CONTAMINANT EXPOSURE OF MASS STRANDED GREEN SEA TURTLES IN AN INDUSTRIAL AREA OFF THE GREAT BARRIER REEF, AUSTRALIA

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Introduction

Australia's Great Barrier Reef (GBR) Marine Park supports among the world's largest remaining congregations of endangered green sea turtles[1]. Seagrass beds in nearshore shallow marine areas of the GBR provide important foraging grounds to this species, to which they display remarkably high fidelity. Between 1 January 2011 and 28 February 2012, 260 green turtle deaths were recorded in the Gladstone area compared to 50-51 per year during 2008-2010[2]. There has also been an increase in the number of other wildlife strandings, as well as outbreaks of diseases in fish in this region[3]. Clinical examination of 56 green turtles in this area revealed that the juvenile turtle population were in poor health, due most likely to chronic malnutrition[4]. Diseases of the digestive, respiratory, and circulatory systems were found and, in most cases, may have developed secondary to chronic debilitation.

Extensive flooding occurred in summer of 2010-2011 across much of Queensland, including the Gladstone region, resulting in increased freshwater and sediment outflow, and reduced seagrass cover[5]. These climatic events are compounded by the Gladstone Harbour region undergoing substantial development of its port resources since May 2011[6]. As major port city for Queensland, Gladstone hosts a variety of industries, including mining and processing of minerals, liquefied natural gas, chemical industry, as well as agricultural activities within the catchment.

In parallel with health assessment of a local green turtle subpopulation[4], a comprehensive contaminant exposure assessment was conducted using predominantly blood of live captured specimens. Of interest were a wide range of inorganic and organic contaminants that may have been brought downstream from the catchment with flood waters, or have arisen from industrial activities. Here we focus predominantly on reporting the levels of persistent halogenated compounds, and present an exposure and risk assessment carried out for dioxins.

Materials and methods

Blood samples (10-25 mL) were obtained from 40 live green turtles during 8-11 July 2011 from the Boyne River Estuary near Gladstone (-23.9 °S, 151.3 °E; central Queensland, Australia). Most animals (n=39) were in their juvenile, benthic foraging life stage (average CCL 46; range 39-62 cm); only one specimen was an adult of unknown gender (CCL 100 cm). A large proportion of the animals were classified to have poor (35%; n=14) or very poor (20%; n=8) body conditions, with the latter showing signs of emaciation[4]. Despite this, blood lipid content did not differ significantly between animals with normal (0.14 ±0.038), poor (0.13 ±0.047) or very poor (0.16 ±0.064) body conditions, suggesting they consisted mainly of fats not used for storage (e.g. lipoproteins, cholesterol). Liver, fat and kidney were collected during necropsy of animals that were euthanized due to poor clinical diagnosis for survival (n=3), or found dead (n=6). One gram of each fat, liver and kidney were pooled for screening analysis. Whole blood was also pooled (1 mL subsample from all 43 live and euthanized animals) for screening analyses.

Analyses:

Analyses of all organic compounds were performed at Eurofins GfA in Hamburg, Germany in accordance with DIN EN ISO/IEC 17025:2005 and are detailed in ref[7]. Analysis for metals and metalloids was undertaken at Entox laboratories according to standardised protocols[7]. Non-target-screening using pooled liver samples was undertaken to identify any high levels of organic contaminants. Approximately 0.5 g was extracted with n-hexane using ultrasonication. The raw extract was injected on an Agilent 6890/5973 GC-MS system using a non-

polar DB5-type capillary column. EI-mode was used to scan a mass range of m/z 50-600. The 10 most abundant peaks were baseline subtracted and manually evaluated against spectra libraries (Wiley 75K; NIST). Target screening was carried out using pooled samples to provide initial information on the type and levels of contaminants to be expected, and inform further prioritisation for subsequent analysis: Fat was analysed for dioxins, and WHO and indicator PCBs; liver for PBDEs, organochlorine pesticides and organotins; and blood for PFOS/PFOA. In a third analytical phase, blood from individual live turtles underwent target analyses prioritised based on the screening results (i.e. based on contaminant type and expected concentrations, taking into account toxic potency). Analyses were carried out and evaluated on a batch-by-batch approach. Where the presence of low levels was consistent, the limited volume for blood samples was prioritised for other analytes. These latter analyses were carried out for organotins (n=7), WHO-PCBs (n=22), PCDD/Fs (n=22), and organochlorine pesticides (n=7); metals and metalloids were also analysed (n=40).

Dioxin and PCB Exposure/risk assessment:
A probabilistic approach was used to estimate the proportion of the turtles exceeding reported LOAELs for various chronic AhR-mediated toxicity endpoints in mammals and birds[8, 9], as no reptile-specific dose-response data is available. Lognormal frequency distributions were fitted to lipid normalised TEQ concentrations in juveniles using mammalian and avian TEFs separately, on both a middle and upper bound basis. TEQ was transformed to body burdens assuming a uniform distribution for total body lipid content (4 and 12% based on ref[10]). As only one adult specimen was obtained, juvenile turtles were assessed separately to the adult. The distribution of TEQ body burdens was then predicted using risk modelling software (Crystalball 2000 Decisioneering Inc.) and compared to body-burden based LOAELs.

Results and discussion
Non-target screening of pooled liver samples did not reveal particular signals that could be traced to environmental contaminants. Using the mass spectra for each of the peaks, the most abundant signals were associated with lipids and sterols naturally occurring in biological samples, or the plastic materials used for blood sampling (diisobutyl phthalate). Similarly, target screening for pesticides, organotins, PBDEs, dioxins and PFOS/PFOA showed their concentrations were mostly near or below the limit of quantification (LOQ) in pooled fat, liver and/or blood samples, and similar to background levels reported in other turtles or marine megafauna[11-13].

Target analysis of individuals further confirmed low levels of organotins and pesticides, which were mostly below LOQ. Middle bound TEQ_{df+pcb} in blood of individual juvenile green turtles from Gladstone averaged 19 pg g^{-1} lw (range <7.1-39; n=21) on a mammalian TEF (Table 1). Using avian TEFs, the levels were 33 pg g^{-1} lw (range 13-62). The TEQ_{df+pcb} levels in Gladstone juvenile specimens were comparable to the lower ranges reported for other juvenile green turtles and similarly low trophic marine mammals (dugongs) in Queensland[10]. The adult specimen contained considerably higher blood TEQ_{df+pcb} (130 pg g^{-1} lw), comparable to the upper ranges reported for higher trophic marine wildlife. The major proportion of this TEQ_{df+pcb} was derived from PCDD/Fs (120 pg g^{-1} lw). This may indicate chronic exposure to elevated levels of dioxins, rather than elevated recent exposure, but more adult specimens would be required to evaluate this.

Assuming that juvenile turtles included in this study are representative of the Gladstone population, the likelihood (% of population) of TEQ body burdens being at or above LOAELs[8, 9] was determined. At middle bound TEQ, up to 6.6% of the juvenile population exceeded LOAELs where biochemical effects may occur in mammals (3 ng kg^{-1} bw). On the more conservative upper bound TEQ basis, this percentage increased to 29%. On an upper bound TEQ basis, up to 5% of the population also exceeded the LOAEL for developmental toxicity effects in avian species (9 ng kg^{-1} bw). While only one adult specimen was obtained, it is noteworthy that the estimated body burden (5.0-15 ng kg^{-1} bw (middle bound; mammalian TEFs) and 4.7-14 ng kg^{-1} bw (middle bound; avian TEFs)) in this specimen exceeded the LOAEL for immunological and developmental effects in both mammals and birds, respectively.

While turtles from other areas in Queensland show similar dioxin (and PCB) concentrations, blood levels of several metals and metalloids were clearly higher in many individuals from Gladstone compared to those

reported for most other sea turtles worldwide. These included arsenic (40-20,000 µg/L), cadmium (8.1-110 µg/L), cobalt (28-440 µg/L), mercury (<0.22-38 µg/L), nickel (0.67-17 µg/L), selenium (84-8,600 µg/L) and vanadium (3.5-38 µg/L). As many of these elements are known to have relatively fast blood clearance half-lives, they are likely to reflect recent (weeks to month) exposure. Metal/metalloid concentrations in some of these animals were also above or near the concentrations where acute adverse health effects have been observed across different vertebrate taxa. Although the sensitivity of sea turtles to these contaminants is mostly unknown, it suggests exposure may have been sufficiently high to cause adverse effects.

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Table 1. Descriptive statistics for dioxin and PCB concentrations (pg g^{-1} lw) and middle bound TEQ (mammalian TEFs-05) in blood from individual (n=22) green turtles (Chelonia mydas).

Compound	Mean	Minimum	Maximum	25th percentile	Median	75th percentile	Standard error
Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs; ppt lw)							
TEQ_{05} ΣPCDD/Fs[†]	16	2.7	120	5.8	13	16	5.0
PCDDs							
2,3,7,8-TCDD	<3.9	<0.69	<12	<1.3	<2.8	<5.5	<0.70
1,2,3,7,8-PeCDD	9.0	<0.89	82	2.7	5.0	8.6	3.5
1,2,3,4,7,8-HxCDD	11	<0.22	90	1.8	3.8	12	4.0
1,2,3,6,7,8-HxCDD	12	2.8	100	4.2	5.9	14	4.3
1,2,3,7,8,9-HxCDD	11	2.7	76	4.1	5.9	13	3.3
1,2,3,4,6,7,8-HpCDD	54	18	180	32	40	65	8.2
OCDD	200	58	(<470)	110	150	260	25
PCDFs							
2,3,7,8-TCDF	11	<1.2	43	3.7	6.4	18	2.2
1,2,3,7,8-PeCDF	2.5	<0.44	6.7	1.0	2.1	3.4	0.41
2,3,4,7,8-PeCDF	3.0	<0.34	7.7	1.2	1.8	5.6	0.55
1,2,3,4,7,8-HxCDF	3.0	<0.32	14	0.93	1.7	3.5	0.71
1,2,3,6,7,8-HxCDF	2.7	<0.31	14	0.84	1.7	3.1	0.64
1,2,3,7,8,9-HxCDF	3.6	<0.42	26	1.3	2.6	3.5	1.1
2,3,4,6,7,8-HxCDF	3.2	0.61	19	0.92	1.8	3.2	0.84
1,2,3,4,6,7,8-HpCDF	11	2.5	26	5.1	9.0	16	1.4
1,2,3,4,7,8,9-HpCDF	5.3	<1.0	33	2.1	3.4	5.9	1.4
OCDF	49	19	98	32	43	62	4.8
Polychlorinated biphenyls (PCBs; ppt lw)							
TEQ_{05} ΣWHO-PCBs[†]	8.2	3.7	18	5.1	7.8	9.5	0.77
Non-Ortho							
PCB 77	<150	<75	<290	<100	<140	<190	<11
PCB 81	<55	<25	<110	<42	<54	<65	<4.2
PCB 126	<120	<50	<290	<69	<110	<140	<12
PCB 169	<150	<75	<300	<100	<150	<190	<12
Mono-ortho							
PCB 105	810	<300	3300	430	640	850	140
PCB 114	<220	<100	<620	<140	<200	<260	<24
PCB 118	3500	<1,500	11000	2100	3000	4200	460
PCB 123	220	<100	440	140	210	280	21
PCB 156	780	<300	2600	410	640	860	120
PCB 157	410	<150	1600	210	330	420	76
PCB 167	620	<250	1800	300	470	590	99
PCB 189	230	<100	520	140	210	280	24