BIOACCUMULATION AND PATHWAYS OF PCDDs IN THE LOWER TROPHIC MARINE SYSTEM

Caroline Gaus[1,2], Olaf Päpke[3], Wendy Blanchard[4], David Haynes[5], Des W. Connell[2] and Jochen F. Müller[1]

[1]Nat. Res. Centre for Environ. Toxicology, 39 Kessels Rd, Coopers Plains 4108, Qld, Australia
[2]School of Public Health, Griffith University, Nathan 4111 Qld, Australia
[3]Ergo Forschungsgesellschaft mbH, Geierstrasse 1, D-22305 Hamburg, Germany
[4]Sea World, PO Box 190, Surfers Paradise 4217 Qld, Australia
[5]Great Barrier Reef Marine Park Authority, PO Box 1379, Townsville, 4810, Qld, Australia

Introduction

Polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) are ubiquitous contaminants in the environment that tend to accumulate in sink areas such as marine sediments. Contaminated sediments can be an important pathway for redistribution of PCDD/Fs into biota. Due to their toxicity and persistent nature, PCDD/Fs are considered a serious threat to ecosystem health in contaminated habitats, particularly with respect to their ability to bioaccumulate and biomagnify in the food chain[1]. While anthropogenic activities undoubtedly present the major sources of PCDD/Fs to the contemporary environment, evidence for their natural formation necessitates a need to evaluate the significance of such "baseline" inputs. Recently, a widespread natural source of PCDDs, in particular OCDD has been postulated for the coastal zone of Queensland, Australia[2, 3]. However, little is known about the extent of this coastal contamination within the marine system and its possible effects on marine biota. Queensland's marine system is known for its unique fauna and flora associated with the Great Barrier Reef Marine Park. These include for example extensive seagrass habitats that represent a major food source for green turtles (*Chelonia mydas*) and dugongs (*Dugong dugon*). Due to increased anthropogenic pressures on coastal habitats in Queensland, green turtles have been declining in numbers[4] whereas dugongs are classified as vulnerable to extinction[5]. This study investigated distribution processes of PCDDs in the marine system of Queensland. Here we focus on dugongs and green turtles since their food specialisation, high fat repositories, and long life span render them potential bioindicators for Queensland's coastal PCDD distribution. This, in combination with their low trophic position as well as the widespread dominance of a specific single source profile, are ideal features to develop a further understanding of the uptake and distribution pathways of persistent organic pollutants in the marine system. Initial results from this study, that also included analysis of marine mammals from higher trophic levels such as the bottlenose dolphin (*Tursiops spp.*), are discussed with respect to bioaccumulation and pathways of PCDDs.

Materials and Methods

Tissue samples from dugongs, green turtles and bottlenose dolphins were obtained from animals stranded or drowned in nets at various regions along the coastline of Queensland (Figure 1). Veterinary post-mortem examinations were carried out on most animals and provided information on possible cause of death, gender, health conditions, estimated age and/or body size. Dugong and bottlenose dolphin samples included tissue from the outer layer of blubber from adult females (n=10 dugongs, n=3 dolphins) and males (n=6 dugongs, n=2 dolphins). Green turtle samples

included carapace fat from adult females (n=4) and males (n=2). Sediment and seagrass (roots, rhizomes and leaves of *Halophila ovalis*, *Zostera capricorni*, *Syringodium isoetifolium*, *Cymodocea serrulata*) samples were obtained from subtidal sites within known dugong and green turtle feeding areas from different regions. Samples were handled using stainless steel or glass materials (except the hand-collection of seagrass) and were stored frozen until processing.

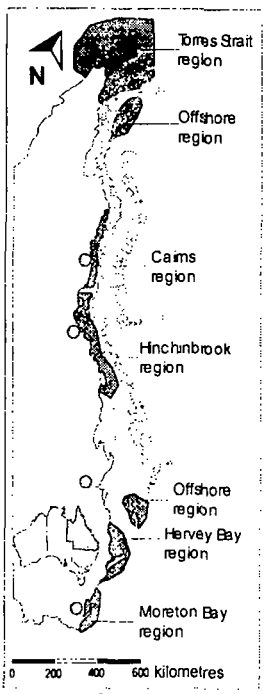

Figure 1. Sampling regions in Queensland, Australia.

Samples were analysed for 2,3,7,8-substituted PCDD/F profiles at Ergo Forschungsgesellschaft mbH or – in the case of some sediments – for OCDD at QHSS laboratories. Blank samples were included in the analysis with each batch of 6 samples. Sediment analyses were carried out using standardarised methods described in reference[2]. Seagrass was washed after collection and carefully cleaned from attached sediment particles using 4 molar HCl followed by fresh water. Samples were then freeze-dried, spiked with ^{13}C-labeled PCDD/F standard and soxhlet extracted using toluene. Blubber from dugongs and carapace fat from turtles was digested using 4 molar HCl at 60°C. Lipid-water partitioning was carried out using hexane and DCM. After gravimetrical lipid determination approximately 3 g of lipid was spiked with ^{13}C-labeled PCDD/F standard. In brief, sample clean up for both seagrass and lipid samples included acidic pre-treatment (H_2SO_4/SiO_2), acid/base activated silica gel, deactivated florisil and carbon/celite fractioning. The final eluent was transferred to vials, and ^{13}C-labelled 1,2,3,4-TCDD recovery standard was added. Analysis of tetra- to octa-CDD/Fs was performed on a GC (DB-5 fused silica column, 60 m, 0.25 mm i.d., 0.1 µm film thickness) interfaced to a VG Autospec mass spectrometer operating on a resolution of approximately 10,000. Identification of 2,3,7,8-substituted PCDD/Fs was performed using retention times of the labeled standards and isotope ratios at M^+ and $M+2^+$. For quality control the retention times of the analyte in a sample had to be within 2 s of the retention times of the internal standards. The limit of quantification was defined by a signal to noise ratio greater than three times the average baseline variation and a substance quantity in the sample greater than 3 times the quantity in the respective blank.

Concentration ratios (CR) (often referred to as "bioaccumulation factors" in the literature) were calculated as the ratio of PCDD concentrations in biota to its predominant source of uptake (note that no water concentration data are available and thus a direct water/biota concentration ratio is not discussed here). Hence, CR discussed here include the seagrass to sediment concentration ratio (SgSCR (in pg g^{-1} dw)/(pg g^{-1} dw)). Similar the CR of the PCDD concentrations in dugongs and turtles (in pg g^{-1} lipid) to those in seagrass (in pg g^{-1}dw, BSgCR) or sediment (in pg g^{-1}dw, BSCR) collected from the region were the animals were found were used to discuss processes.

Results and Discussion

PCDD/Fs, including non-lateral substituted isomers were detected in all samples analysed. The 2,3,7,8-congener profiles were consistently dominated by OCDD and, to a lesser extent, HpCDD (Figure 2A-B). In general, PCDD concentrations were found to decrease with decreasing degree of chlorination whereas PCDFs were present in relatively low concentrations and often below the limit of detection/quantification. The 2,3,7,8-congener profiles observed in biota differ from those observed in marine mammals from elsewhere[6, 7] and reflect the profiles observed in marine sediments from the entire Queensland coastline[2] (Figure 2A). These results suggest that PCDD source(s) to sediment and biota are identical along Queensland's coast.

Concentrations of ΣPCDD/F ranged from 49 to 2400 pg g^{-1} lipid in dugong blubber (average 660 pg^{-1} lipid), from 19 to 770 pg g^{-1} lipid in turtle fat (average 200 pg^{-1} lipid) and from 96 to 162 pg g^{-1} lipid (average 132 pg g^{-1} lipid) in dolphin blubber. WHO-TEQ values ranged from 1.5 to 135 pg g^{-1} TEQ among all dugong sampes (average 34 pg^{-1} lipid), 0.8 to 20 pg g^{-1} TEQ among turtle fat tissue (average 6.9 pg g^{-1} lipid) and 1.1 to 4.3 pg g^{-1} TEQ in dolphins (average 2 pg g^{-1} lipid). Compared to marine mammals from New Zealand or even relatively polluted areas in the Mediterranean, the PCDD/F concentrations and their respective TEQ values observed in dugongs and turtles in Queensland are relatively high[6, 7]. This is remarkable considering that Queensland is predominantly rural and not a heavily industrialized state, and both dugongs and turtles are representatives of the lower trophic level within the marine food chain.

Regional differences in PCDD concentrations of both dugongs and turtles were observed with lowest levels in dugongs from the Torres Strait region and offshore turtles compared to highest concentrations from inshore animals. Preliminary evaluation of our existing data show that average sediment OCDD concentrations within a region correlate well with PCDD/F concentrations in adult male dugongs found stranded in the respective regions (Figure 2C). No significant correlation was found between the ΣPCDD/F concentrations in adult female dugongs and OCDD sediment concentrations from the respective regions. This is most likely due to fluctuations in ΣPCDD/F concentrations often observed in female mammals as a result of contaminant transfer during lactation and gestation[8]. Since both dugongs and turtles ingest sediment particles that are attached to seagrass roots and leaves, sediment PCDD/F uptake is likely to present a direct PCDD/F uptake pathway for these animals[9]. However, cleaned seagrass analysed during this study showed similar ΣPCDD/F concentrations compared to sediment ΣPCDD/F concentrations while differences in 2,3,7,8-congener profiles between sediments and seagrass (Figure 2A) were observed. Seagrass sediment concentration ratios (SgSCR) demonstrate these differences (Figure 2D) and suggest that seagrass may have the capacity to accumulate specific PCDD/Fs congeners, which, in addition to contaminated adsorbed sediment particles, add to their PCDD/F burden but result in a shift of congener distribution towards lower chlorinated PCDDs in the plants. Accumulation of PCDD/Fs in aquatic plants has been reported previously and leaves were found to have considerably lower PCDD/F concentrations compared to the fibrous roots[10]. Although no data on leave/root comparisons is yet available for seagrass, it is interesting that concentration ratios calculated for ΣPCDD/F from adult turtles are lower (average BSgCR 2.1) compared to those from adult dugongs (average BSgCR 5.8). This may be related to differences in feeding behaviour between turtles (i.e. feed predominantly on seagrass leaves) and dugongs (i.e. uproot their food and consume both the root/rhizome system and leaves).

Similar to SgSCR, decreasing CR (BSgCR and BSCR) values with increasing degree of chlorination were observed in dugongs (3.2 to 217 for BSgCR) and turtles (1.5 to 41 for BSgCR). Similar trends have been observed in other biota and are thought to be the result of decreased uptake efficiencies of the higher chlorinated congeners due to physico-chemical characteristics[11, 12]. However, BSgCR and BSCR values for lower chlorinated PCDDs in dugongs and turtles are considerably higher compared to CR calculated for other, even high trophic organisms[12, 13]. Typically, biomagnification of persistent organochlorines through the food chain results in higher concentrations in high trophic marine mammals compared to lower trophic animals[14]. Results on bottlenose dolphins from Queensland however, indicate lower PCDD/F concentrations in these higher trophic animals compared to dugongs/turtles from the same region. This may be related to the OCDD dominance of Queensland's PCDD source, for which a

biodepuration, rather than biomagnification has been observed[15] however, even lower chlorinated PCDD concentrations were relatively low in dolphin compared to dugong/turtle tissue.
These results indicate that along the east coast of Queensland, the habitat location (i.e. proximity to the coastline) may have a strong influence on biota PCDD/F concentrations that may also supersede the influence of trophic position. More work is presently underway to evaluate interspecies and trophic transfer processes on an isomer specific level.

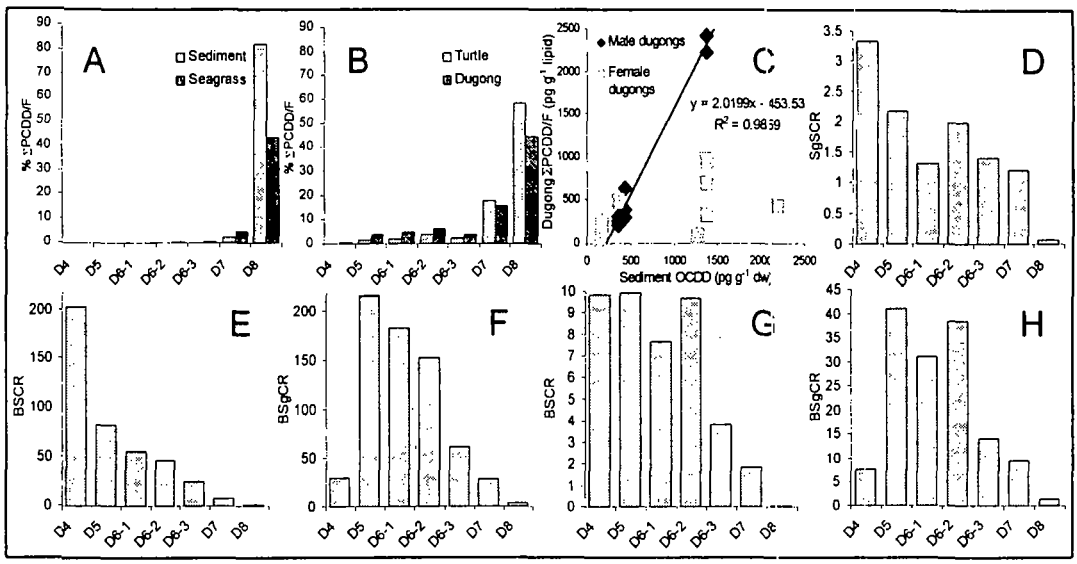

Figure 2. Typical 2,3,7,8-PCDD congener profiles in A. sediment and seagrass, B. adult male turtles and dugongs. C. Correlation between sediment OCDD concentration and ΣPCDD/F concentrations in adult male dugongs (includes data from reference[9]). D-E. CR for individual 2,3,7,8-congeners from D. seagrass vs sediment, E. adult male dugongs vs sediment, F. adult male dugongs vs seagrass, G. adult male green turtles vs seagrass, H. adult male green turtles vs sediment. (Note that due to low concentrations in all samples PCDFs are not shown in A and B).

References

1. De Boer, J., Stronck, C. J. N., Traag, W. A. and Van der Meer, J. (1993) Chemosphere 26.
2. Gaus, C., Päpke, O., Dennison, N., Haynes, D., Shaw, G. R., Connell, D. W. and Müller, J. F. (2001) Chemosphere 43, 549.
3. Gaus, C., Päpke, O., Brunskill, G. J. and Müller, J. F. (2000) Organohalogen Compounds 46, 15.
4. Bjorndal, K. A. (1980) Marine Biology 56, 147.
5. IUCN (1990) Gland, Switerland (1990).
6. Buckland, S. J., Hannah, D. J. and Taucher, J. A. (1990) Chemosphere 20, 1035.
7. Jimenez, B., Gonzalez, M. J., Jimenez, O., Reich, S., Eljarrat, E. and Josep, R. (2000) Env. Sci. & Technol. 34, 756.
8. Safe, S., Brown, K. W., Donnelly, K. C., Anderson, C. S., Markiewicz, K. V., McLachlan, M. S., Reischl, A. and Hutzinger, O. (1992) Env. Sci. & Technol. 26, 394.
9. McLachlan, M. S., Haynes, D. and Müller, J. F. (2001) Environmental Pollution 113, 129.
10. Wu, W. Z., Schramm, K.-W. and Kettrup, A. (2001) Water Research 35, 1141.
11. Connell, D. W. (1990) Bioaccumulation of xenobiotic compounds, CRC Press, Inc., Boca Raton, Florida.
12. Niimi, A. J. (1996) The Science of the Total Environment 192, 123.
13. Segstro, M. D., Muir, D. C. G., Servos, M. R. and Webster, G. R. B. (1995) Env. Tox. & Chem. 14, 1799.
14. Jarman, W. M., Hobson, K. A., Sydeman, W. J., Bacon, C. E. and McLaren, E. B. (1996) Env. Sci. & Technol. 30, 654.
15. Broman, D., Naef, C., Rolff, C., Zebuehr, Y., Fry, B. and Hobbie, J. (1992) Env. Tox. & Chemistry 11, 331.