

## EVALUATING SPATIAL PATTERNS OF DIOXINS IN SEDIMENTS TO AID DETERMINATION OF POTENTIAL IMPLICATIONS FOR MARINE REPTILES

Siobhan Hermanussen<sup>1</sup>, Colin J. Limpus<sup>2</sup>, Olaf Pöpke<sup>3</sup>, Wendy Blanshard<sup>4</sup>, Des Connell<sup>5</sup>,  
Caroline Gaus<sup>1</sup>

<sup>1</sup>National Research Centre for Environmental Toxicology, Brisbane

<sup>2</sup>Queensland Environmental Protection Agency, Brisbane

<sup>3</sup>Ergo Forschungsgesellschaft mbH, Hamburg

<sup>4</sup>Sea World, Gold Coast

<sup>5</sup>School of Public Health, Griffith University, Brisbane

### Introduction

Recent investigations have identified elevated concentrations of polychlorinated dibenzo-*p*-dioxins (dioxins) in marine sediments and wildlife of Queensland, Australia<sup>1, 2</sup>. While it has been demonstrated that the contamination is widespread and predominantly land-based, limited information exists on the pathways and fate of these compounds within the near-shore marine system. This environment supports unique and threatened species including green sea turtles (*Chelonia mydas*). Adult green turtles are predominantly herbivorous, feeding on seagrass and algae. Apart from initial migration to feeding grounds (at ~10 years of age) and intermittent migrations to breeding grounds (at ~30-50 years and thereafter), green turtles remain and feed within relatively small home ranges<sup>3, 4</sup>. Long life-span (50 years or more), near-shore feeding grounds and highly specialized food requirements render green turtles potentially vulnerable to contaminant exposure. Recent studies have shown a relationship between PCDD/F concentrations found in herbivorous marine wildlife and concentrations in sediments of their habitats<sup>1</sup>. Hence, the spatial evaluation of sediment PCDD/F distribution may assist the assessment of green turtle exposure and its potential implications. The present study provides baseline information on green turtle PCDD/F concentrations in Queensland, Australia and investigates exposure pathways. In addition, spatial distribution of PCDD/Fs in sediments from known green turtle feeding regions is assessed using geographic information systems. This represents the first stage of a large scale investigation into the exposure and sensitivity of marine reptiles to dioxins and dioxin-like compounds and to evaluate whether poor health status observed in some populations may be related to contaminant exposure.

### Materials and Methods

Moreton Bay is a semi-enclosed embayment (approximately 1523 km<sup>2</sup>), located off Queensland's capital city Brisbane. Its catchments consist of extensive agricultural and grazing areas and the urban metropolitan area supports a population of 1.6 million people. The sub-tropical climate of this region is characterized by heavy rainfall events in the summer months resulting in regular

flooding and extensive plumes in the Brisbane River and its tributaries, carrying significant sediment yields into Moreton Bay. Modeled estimates of sediment exports suggest that 329,937 tonnes are released into Moreton Bay per year<sup>5</sup>.

A 3-6 kilometer resolution grid-based sampling schedule was employed in this study from West to East across the Bay. Additional sites were selected between grid-points in areas of high habitat variability (e.g. bathymetry, macroflora coverage, river discharges). Estuarine and marine sediment samples were collected using a modified Van Veen grab sampler at the grid points. Three separate samples from the top 5-10 cm of sediment were collected and homogenized forming one composite sample. Samples were freeze-dried and sieved discarding the fraction <2.3cm (shells and stones) prior to analysis. Green turtle carapace fat tissue was obtained from two juvenile females (T400, T403), one subadult male (T208) and one adult male (T209) stranded in Moreton Bay. Information on the cause of death of T400 and T403 could not be ascertained, however, necropsies on T208 and T209 revealed chronic and acute causes of illness, respectively. T208 appeared to have been affected by circulatory compromise secondary to spirorchid fluke infestation and T209 succumbed to shock and toxemia resulting from intestinal intussusception. Physical body condition was good for T209 and T400, whereas both T208 and T403 were noted to have low fat reserves.

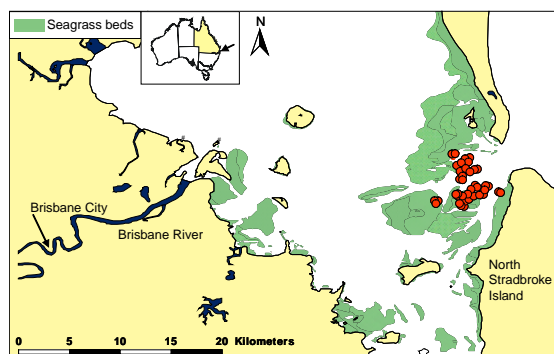
Sediments were analyzed for OCDD concentrations (PCDD/F screening method, using GC/ECD) at the Queensland Health Scientific Services (QHSS) laboratories according to standardized methods<sup>6</sup>. Full PCDD/F profiles using high resolution GC/MS (VG Autospec) were determined in a subset of sediment samples and for all turtle fat samples at Ergo Forschungsgesellschaft mbH (ERGO) in Germany using certified methods described in<sup>1</sup>. In all analysis, blank samples were included with each batch of 6 samples and quantification was performed using either native HpCDF internal standards (for OCDD screening) or <sup>13</sup>C-labelled 2,3,7,8-substituted PCDD/F standards (at ERGO). Details of these methods and standard quality control procedures have been reported previously<sup>7</sup>. Total organic carbon (TOC) was determined at the QHSS laboratory according to a standardized procedure<sup>8</sup>. Inorganic carbonates were removed using acid catalyzed digestion (10 % HCl, 1 % FeCl<sub>2</sub> at 70°C). The remaining material was dried and combusted in the LECO induction furnace with subsequent detection of CO<sub>2</sub> (LECO WR12 CO<sub>2</sub> detector).

Spatial distribution of OCDD concentrations (and % total organic carbon (TOC)) in sediments was analyzed using Geographic Information System Software (ArcView 3.2a). OCDD concentrations (and % TOC) at each sample grid were surface interpolated using Inverse Density Weighted (IDW) analysis. The model assumes a localized influence of each site that diminishes with distance. The sites closer to the processing cell have greater weight compared to those more distant. The IDW assigns values for each point between the grid sites by weighing the OCDD concentrations of neighboring sites (n=12) by the distance (decreasing by the power of 4) these sites are from the cell and then averaging the values. The output represents a best estimate of OCDD concentrations (or % TOC) between sampling points and was validated using six sites (5 in the western, 1 in the eastern bay) that were located between two grid sites 3 km apart. The OCDD concentration ranges of the validation points were predicted accurately for four sites. Two sites close to the Brisbane River mouth in the western bay were overestimated by a factor of ~1.5 (1000-1500 predicted - 675 pg g<sup>-1</sup> actual) and ~6 (500-1000 predicted - 76 pg g<sup>-1</sup> actual), respectively. In addition to the samples collected and analyzed for the present study, results from previously analyzed sediment

samples with known coordinates and collected using composite sub samples were included in the spatial analysis. These include 3 sites within and near the Brisbane River<sup>9</sup> and 4 samples in the western bay<sup>10</sup>.

## Results and Discussion

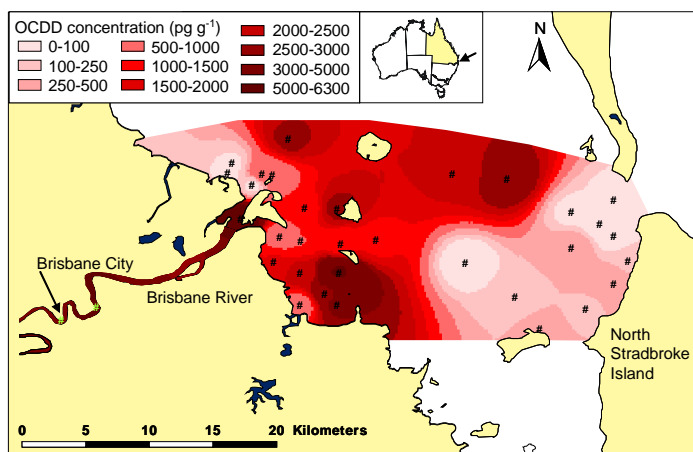
$\Sigma$ PCDD/F concentrations in herbivorous green turtles were 170 (T209), 770 (T208), 1400 (T400) and 5700 (T403)  $\text{pg g}^{-1}$  lipid weight (lw). Corresponding TEQ values (calculated using WHO 1998 TEFs<sup>11</sup>) were 11, 20, 4.7 and 140  $\text{pg g}^{-1}$  lw, respectively. It should be noted that the two juvenile female and subadult male turtles had the highest concentrations and TEQ levels compared to the adult male specimen. To date, only limited information is available on PCDD/Fs in marine reptiles and among turtles only freshwater species have been reported. Compared to liver tissue from the omnivorous yellow-blotched map turtle reported from the Pascagoula River drainage, Mississippi (maximum  $\Sigma$ PCDD/F 16  $\text{pg g}^{-1}$  lw; TEQ 0.89  $\text{pg g}^{-1}$  lw)<sup>12</sup>, concentrations and TEQs observed in Queensland green turtles were elevated. PCDD/F concentrations comparable to the present study were reported in pooled egg samples from piscivorous snapping turtles in the Great Lakes<sup>13</sup> where 2,3,7,8-PCDD/F concentrations ranged from 4.9 to 800  $\text{pg g}^{-1}$  lw (calculated using average %lipid values reported) with TEQ<sub>WHO98</sub> values ranging from 0.00049 to 252  $\text{pg g}^{-1}$  lw (calculated using average % lipid values reported). The St. Lawrence River Basin sites included both remote reference sites and contaminated sites near heavy industry and PCB-contaminated landfills. A significant increase in abnormal development was found in turtle hatchlings from these areas with increasing PCB, and in particular with increasing PCDD/F concentrations<sup>13</sup>.



**Figure 1. Map of Moreton Bay, Queensland, showing seagrass distribution and satellite tracked foraging activities of one adult male green turtle over a period of 4 months ( ).**

Within Moreton Bay, information on PCDD/Fs in wildlife is limited to the marine mammal dugong ( $n=6$ )<sup>1</sup>. Both dugongs and green turtles are herbivorous, feeding preferentially on seagrass. Despite the similar food source, both  $\Sigma$ PCDD/F concentrations and TEQs in green turtles were on average 10 fold higher (range 0.6-19 fold) compared to dugongs. Both species source food within the bay. Green turtle feeding areas are highly conserved and localized (few km in diameter) (Figure 1.) and are located in eastern or western areas of the Bay. Dugongs, however, prefer to feed within the Bays' eastern areas. It is noteworthy in this respect that higher incidence of poor health in green turtles from southwestern feeding grounds compared to the northeastern populations has been observed. For example, recent investigations showed that a gradient is present in the prevalence of fibropapilloma (the growth of tumours on turtles that can eventually lead to death) with approximately 70% of turtles affected in the Southwestern (riverine influenced) part of Moreton Bay compared to approximately 20% in the northeastern (less impacted) part of the Bay<sup>17</sup>. The elevated PCDD/F concentrations observed in green turtles (in combination with potentially higher concentrations in juvenile compared to adult animals) raise the question of whether localized contaminant patterns within green turtle western and eastern sites are

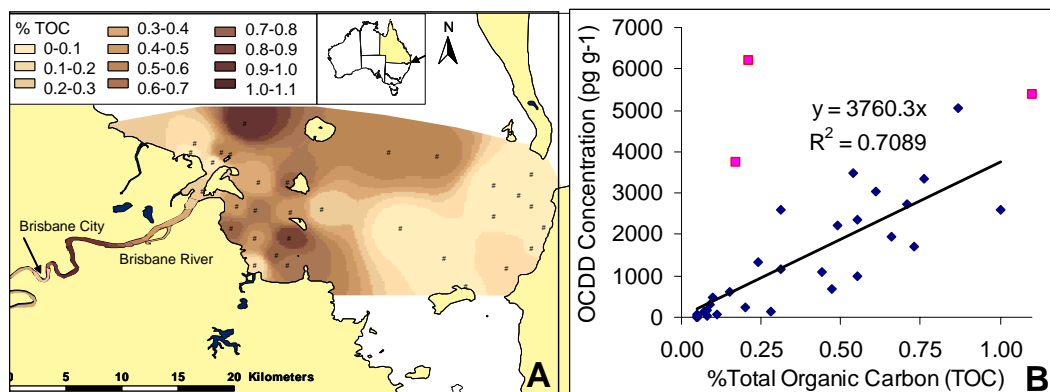
correlated to these findings and whether these may be implicated in the incidence of morbidity incidence observed in green turtles.



**Figure 2. Inverse Density Weighted spatial analysis of OCDD concentrations (pg g<sup>-1</sup> dw) in Moreton Bay, Queensland. (" Indicate sampling locations).**

In sediments, the PCDD/F profile was characterized by the dominance of OCDD, decreasing concentrations towards lower chlorinated PCDDs and low (mostly below the LOD) levels of PCDFs. This profile is consistent to other marine sediment samples from Queensland and characteristic for the coastal zone<sup>14</sup>. Using previous and present data from Queensland sediments, an average of 82%±9.6 (range 66-98%, median 80%) of the total PCDD/F concentration can be attributed to OCDD, and a significant linear correlation exists between

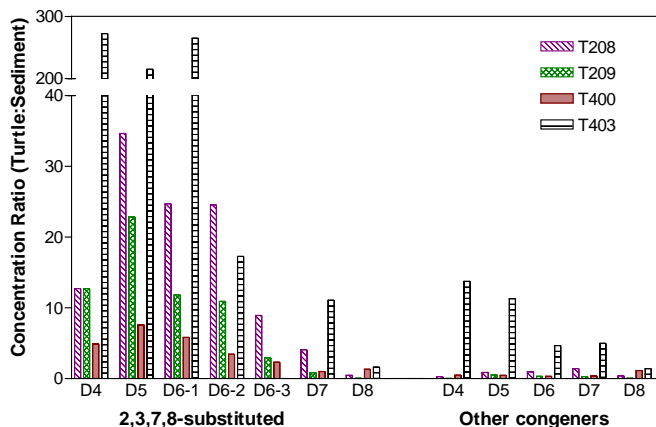
the two parameters ( $PCDD/F = 1.090 \times OCDD$ ;  $R^2=0.9821$ ). Hence, OCDD concentration provides a cost-effective screening tool for  $\Sigma PCDD/F$  concentration in sediments<sup>6</sup>. OCDD concentrations varied between sampling sites, ranging from 50 pg g<sup>-1</sup> to 6200 pg g<sup>-1</sup> dw (Figure 2.). Generally, higher concentrations were found in the river sediments and western bay sites compared to the eastern bay, with an average 5.4-fold increase in OCDD concentration from west to east (Figure 2.). Similar results were found from other regions in Queensland and indicate a predominant riverine transport of sediment/soil associated PCDD/Fs into the marine system<sup>16</sup>. A similar trend was observed when comparing samples analyzed for  $\Sigma PCDD/F$  concentrations (average  $\Sigma PCDD/F = 350$  pg g<sup>-1</sup> in eastern versus 2400 pg g<sup>-1</sup> in western sediments; n=8). Correspondingly, relatively low TEQ<sub>WHO98</sub> were observed at eastern sites (ranging from 0.24-0.54 pg g<sup>-1</sup> dw) compared to western sites (ranging from 0.21-4.9 pg g<sup>-1</sup> dw). However, concentration differences between west and east were not solely responsible for the TEQ increase in western sites. Despite the overall consistency of the PCDD/F profiles in the bay (considering the fingerprint patterns of all 210 PCDD/Fs), differences in the percent contribution of the different congeners were observed between western and eastern sites. A trend towards increased lower chlorinated PCDDs was apparent in the western, compared to the eastern sediments (e.g. 18-fold increase in  $\Sigma TCDDs$ ). While at this stage only limited data (n=5 western sediments and n=4 eastern sediments) are available for this comparison, this trend was significant ( $p > 0.0005$ ) and may indicate the loss of lower chlorinated PCDDs along their transport pathways from west to east (e.g. degradation, burial). Further investigations are underway to evaluate the relevance of such processes to the PCDD/F exposure of green turtles in western habitats compared to the eastern populations.



**Figure 3. A. Inverse Density Weighted spatial analysis of % TOC levels in Moreton Bay, Queensland. (● Indicate sampling locations) B. Linear regression of OCDD concentrations (pg g<sup>-1</sup> dw) and TOC (%). Note: red points indicate samples excluded from regression analysis (see text).**

OCDD concentrations were relatively low and homogenous within the eastern bay, and elevated concentrations were observed in defined “hotspot” areas in the mid and western bay areas (Figure 2.). In contrast to the eastern sites, high variability was observed in the western bay, in particular near the Brisbane River mouth (Figure 2.). Highest OCDD concentrations were observed within the river (3700 and 5400 pg g<sup>-1</sup>) and the river mouth (6200 pg g<sup>-1</sup>), whereas <700 pg g<sup>-1</sup> were detected at sites approximately 3 -5 km from the river mouth. Since PCDD/Fs have a high affinity for the organic fraction in sediments, the relationship of OCDD and organic carbon distributions within the bay was investigated. Figure 3A shows the spatial distribution of total organic carbon (% TOC) in Moreton Bay which generally reflects the distribution of OCDD concentrations (Figure 2.). When all sediment samples are included in a regression analysis, however, the correlation between the two parameters is low ( $R^2 = 0.4869$ ). This is predominantly due to relatively low TOC, but high OCDD contents in Brisbane River sites (Figure 3B). Removing these river sites from the regression analysis results in a higher correlation between % TOC and OCDD concentrations ( $R^2 = 0.7089$ , see Figure 3B). This indicates that the OCDD distribution within the bay is governed by the distribution of organic particles, whereas additional factors may influence its distribution within riverine systems.

While the TEQ levels and PCDD/F concentrations in sediments of Moreton Bay could be considered negligible compared to polluted areas in the Northern Hemisphere, concentrations in green turtles are comparable to those found in reptiles of the Great Lakes and St Lawrence basins. Extensive seagrass beds in the western and eastern bay regions provide an efficient trap for terrigenous sediments (sediment retention is up to 15 times higher in seagrass beds compared to bare sediment<sup>15</sup>). With the accretion of fine particles, PCDD/Fs can accumulate to elevated concentrations in these areas. In addition, seagrass beds provide a lipid surface area for sequestering PCDD/Fs and previous studies have demonstrated a preferred bioaccumulation of lower chlorinated PCDD/Fs on seagrass compared to the sediment, thereby increasing the more toxicologically relevant congeners<sup>1</sup>. For the present study, bioaccumulation of PCDD/Fs congeners in turtles from Moreton Bay was investigated using turtle to sediment concentration ratios (Figure 4.). A clear trend of increasing concentrations with decreasing degree of chlorination



**Figure 4. Concentration ratios of PCDD congeners in stranded green turtles to average sediments from Moreton Bay.**

observed in one green turtle (T403, up to 350 fold increase of 2,3,7,8-TCDD compared to sediments) may be associated with its relatively low fat reserves and the remobilization of PCDD/Fs during weight loss or foraging within areas of higher contamination to that observed to date. These results highlight that transfer pathways and processes within the soil/sediment-biota system can have considerable influences on the toxicological relevance of the PCDD/F source. In addition, it should be noted that while OCDD has relatively low toxicological relevance in mammals and birds, no information exists to date on the sensitivity of reptiles to dioxins and dioxin-like compounds. Other studies<sup>13</sup> have highlighted that individual PCDD/F congeners were important in toxicity to snapping turtle eggs, however, TEQs were not highly predictive in this species, indicating that this reptiles' sensitivity to and metabolism of PCDD/Fs may differ compared to those observed in mammals and birds.

Future studies within the present project will include the evaluation of spatial relationships among green turtle PCDD/F exposure and health status from tagged animals with known feeding grounds and extensive biometric data, in combination with investigations of sensitivity and metabolism of PCDD/Fs in these animals. The consideration of spatial PCDD/F distributions, as presented in this study, represents a useful method for identifying "hotspots" and prioritizing further evaluations.

### Acknowledgements

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was observed in the animals compared to the sediment. Similar results were reported previously from other areas in Queensland, including preliminary data on green turtles and dugongs (Gaus et al. 2001). This selective bioaccumulation and retention results in 5 to 35 times higher concentrations of 2,3,7,8-substituted TCDD and PeCDD congeners in three of the green turtles analyzed, compared to the average sediment concentrations across the bay (excluding rivers). Extremely high concentration ratios

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