Dioxins and Dioxin-Like PCBs in Marine Mammals from Australia

Caroline Gaus[1], Ray Correll[2], Jochen Mueller[1], Eva Holt[1], David Ellis[2], Joelle Prange[1], Melanie Shaw[1], Ulrike Bauer[1], Robert Symons[3], Debbie Burniston[3]

[1]National Research Centre For Environmental Toxicology
[2]CSIRO
[3]National Measurement Institute

Introduction

The marine system represents the ultimate environmental sink for persistent organic pollutants such as dioxins and dioxin-like PCBs. Due to their resistance to degradation and metabolism, these contaminants tend to accumulate in the marine environment, often to elevated concentrations. Marine biota can be exposed to persistent organic pollutants through various pathways, predominantly by the intake of food and/or exchange of water. High lipophilicity of dioxin-like compounds and relatively low metabolic capacities for these in most animals contributes to accumulation in biota over a life time. In addition, biomagnification can occur, resulting in elevated concentrations of toxicologically relevant compounds in higher trophic level animals. The majority of marine mammals represent long-lived, higher trophic wildlife, with large fat repositories, and are among the most vulnerable to contamination of their habitat.[1,2] However, only limited information exists on the levels, exposure, sensitivity or metabolism of these animals to dioxins and dioxin-like compounds in general, and only few data are available from Australia. This is partially a result of the logistical difficulties involved with executing rigorous and extensive sampling regimes in the marine environment to resolve data for the numerous factors that influence contaminant levels and pathways, in particular biometric parameters such as age, gender, habitat location and trophic level.

The Australian Government Department of Environment and Heritage (DEH) implemented a National Dioxin Program (NDP) in 2001. Under this program, information on the current levels of dioxins and dioxin-like compounds in Australia were gathered to provide data for risk assessment and the development of reduction, and where feasible, elimination measures for dioxins in Australia. The present study formed part of the NDP Ambient Environmental Levels data gathering phase, with the overall objective to determine levels of dioxins in Australian fauna across a range of urban, agricultural and remote reference areas. Marine mammals were included as a small part of this study to provide an understanding on wildlife contamination in the marine environment.

Materials and Methods

Fat tissue samples were obtained opportunistically from 13 marine mammals stranded along the coastlines of the Northern Territory, South Australia and Tasmania. Since sampling was reliant on stranded animals within a relatively short time period, the samples obtained were non-randomized. The majority of animals (n=9) represented whales sampled from Tasmania, including sperm whales (n=7), long-finned pilot whale (n=1) and beaked whale (n=1). A sea lion (n=1) and bottlenose dolphin (n=1) were obtained from South Australia and two samples were obtained from the Northern Territory, including a humpback dolphin (n=1) and dugong (n=1).

Marine mammals were classified into trophic levels according to standardised diet compositions derived from published accounts of stomach contents and morphological, behavioral and other information.[3] Unlike most terrestrial fauna, however, there is only limited information available on diet composition of many marine mammals and trophic level estimates vary accordingly in the literature. Among the marine mammals analysed for this study, the dugong, as a primary consumer (predominant food source: seagrass), represented the lowest trophic level. Among the remaining animals, the humpback dolphin, and sea lion represented the lowest trophic level, followed by the beaked whale and finally the sperm and long-finned pilot whales at the highest trophic level.

Samples were analysed by the National Measurement Institute (Sydney). In brief, thawed blubber, mixed with hydromatrix, was extracted with ethanol:toluene (68:32) using an ASE 100 (Dionex). Approximately 1-5 g of extracted lipid was spiked with a known amount of $^{13}C_{12}$ surrogate spiking solutions. Lipid was cleaned up using multiple

extractions with concentrated sulfuric acid. Extracts were concentrated prior to clean-up on the Power-PrepÔ system, including multi-layer silica and alumina column and carbon column. Two fractions were eluted from the carbon column, the first containing the mono-ortho and di-ortho PCBs, the second fraction containing the non-ortho PCBs and all of the PCDD/F congeners. Both fractions were concentrated to almost dryness and recovery standards added to a final volume of 10 µL. All analysis was carried out on a MAT95XL HRMS (ThermoFinnigan) coupled to an Agilent 6890 GC equipped with a CTC A200S autosampler. A DB-5 capillary column (60m x 0.25mm i.d., film thickness 0.25µm) was used as the primary analytical column. Resolution was maintained at 10,000 throughout the sample analysis. Multiple ion detection (MID) was performed in the electron impact mode with monitoring of the masses of appropriate ions for native and labelled compounds. Individual congeners were identified using the GC retention time and ion abundance ratios with reference to internal standards. TEQs were calculated using Toxic Equivalency Factors (TEFs) assigned by the World Health Organization.[4] Compounds that were below the limit of detection (LOD) were incorporated into the TEQ as 50% of the reported values.

Results and Discussion

PCDD/F and PCB concentrations in the thirteen marine mammals ranged from 9.8 to 140 pg g^{-1}lipid and 490 to 2,800,000 pg g^{-1} lipid, respectively (Table 1). In the majority of animals, PCDDs and mono-ortho PCBs contributed the highest concentrations among their compound groups, respectively. Sum TEQ levels ranged from 1.1 to 590 pg g^{-1} lipid (Table 1). PCBs contributed the greatest proportion (64-99%) to the total TEQ in all animals except the dugong (27% of total TEQ). Congeners of note included PCB 126, 169, 156 and 118 in whales, PCB 156, 118 and 105 in dolphins, and PCB 126 and 118 in the sea lion. Only in two animals did PCDDs (70% in the dugong and 35% in the sea lion) contribute considerable proportions to the total TEQ.

Considering all available data for marine mammals from Australia[5-10], whale species generally appear to have relatively low TEQ levels, whereas elevated levels have been found in dolphins, in particular from South Australia and the Northern Territory. $TEQ_{PCDD/Fs}$ obtained for whales during this study are comparable to other whale species analysed from Queensland[9], and relatively low compared to those reported elsewhere. Similarly, $TEQ_{PCDD/Fs}$ in dolphins analysed for this study were relatively low and comparable to previous reports from dolphins in Queensland[6,9] and South Australia.[8] In contrast, however, the TEQ_{PCBs} in the dolphins analysed for this study (from Port Adelaide: 590 pg g^{-1} and Darwin: 150 pg g^{-1} lipid) were elevated, and similar high TEQ_{PCB} levels (280 and 440 pg g^{-1} lipid) were reported previously from two bottlenose dolphins from Port Adelaide.[8] Interestingly, two bottlenose dolphins from the nearby Spencer Gulf[8], as well as the sea lion obtained for this study (200 km SW of Port Adelaide, on the "clean" offshore site near Kangaroo Island), contained an order of magnitude lower TEQ levels (dolphins: 10 and 37; sea lion: 20 pg g^{-1} lipid) compared to the Port Adelaide samples. This indicates the presence of a significant PCB point source into the bay of Port Adelaide. On an international scale, TEQ levels in bottlenose dolphins from Port Adelaide are comparable to those reported in cetaceans from areas considered relatively polluted, such as the Mediterranean (Risso's and bottlenose dolphins, average 300 pg g^{-1} lipid, n=8[11]) or British Columbia (killer whale, average 660 pg g^{-1} lipid.[12] The dugong (originating from Darwin) analysed for the present study represented the animal with the lowest TEQ levels, which is in accord with their low trophic position as herbivorous mammals, however, a dugong analysed previously from the same area contained more than 30 times higher TEQ levels (33 pg g^{-1} lipid).[7] Such variability within a region is most likely attributable to biological parameters (e.g. age, gender, parity), since congener profiles are similar for these dugongs, indicating that sources and pathways are comparable. In addition to individual variability due to biological parameters, regional contamination in Queensland represents an important factor that can considerably influence contaminant levels in marine mammal populations.[7] For example, while dugongs from far northern Australian regions have relatively low PCDD/F and TEQ concentrations, dugongs from different regions within the state of Queensland have been found to contain 5-170 times (depending on habitat region) higher (n=35) TEQ levels. These differences have been suggested to be the consequence of considerable differences in riverine PCDD/F inputs into the near shore habitats, in combination with low metabolism of toxicologically relevant congeners.

PCB congener profiles were relatively similar among the different marine mammal species. PCB 118 dominated the profile (56-73%) in the majority of animals. PCB 105 contributed 16-21% to the sum of the 12 PCB congeners analysed, followed by PCB 156 (2.2-12%) and PCB 167. In contrast to PCB congener distributions, the PCDD/F

profiles showed marked differences between some of the animals analysed. In particular, the bottlenose dolphin and sea lion were found to have markedly different congener profiles with higher contributions of TCDF and PeCDD, respectively. Both samples originated from South Australia and feed on fish as a predominant food source. However, the two animals originated from different habitats, including a habitat from Port Adelaide (bottlenose dolphin) and a relatively clean oceanic site with few direct urban and industrial influences near Kangaroo Island (sea lion). Unusually elevated concentrations of PCDFs (in addition to elevated PCB levels) were evident in the bottlenose dolphin, suggesting local PCB contamination in Port Adelaide. In contrast, the PCDD/F congener profile of the sea lion was dominated by PCDDs, and TEQ levels were approximately 30 fold lower compared to the dolphin (Table 1).

Different trophic level animals for the same location were only available from Darwin in the Northern Territory, and included the carnivorous humpback dolphin (sum PCBs = 900,000 pg g^{-1}; sum PCDD/Fs = 140 pg g^{-1}) and the herbivorous dugong (sum PCBs = 490 pg g^{-1}; sum PCDD/Fs = 9.8 pg g^{-1}). A comparison of the congener profiles between these two species show similar patterns, suggesting that both were exposed to similar sources. However, higher contributions of PCDD/F congeners with no chlorines in the 1,4 and/or 1,9 positions to the total PCDD/F concentration were apparent in the humpback dolphin (27%) compared to the dugong (12%). These congeners have been demonstrated to biomagnify through food webs[13], and represent the congeners with the highest TEF factors.[4] Correspondingly, TEQ levels in the humpback dolphin are approximately 14 fold higher compared to the dugong (sum TEQ = 170 and 1.1 pg g^{-1} lipid, respectively). An initial comparison of trophic levels with analyte concentrations and TEQ levels obtained for all marine mammals analysed for this study did not reveal any correlations. A previous investigation in Queensland, Australia, showed that in particular coastal versus offshore habitat locations can have strong impacts on PCDD/F contaminant levels in marine mammals (due to terrestrial runoff of PCDD/Fs and corresponding accumulation predominantly in the near shore environment).[7,9] Considering this, a separate comparison of trophic levels with TEQ levels was undertaken for coastal and pelagic/offshore species analysed for this study. This analysis indicated a trend of increasing TEQ with increasing trophic position, and more than 10 fold higher TEQ levels in coastal animals compared to those with offshore/pelagic habitats.

In conclusion, the information gained from this and previous studies suggest that offshore and pelagic marine mammals from Australia are exposed to relatively low PCDD/F and PCB concentrations, whereas species and populations that rely on near shore habitats can be exposed to elevated levels of these compounds. The extent of the latter is strongly determined by biological, physical and chemical parameters, in particular the animals' age, gender, trophic position, and importantly, point source inputs from terrestrial runoff and riverine systems. This should be taken into account for any evaluations on the risks for marine wildlife from dioxins and dioxin-like compounds.

Table 1. PCDD/F and PCB concentrations (pg g^{-1} lipid) in marine mammals from Australia (values in italics represent ½ the values reported D-dugong, HD-humpback dolphin, BD-bottlenose dolphin, SW-sperm whale, BW-beaked whale, PW-pilot whale, SL-sea lion, NT-Northern Territory, SA-South Australia, TAS-Tasmania.

	D (NT)	HD (NT)	BD (SA)	SL (SA)	SW (TAS)	SW (TAS)	SW (TAS)	SW (TAS)	SW (TAS)	SW (TAS)	SW (TAS)	BW (TAS)	PW (TAS)
TCDD	0.15	1.1	0.51	1.2	0.3	0.3	0.15	0.5	1	1	0.5	0.1	0.2
PD	0.45	9.5	0.75	5.4	1.8	1	1.5	1.8	1	1	0.5	0.1	0.1
H1D	0.35	7.6	0.24	0.61	1	2.3	1	0.5	0.5	1	0.5	0.1	0.035
H2D	0.4	16	0.55	1.7	4.5	5.7	2.5	4.2	3.4	6.1	3.3	0.1	0.1
H3D	0.68	4.1	0.1	0.32	0.35	0.76	0.35	0.3	0.15	0.2	0.15	0.1	0.05
HpD	1.9	16	1.1	0.88	5.5	4.2	3.5	1	1	9.1	2.9	1	1.2
OCDD	5.6	73	6.9	3.3	35	6.2	9.9	16	4.1	88	11	47	13
TCDF	0.05	5.6	10	0.35	0.2	0.2	0.5	0.2	0.2	0.45	0.5	1	0.35
P1F	0.05	1.7	2.7	0.19	0.2	0.2	0.35	0.2	0.1	0.15	0.2	0.045	0.05
P2F	0.05	3.1	3.5	0.25	2.7	2.7	3.3	2.3	1.5	2.7	2.2	0.35	0.45
H1F	0.01	0.61	2	0.15	1.4	1.9	1	0.5	1	0.5	0.45	0.1	0.15
H2F	0.01	1.1	0.045	0.05	0.15	0.15	0.1	0.05	0.05	0.15	0.1	0.05	0.04
H3F	0.015	0.43	0.05	0.025	0.2	0.25	0.2	0.15	0.2	0.3	0.25	0.25	2.5
H4F	0.01	0.03	0.02	0.015	0.15	0.025	0.02	0.03	0.02	0.035	0.05	0.045	0.025
Hp1F	0.05	0.1	0.28	0.045	0.25	0.25	0.15	0.15	0.15	0.5	0.2	0.2	0.2
Hp2F	0.025	0.15	0.02	0.02	0.15	0.1	0.1	0.1	0.045	0.05	0.05	0.1	0.05
OCDF	0.035	0.05	0.1	0.05	0.5	0.35	0.05	0.5	0.15	2.8	0.25	1.2	0.35
Sum PCDD/F	9.8	140	29	15	54	27	25	28	15	110	23	52	19
PCB 77	2.9	280	550	11	6.6	8.6	6.6	11	6.1	5.9	14	98	48
PCB 81	0.67	120	440	8.1	8.4	11	11	7.2	7.8	9.4	14	47	34
PCB 126	2.3	110	1700	38	180	230	250	160	140	200	120	140	64
PCB 169	0.5	1100	410	2.8	410	490	480	360	330	480	230	110	500
PCB 105	95	190000	600000	16000	4300	6000	6700	4600	3100	6300	3400	8100	13000
PCB 114	2	10000	29000	480	390	540	610	440	320	500	280	740	930
PCB 118	300	570000	1900000	56000	14000	23000	23000	16000	11000	20000	10000	32000	39000
PCB 123	3.5	6800	44000	720	960	1200	1200	400	300	1000	570	1600	1000
PCB 156	38	75000	210000	1700	3100	4200	4400	3400	2700	4300	1800	5600	6800
PCB 157	3	25000	73000	1100	560	640	840	620	500	790	400	1200	1800
PCB 167	43	20000	5000	450	1000	1000	1500	1000	500	1000	500	1500	2500
PCB 189	2.6	5300	27000	56	290	340	440	350	260	410	200	400	610
Sum PCB	490	900000	2800000	77000	25000	38000	39000	27000	19000	35000	18000	52000	66000
TEQ	1.1	170	590	20	30	37	40	28	24	35	20	24	22

Acknowledgements

This study was funded by the Australian Government Department of the Environment and Heritage (under the NDP). The views expressed herein are not necessarily those of the Commonwealth of Australia. EnTox is co-funded by Queensland Health. Thanks to the staff at the National Measurement Institute's Dioxin Analysis Unit for analysis, and the NT Museum, Tasmanian Museum and Art Gallery and SA Museum for provision of samples.

References

1. Tanabe S, Watanabe S, and Tatsukawa R (1988) *Marine Mammal Science* 4: 103-124.

2. Borrell A, Aguilar A, Corsolini S, and Focardi S (1996) *Chemosphere* 32: 2359-2369.

3. Pauly D, Trites AW, Capuli E, and Christensen V (1998) *ICES Journal of Marine Science* 55: 467-481.

4. Van den Berg M, Birnbaum L, Bosveld ATC, Brunstroem B, et al. (1998) *Env. Health Persp.* 106: 775-792.

5. Haynes D, Mueller JF, McLachlan MS (1999) *Chemosphere* 38: 255-262.

6. Gaus C, Päpke O, Blanchard W, Haynes D, Connell DW, and Müller JF (2001) *Organohalogen Compounds* 52: 95-99.

7. Gaus C, O'Donohue M, Connell D, Mueller J, Haynes D, and Paepke O (2004) *Organohalogen Compounds* 66: 1559-1566.

8. Ruchel M (2001) Greenpeace Australia-Pacific Pty Ltd, Sydney.

9. Gaus C (2002) School of Public Health, Department of Health, Griffith University, PhD Thesis

10. Symons RK, Burniston D, Jaber N, Piro N, Trout M, Yates A, Gales R, et al. (2003) *Organohalogen Compounds* 62: 257-260

11. Jimenez B, Gonzalez MJ, Jimenez O, Reich S, Eljarrat E, and Josep R (2000) *Env. Sci. Technol.* 34: 756-763.

12. Ross PS (2000) *Human and Ecological Risk Assessment* 6: 29-46.

13. Broman D, Naef C, Rolff C, Zebuehr Y, Fry B, and Hobbie J (1992) *Env. Tox. Chem.* 11: 331-345.