1,000 ± 1,400	5,700 ± 9,600	49 ± 29	5 ± 3	210 ± 310	7,300 ± 6,800
Neonate female	F	750 ± 960	76 ± 85	970 ± 1,200	18 ± 8	0 ± 0	37 ± 11	2,800 ± 1,200
	L	1,400 ± 1,800	110 ± 120	1,700 ± 2,000	33 ± 4	3 ± 4	49 ± 28	3,600 ± 530
	K	1,100 ± 1,300	78 ± 89	990 ± 1,200	25 ± 6	2 ± 1	33 ± 23	2,200 ± 34
	M	900 ± 1,100	61 ± 56	770 ± 830	32 ± 3	3 ± 3	32 ± 6	2,400 ± 1,100

Table 2. Comparison of POP and MeO-PBDE results between dolphins in the present study and various cetaceans from Australia. Baleen whales (Mysticeti) and dugongs are not included due to the difference in diet. Please note that sums are not necessarily composed of the same congeners in all studies. All results are in blubber and expressed in ng/g lw: for results given in ww, an average lipid percentage (if not mentioned in the respective study) of 69% was used. 'Various species' include seals and odontocetes other than delphinids.

Species	DDXs	PCBs	CHLs	HCB	HCHs	PBDEs	PCDD/Fs	MeO-PBDEs	Ref
Tursiops aduncus	330 - 34,000	97 - 25,000	55 - 10,000	23 - 160	0.30 - 8.0	0.80 - 91	0.006 - 0.017	580 - 26,000	This study (YI group)
Tursiops aduncus	89 - 46,000	67 - 50,000	6.6 - 7,800	2.9 - 59	< 2.2	23 - 1,900	0.003 - 0.054	360 - 42,000	This study (YII group)
Various species*	43 - 41,000	72 - 5,600	10 - 68	420 - 590					Kemper et al. (1994)
Delphinus delphis	14,000	260							
Tursiops spp.	Up to 54,000	87							
Stenella attenuata	1,700	1,200	3						
Sousa sahulensis	1,800-17,000	1,600-370,000	270 - 1,000	9.4 - 17	1.7 - 15	150 - 440	0.01 - 3.0		Weijs et al. (2016)
Sousa sahulensis	310 - 6,200	780 - 94,000		<0.10 - 46					Cagnazzi et al. (2013)
Orcaella heinsohni	180 - 16,000	170 - 21,000		<0.10 - 50					
Tursiops spp.		760 - 39,000							EPA (2000)
Various species*						4.3 - 50			Symons et al. (2004)
Tursiops spp.						80-210			Law et al. (2003)
Peponocephala electra						230			
Tursiops spp	680 - 53,000	790 - 26,000	9.3 - 470	5.9 - 23					Vetter et al. (2001)
Delphinus delphis	580	630	24	40					
Sousa sahulensis		900					0.14		Gaus et al. (2005)
Tursiops spp.		2,800					0.029		
Various species*		18 - 77					0.015 - 0.11		
Globicephala melas	92 - 4,700	59 - 1,400	12 - 530	15 - 380		2 - 54		59 - 1,600	Weijs et al. (2013)

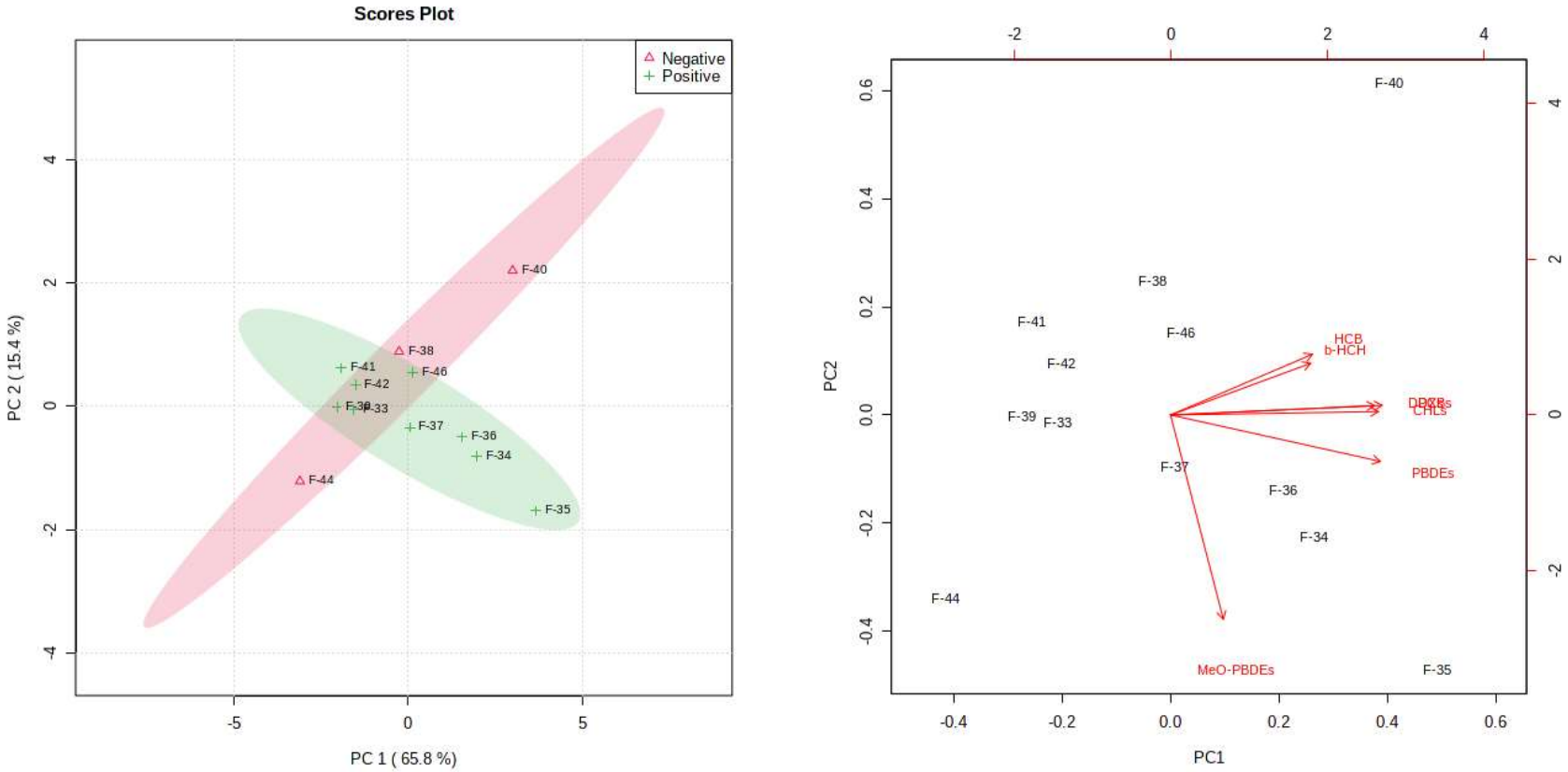
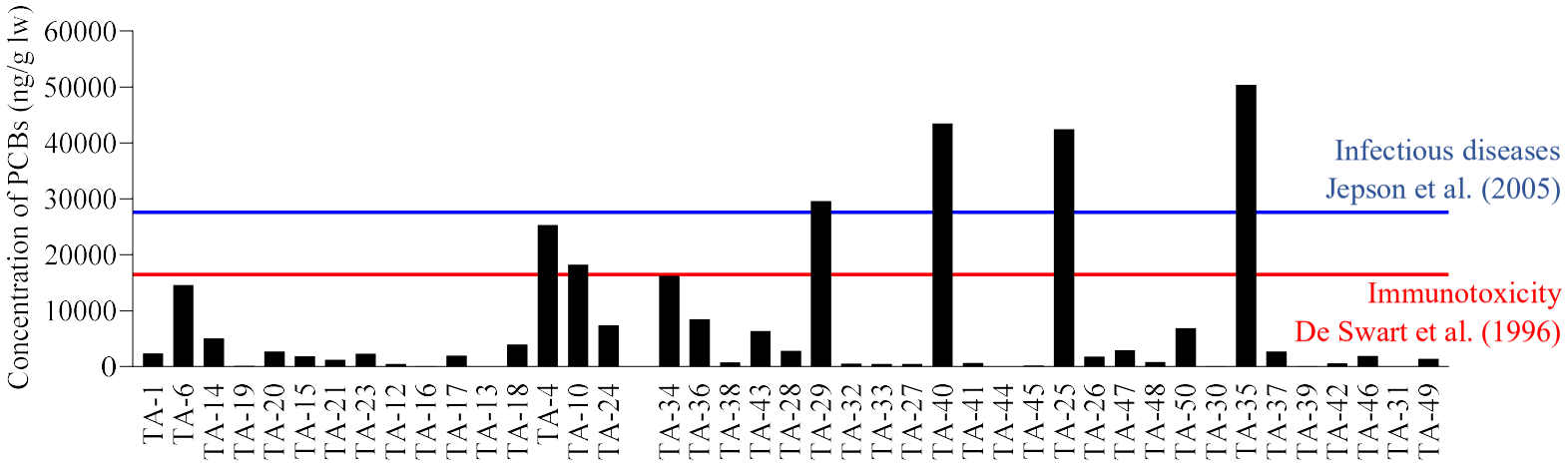
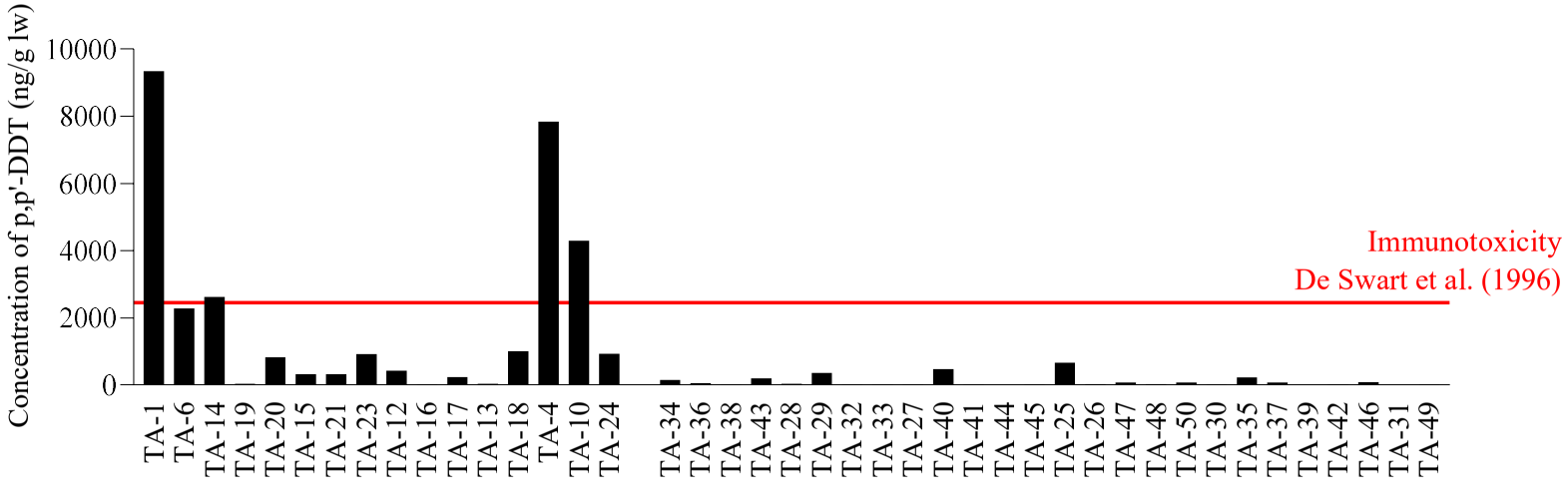


Figure 1. Principal component analysis of all compound groups for the animals that died during the unusual mortality event in 2013 (n = 12 in total; n = 9 animals are morbillivirus victims (positive IHC morbillivirus test) and n = 3 animals are morbillivirus carriers (negative IHC morbillivirus test but PCR positive)). Results given for blubber; results for liver, kidney and muscle can be found in Fig S4. Label numbers in Figures 1 and S4 refer to the TA-codes, label letters refer to the tissue type (for example: F-43 is blubber tissue of individual TA-43).

A


Concentration of PCBs (ng/g lw)
60000
50000
40000
30000
20000
10000
0
Infectious diseases
Jepson et al. (2005)
Immunotoxicity
De Swart et al. (1996)
TA-1 TA-6 TA-14 TA-19 TA-20 TA-15 TA-21 TA-23 TA-12 TA-16 TA-17 TA-13 TA-18 TA-4 TA-10 TA-24 TA-34 TA-36 TA-38 TA-43 TA-28 TA-29 TA-32 TA-33 TA-27 TA-40 TA-41 TA-44 TA-45 TA-25 TA-26 TA-47 TA-48 TA-50 TA-30 TA-35 TA-37 TA-39 TA-42 TA-46 TA-31 TA-49

B


Concentration of p,p'-DDT (ng/g lw)
10000
8000
6000
4000
2000
0
Immunotoxicity
De Swart et al. (1996)
TA-1 TA-6 TA-14 TA-19 TA-20 TA-15 TA-21 TA-23 TA-12 TA-16 TA-17 TA-13 TA-18 TA-4 TA-10 TA-24 TA-34 TA-36 TA-38 TA-43 TA-28 TA-29 TA-32 TA-33 TA-27 TA-40 TA-41 TA-44 TA-45 TA-25 TA-26 TA-47 TA-48 TA-50 TA-30 TA-35 TA-37 TA-39 TA-42 TA-46 TA-31 TA-49

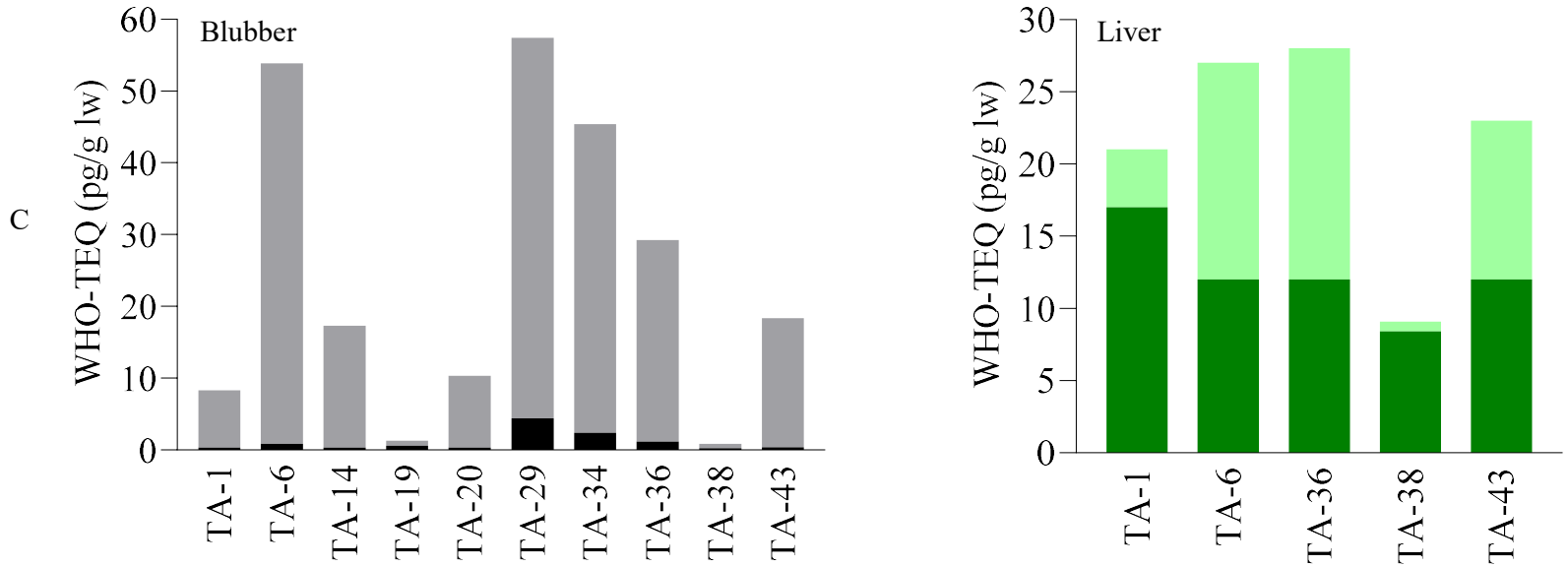


Figure 2. Potential health impacts of PCBs, *p,p'*-DDT, and PCDD/Fs in the dolphins investigated in the present study. A) PCB levels in blubber (present study) vs PCB toxicity thresholds from the literature, B) *p,p'*-DDT levels in blubber (present study) vs *p,p'*-DDT toxicity thresholds from the literature, C) WHO-TEQ values for PCDD/Fs and dioxin-like PCBs in blubber (n=10; grey for WHO-TEQ of dioxin-like PCBs, black for WHO-TEQ of PCDD/Fs) and liver (n=5; dark green for WHO-TEQ of PCDD/Fs, light green for WHO-TEQ of dioxin-like PCBs). WHO-TEQ values were calculated using the TEF values from Van den Berg et al. (2006). Dioxin-like PCBs included in Fig C were PCBs 105, 118 and 156. The red line represents the toxicity threshold for immunotoxicity in harbour seals (De Swart et al., 1996); the blue line represents the toxicity threshold for infectious diseases in harbour porpoises (Jepson et al., 2005).