FORAGING HABITAT CONTAMINATION INFLUENCES GREEN SEA TURTLE PCDD/F EXPOSURE

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Introduction

Previous investigations within Queensland, Australia have identified polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDD/Fs) within soils, sediments and biota.[1,2] While it has been shown that PCDD/Fs are present along the entire 2000 km coastline, these studies were undertaken on a relatively broad scale with the aim of identifying future prioritisations. More recently, a high-resolution sampling approach was applied to sediments within Moreton Bay, Queensland with the aim of investigating the pathways and fate of these compounds within the near-shore marine system[3], which is generally considered the final sink environment for PCDD/Fs. Moreton Bay is a semi-enclosed embayment (1523 km^2) located in the south-east region of Queensland, Australia (Figure 1). The surrounding catchment area that drains into Moreton Bay is dominated by agriculture, grazing and urban land uses. Sub-tropical climatic conditions produce heavy rainfall events during the summer months leading to periodic flooding and associated soil-runoff into the bay. Modelled sediment loads into this marine system are estimated at 329,937 tonnes per year.[4] This near-shore environment supports green sea turtle populations (*Chelonia mydas*), a threatened species potentially vulnerable to contaminant exposure due to their long life span (50 years or more), specialised food requirements, complex life cycle and long maturation process (approximately 30-50 years until breeding commences). Apart from the open-water (pelagic) developmental phase (until ~8 years of age) and intermittent migrations to breeding grounds (during which time little to no feeding occurs), green turtles remain and feed within relatively small home ranges.[5, 6] During their pelagic phase, green turtles are omnivorous but quickly shift to a predominantly herbivorous diet of seagrass and algae upon arrival at their home-range habitats. The present study aimed to provide a better understanding of exposure and exposure pathways of green turtles to PCDD/Fs. Specifically, the relationship between sediment contamination zones and tissue levels in green turtle populations is described in detail.

Materials and Methods

Approximately 100 sediment samples were collected using a grid-based sampling schedule across Moreton Bay and prepared for analysis in accordance to previously described methods.[3] Sea turtle blood samples (approximately 25-50mls depending on the individual's size) were collected from live healthy turtles caught either by net or "rodeo" style in conjunction with the Queensland Turtle Conservation Programme (Queensland Parks and Wildlife Service) (Ethical Clearance ENTOX/762/04/ARC and EPA Permit WITK02868705). 29 green sea turtles, foraging and residing within western and eastern Moreton Bay (Figure 1), were sampled for blood (from the dorsocervical sinus) and biometric data was collected (including size, age class, weight, gender, breeding status and health status). Whole blood was stored in solvent-washed glass jars containing heparinised saline and the fixative potassium dichromate and stored at 4°C for a maximum of 5 days, and then -20°C until analysis.

All sediments were analysed for OCDD by GC/ECD techniques using a previously established screening method at EnTox/Queensland Health Scientific Services laboratories, previously described in detail.[3,7] PCDD/Fs were analysed by using high resolution GC/MS (VG Autospec and Finnigan) in a subset of these sediment samples (n= 30) and for all turtle blood samples at Eurofins-ERGO Forschungssesellschaft mbH. For sediment analysis, detailed methods and standard quality control procedures have been reported previously.[1] Methods for analysis of turtle whole blood were developed based on standard ERGO techniques for extraction, clean-up and PCDD/F analysis of human whole blood.[8] For all analysis, a blank was included with each batch of 7-12 samples and quantification was carried out using either native HpCDF internal standard (for OCDD screening) or ^{13}C-labelled 2,3,7,8-substituted PCDD/Fs by the isotope dilution method (for PCDD/F analysis at ERGO).[9] All ERGO

analysis was undertaken on a VG Autospec or Finnigan HRGC/MS using a DB5 column; 60m, 0.1µm FT, 0.25mm ID with resolution between 8000-9000 and DB5ms; 60m, 0.25 µm FT, 0.25mm ID with resolution between 6000-7000, respectively.

For chromatogram integration, the ratios for the area of the two most abundant isotopes had to be within 20% of their calculated values. The detection limit for PCDD/Fs was defined as a signal–to-noise ratio greater than 3 times the average baseline variation. The quantification limit was defined as analyte concentration 3 times the concentration found in the batch blank. All toxic equivalencies (TEQs) were calculated using mammalian WHO-TEFs[10] and are reported using lower bounds limits. Spatial distribution of OCDD concentrations in sediments was determined using Geographical Information System Software (ArcView 3.2) inverse density weighted spatial analysis, which was reported in detail previously.[3]

Results and Discussion

The PCDD/F profile of the majority of soil and sediment samples analysed from Queensland is dominated by OCDD (average 82%± 9.6 of the total PCDD/F concentration), lending itself as a useful "marker" of total PCDD/Fs in the local environment.[7,11] OCDD was detected in all sediment samples analysed from Moreton Bay and ranged from "background" to relatively high concentrations (7 to 8100 pg/g dry weight (dw)). The spatial distribution of OCDD across the Bay displayed high geographical variability. In general, OCDD concentrations were relatively low and homogenous within the eastern banks area, and elevated but often highly variable between sites in the western, mid and southern Bay regions (Figure 1).

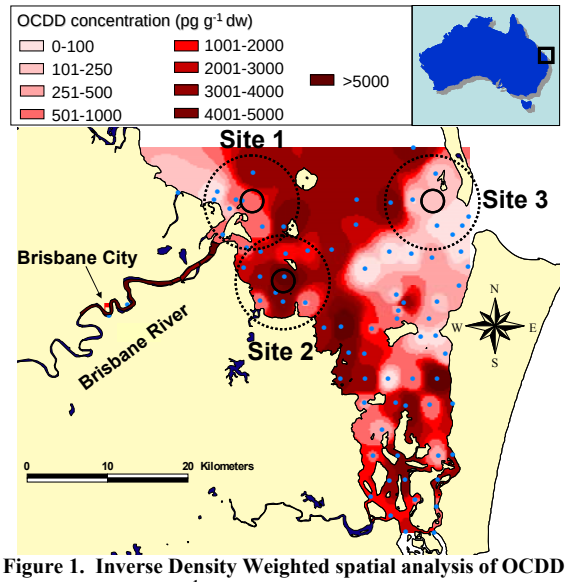

Figure 1. Inverse Density Weighted spatial analysis of OCDD concentrations (pg g^{-1} dw) in Moreton Bay, Queensland. Sampling sites depicted by (•). Target marks (labeled site 1-3) represent rough schematic of estimated sea turtle home range (see text).

Previous satellite tracking studies undertaken by Qld EPA found that green turtle feeding areas are highly conserved and localised (2-10 km^2) within Moreton Bay.[3] This, in combination with sediment PCDD/F contamination gradients in Moreton Bay provides an ideal opportunity to investigate the influence of different contamination zones on the PCDD/F exposure of marine wildlife. Turtles investigated for this study originated from three locations (Figure 1) characterised into three different sediment contamination zones. Site 1 displayed "variable" contamination levels of OCDD (42 to 3700 pg g^{-1} dw), Site 2 was characterised by more consistently elevated OCDD levels (590 to 5600 pg g^{-1} dw) whereas OCDD levels at Site 3 were relatively low and homogeneous (9 to 200 pg g^{-1} dw), and comparable to background levels found elsewhere.[12]

PCDD/Fs were detected in all of the green turtle blood samples analysed. ∑ 2,3,7,8 PCDD/F concentrations ranged from 160 to 1900 pg g^{-1} lipid weight (lipid) at Site 1 (n=6), 510 to 1400 pg g^{-1} lipid at Site 2 (n=7) and 30 to 200 pg g^{-1} lipid at Site 3 (n=16) (see Figure 2). Average PCDD/F concentrations were higher for those sub-populations proximal to river inputs (Site 1 and 2), when compared to greens foraging in the eastern area (average 580 and 910 compared to 90 pg g^{-1} lipid, respectively). Similarly, TEQ values in turtle blood from animals with near-shore feeding grounds were elevated (2.7 to 160 and 20 to 79 pg g^{-1} lipid for Site 1 and 2, respectively) compared to those from eastern sampling location (0.70 to 15 pg g^{-1} lipid at Site 3) (Figure 2). PCDD's contributed the largest proportion of the total TEQ (67 to 96%, average 86%), with the exception of two individuals (20 and 30%). Among the PCDDs, 1,2,3,7,8-Penta-CDD contributed the greatest proportion (40 to 88%) towards the total TEQ in 90% of animals analysed as part of this study. To date, no information is

available on PCDD/F exposure in marine turtles from other locations. Comparisons to other marine wildlife are limited by the low trophic level of green turtles, which limits biomagnification steps and results generally in lower TEQ levels compared to higher trophic animals. In general, however, TEQ levels in green turtles from Site 3 are relatively low compared to those reported for many other marine wildlife.[12, 13, 14, 15] In contrast, TEQ levels in animals from Site 1 and 2 are elevated compared to other, higher trophic marine wildlife, (e.g. seals from Greenland (4.1 to 12 pg g^{-1} lipid),[13] and even from relatively polluted locations (e.g. dolphins from the Mediterranean (2.9 to 64 pg g^{-1} lipid),[14] and Baikal seals from Russia (85 to 110 pg g^{-1} lipid).[15] Such variation between green turtle populations is remarkable considering they all reside within the same Bay and consume similar food sources. The only clear difference is that these animals forage about 20-30kms apart in distinct habitat contamination zones, highlighting the significant influence habitat location has on sea turtle PCDD/F exposure.

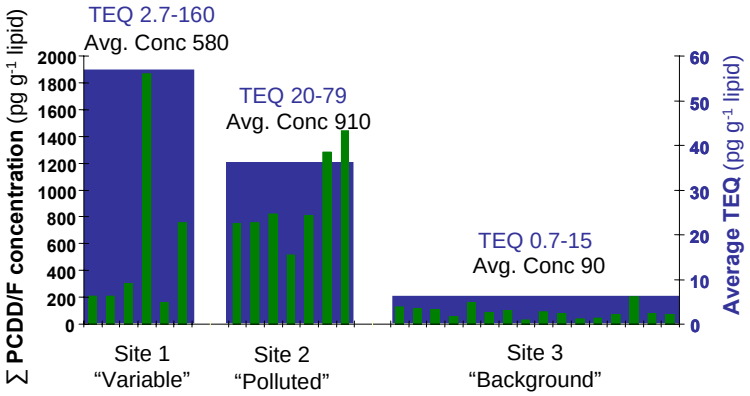

Figure 2. ∑ 2,3,7,8 PCDD/F concentration and average TEQ levels in pg g^{-1} lipid for Green sea turtles caught and sampled from three habitat contamination zones (Site 1 "variable", Site 2 "polluted", Site 3 "background"). Within zones, results are plotted according to ascending animal size (curved carapace length (ccl) cm)

In general, PCDD/F congener profiles in green sea turtle blood reflected that observed in sediments, with a dominance of PCDDs, especially OCDD. Compared to sediment, however, the contribution of OCDD to sum PCDD/F concentration was lower in turtle blood (40 to 78%, average 57%), whereas contributions of lower chlorinated PCDDs (and more toxicologically relevant congeners) were generally increased. This reflects bioaccumulation processes from sediment-seagrass-turtle and has been described previously for a range of biota, including green turtles.[3,12] This shift in profile and the concurrent increase in TEQ levels in green turtles highlight that elevated TEQs may be present even in low trophic biota despite low TEQ levels in sediments. For example, while 2,3,7,8-TCDD and PnCDDs were often below the limit of detection in sediments in the current study, the latter were biomagnified in green turtles to contribute the majority to the TEQ. Among the green sea turtle sub-populations, the PCDD/F congener profile altered marginally between sites. Contributions of lower chlorinated (and more toxic) PCDDs were slightly higher in populations proximal to the river inputs compared to those from the eastern sampling site. This may be due to a) slight differences in local habitat contamination (including the influence of transport processes from the western to eastern sediments) and/or b) intake of higher trophic dietary supplements by green turtles foraging in certain regions (i.e. sourcing an opportunistic omnivorous diet where seagrass is not as readily available). These slight profile shifts result in markedly higher TEQs for green turtles foraging in closer proximity to the river inputs.

To date, it is uncertain whether the levels found in the present study have the potential to result in adverse effects for green sea turtles, since there is currently no data pertaining to the sensitivity of reptiles to PCDD/Fs. Interpretation of sea turtle toxicity data, using mammalian TEFs, simply provides a rough indication of the potential health implications these animals face and allows for inter-species comparisons with other marine wildlife. From these comparisons, it is clear that certain populations of green sea turtles, in particular, those foraging in close proximity to land-based secondary sources may be among the higher risk groups in terms of marine wildlife exposure to PCDD/Fs. Therefore, it is important that future studies aim to investigate reptilian sensitivity to and metabolism of PCDD/Fs, to allow for a better understanding of exposure data.

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References

1. Gaus C, Päpke O, Blanchard W, Haynes D, Connell D, Müller JF. *Organohalogen Compounds* 2001; 52:95.
2. Gaus C, Päpke O, Dennison N, Haynes D, Shaw G, Connell D, Müller JF. *Chemosphere* 2001; 43:549.
3. Hermanussen S, Limpus CJ, Päpke O, Blanchard W, Connell D, Gaus C. *Organohalogen Compounds* 2004; 66: 95
4. Marston F. *CSIRO Land and Water Technology Report* 27/00 2000.
5. Limpus CJ, Cooper PJ, Read MA. *Memoirs of Queensland Museum* 1994; 35:139.
6. Limpus CJ, Chalaupka M. *Marine Ecology – Progress Series* 1997; 149:27.
7. Müller J, Dennison N, Gaus C, Haynes D, Manonmanii K, Päpke O, McLachlan M. In *17th Conference of Residue Chemists*: Armishaw P, Millar R, McLay P. (Eds). Perth, WA, Australia. 1999.
8. Päpke O, Ball M, Lis ZA, Scheunert K. *Chemosphere* 1989; 19: 941.
9. Gaus C, Brunskill GJ, Weber R, Päpke O, Müller JF. *Environmental Science and Technology* 2001; 35:4597.
10. Van den Berg M, Birnbaum L, Bosveld ATC, Brunstroem B, Cook P, Feeley M, Giesy JP, Hanberg A. *Environmental Health Perspectives* 1998;106:775.
11. Gaus C, Brunskill GJ, Connell DW, Prange J, Müller JF, Päpke O, Weber R. *Environmental Science and Technology* 2002;36:3542.
12. Gaus C. School of Public Health, Department of Health, Griffith University, Queensland, Australia; PhD Thesis: 2002.
13. Oehme M, Biseth A, Schlabach M, Wiig Q. *Environmental Pollution* 1995; 90:401.
14. Jimenez B, Gonzalez M, Jimenez O, Reich S, Eljarrat E, Rivera J. *Environmental Science and Technology* 2000; 34: 756.
15. Tarasova EN, Mamontova AA, Mamontova EA, Klasmeier J, McLachlan MS. *Chemosphere* 1997; 34: 2419.