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PCB LEVELS IN TISSUES OF THE STRICTLY HERBIVOROUS AUSTRALIAN DUGONG (DUGONG DUGON)

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Introduction

The marine mammal dugong (*Dugong dugon*) is classified as 'vulnerable' on the IUCN Red List of Threatened Species (1). They are the most widespread Sirenians and occupy shallow, coastal waters in the Indian Ocean and Pacific Ocean covering several continents including Australia. As inhabitants of coastal waters, they face numerous anthropogenic (e.g. boat strikes, tourism, pollution) and natural stressors (e.g. cyclones, storms) that can have a direct as well as an indirect impact on the animals. Dugongs are strictly herbivorous and rely on sea grass beds as food source. Among all potential stressors, pollution in general has been recognised as important as it can impact both the seagrass beds as well as the health of dugongs. Pollution is, however, still among the least characterised threat (2). In the past, levels of several contaminant classes were reported in dugongs in Asia and Australia (3-8), however, PCBs (polychlorinated biphenyls) were only targeted in two studies, Kumar et al. (3) for dugongs from Thailand and Vetter et al. (7) for dugongs from Queensland (QLD). PCBs are legacy organochlorine pollutants that are known to bioaccumulate in marine mammal tissues. These compounds were recently found to be present at high levels in humpback dolphins (*Sousa sahalensis*) from coastal waters in southeast Queensland, Australia (9), and were therefore the focus of this study.

Materials and methods

Samples. Liver, muscle and blubber tissue of 5 males and 5 females (n = 9 for liver, n = 5 for muscle, n = 6 for blubber) were analysed as well as a faeces sample of one individual. All males were from central Queensland (Townsville), collected during 1998-1999. All females were from southeast Queensland (Moreton Bay), Queensland, collected during 2001-2005. All samples were archived at -20 °C. Biological information can be found in Table 1.

Sample preparation, clean-up and analysis. Target analytes were PCBs 28, 52, 101, 118, 153, 138, and 180. Blubber was extracted using an acid digestion method. Liver and muscle samples were freeze-dried, homogenized with coarse Na₂SO₄ and extracted with hexane/acetone (1/1). Lipid contents were determined gravimetrically. Extracts were spiked with isotopically labelled target compounds (dioxins/furans, dioxin-like PCBs and marker PCBs), pre-treated with acid silica and cleaned up using acid, neutral and basic silica columns followed by fractionation on activated carbon (ENVI-Carb Supelco). The dioxin/furan fraction is not discussed here. For the PCB fraction, chromatographic separation was achieved on a gas chromatograph (Thermo Scientific Trace 1300) equipped with a DB-5-MS column (30 m × 0.25 mm i.d.) (Agilent Technologies, Santa Clara, USA). Mass spectrometry was performed on a high resolution mass spectrometer (Thermo Scientific DFS) operated in electron impact (EI) mode at mass resolution R ≥ 9,000.

QA/QC. Quantification was carried out over two recorded masses by isotope dilution for all analytes against the added ¹³C₁₂-labelled quantification standards. Blanks, a reference sample (humpback dolphin blubber analysed repeatedly, 9), calibration standards were included to ensure data reliability.

Statistics and data handling. Because of the overall low sample size, the different locations and years for males and females, the different tissue types and the different age classes, no statistical tests were performed. Across all genders and age classes, the lipid percentages varied considerably and ranged from 3.0 to 89.7 % in blubber, from 2.5 to 6.1 % in liver, and from 0.2 to 2.0 % in muscle. The sample of faeces had a lipid percentage of 2.0 %. In the present study, all results were normalized to their lipid contents and are expressed in pg/g lipid weight (lw) (Table 1).

Results and discussion

Compared to other areas across the globe, PCBs have never been extensively monitored in Australian marine wildlife. A recent study, however, found that PCB concentrations in blubber of several Australian humpback dolphins were well above toxicity levels associated with immunotoxic and reproductive effects in marine mammal species (9). These findings fuelled the present study in which another coastal

species – dugongs – was investigated. Despite sharing a similar habitat, dugongs are strictly herbivorous in contrast to the piscivorous humpback dolphins. As such, dugong exposure to PCBs would be expected to be considerably lower. However, partitioning processes between sediment, water and plants can concentrate some POPs on seagrasses, and uprooting sea grass brings dugongs close to sediments and associated pollutants thereby potentially contributing to the total pollutant loads in this species.

Levels, patterns and potential toxicity. Levels of Σ PCBs ranged from 1,620 to 10,920 pg/g lw in liver (median 4,662 pg/g lw), from 1,008 to 12,537 pg/g lw in blubber (median 8,083 pg/g lw), and from 1,486 to 42,284 pg/g lw in muscle (median 18,631 pg/g lw). The observation of muscle containing considerably higher lipid normalised PCB levels is unexpected for lipophilic and persistent compounds when exposure has occurred over a lifetime. However, this may be due to the low sample muscle mass available for some muscle samples, and their generally low lipid content and associated error in gravimetric measurements; muscle lipid contents ranged from 0.2 to 2.0 %.

PCB profiles were similar among tissue types, but differed across matrices. Muscle and liver were dominated by lower chlorinated PCBs (PCB 28 and 52; contributing up to 63% to Σ PCBs) while blubber showed higher contributions of higher chlorinated PCBs (PCB 153, 138; up to 70% of the sum) (Fig 1). The PCB levels found in the present study are orders of magnitude lower than those associated with impaired health effects in other marine mammals (9). Although only few samples were analysed and the sensitivity of dugongs to POPs is generally unknown, our findings suggest that contamination with PCBs was unlikely a major risk to the health of dugongs from 1998 to 2005 in central and southeast QLD.

Interspecies comparisons. Levels of PCBs are typically difficult to compare to other studies due to the different number of PCBs included in the sums. Usually, this can be addressed by comparing PCB 153 levels as this is the most dominant PCB congener across tissues in most marine mammal species. Our results, however, show that PCB 153 is only useful for comparing blubber as this congener contributes only a minor proportion to the sum of PCBs in liver and muscle. The highest PCB 153 level found in blubber in this study (4,954 pg/g lw) is approximately 34,000 times lower than the highest PCB 153 level found in blubber of a humpback dolphin from the same area (Moreton Bay, Queensland; 9). In contrast, Σ PCBs in muscle of dugongs from Queensland (sum of 7 congeners) is higher than in muscle of dugongs from 1999-2001 from Thailand (3; sum of 14 congeners).

Toxicokinetics and future work. The present study investigated PCB levels in multiple tissues of a limited number of animals from different locations, gender, age class, body size, and sampling years. From a monitoring perspective, it is very difficult to draw conclusions from such a dataset as it is well-known that exposure differs spatially, that females have an additional elimination pathway compared to males thereby lowering their pollutant body burdens, and that persistent pollutants such as PCBs accumulate with age in animals. For this reason, toxicokinetic models can be valuable tools to shed some light on the distribution as well as the age-dependent accumulation of PCBs within the individuals. Such models can also give more insights on how the levels of PCBs have changed over time which is especially useful considering the high PCB levels recently found in archived humpback dolphins sampled from 2002 up to 2014 and the intensive developments along the Queensland coasts.

Acknowledgements

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Table 1. Levels of selected PCB congeners in liver (L), muscle (M), blubber (F), and faeces (FC) of dugongs (*Dugong dugon*) from Queensland, Australia.

Sample ID #	14			6		13			11	17			60			57		64		41		66
Tissue	L	M	F	L	M	L	M	F	L	M	L	M	F	L	L	FC	F	L	L	F	F	
Location	Hinchinbrook					Townsville						Moreton Bay										
Year	1999			1998		1999			1999	1999			2004			2004		2005		2001		2005
Gender	Male											Female										
Age class	Adult			Immature		Juvenile			Immature	Adult			Adult			Adult		Adult		Adult		
Size	235			117		194			130	220			294.5			265		227		308		320
Lipid %	4.7	2.1	82.8	6.1	1.7	2.5	0.2	8.4	5.4	0.4	2.8	0.2	3.0	5.0	2.7	2.0	42.2	2.6	4.0	89.7	80.3	
PCB 28	643	317	46*	2988	1839	552	2211	707	1967	4387	789	9120	416*	619	4221	9500	295	955	347	171	90	
PCB 52	204	202	125	1313	621	286	1854	306*	784	1413	419	6173	194*	270	1785	3175	1816	306	304	222	109	
PCB 101	191	328	337	976	702	491	3238	310*	504	1521	700	7061	315*	195	1724	1738	1342	384	435	413	138	
PCB 118	137	189	279	533	638	568	2684	1235	431	2462	1691	4589	1137*	273	1022	1239	983	1073	496	846	151	
PCB 153	202	200	1124	428	837	413	7765	2755	438	4349	2247	8430	4954	405	707	1481	1326	1221	893	3041	203	
PCB 138	206	224	450	441	586	896	4225	2494	494	3697	2521	5498	3728	763	775	1622	2049	6342	767	1979	266	
PCB 180	36*	26*	233	66	77	89	1262	653	44	802	587	1413	1792	360	61	1100	264	640	214	1418	50*	

* - values are < 3 times the blank value

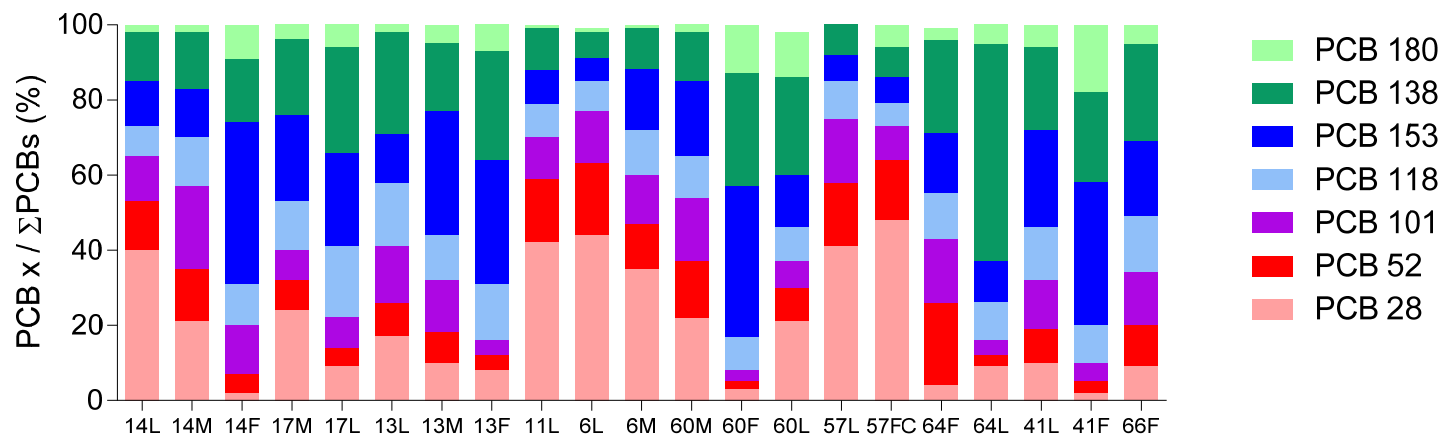


Fig 1. Relative contributions of each PCB congener to the sum of PCBs in several tissues (liver (L), blubber (F), muscle (M) and faeces (FC)) of dugongs from Australia.