MATERNAL TRANSFER OF PCDD/Fs AND PCBs IN MARINE TURTLES

Muusse M[1,4], Hermanussen S[1], Limpus CJ[2], Päpke O[3] and Gaus C[1]

[1]National Research Centre for Environmental. Toxicology (EnTox), 39 Kessels Road, Coopers Plains, Brisbane, Australia; [2]Queensland Environmental Protection Agency, 160 Ann St, Brisbane 4000, Qld, Australia; [3]Eurofins-Ergo Forschungsgesellschaft, Geierstrasse 1, 22305 Hamburg, Germany; [4]Institute for Risk Assessment Sciences, Utrecht University, PO Box 80176, 3508 TD Utrecht, The Netherlands

Introduction

Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) and polychlorinated biphenyls (PCBs) are contaminants that have the potential to accumulate to elevated levels in the environment. Their physico-chemical properties, including their high lipophilicity and strong resistance to metabolic degradation, allow such persistent organic pollutants (POPs) to bioaccumulate and biomagnify in organisms and transfer from mothers to offspring.[1;2] A growing embryo requires energy in the form of lipid which is obtained via blood during gestation in mammals, or stored in eggs in birds and reptiles. Given that PCDD/Fs and PCBs are lipophilic, these compounds may be transferred to the embryo via such lipid pathways.[1] It has been observed that during such processes, a preferred transfer of the lower chlorinated, toxicologically more potent, compounds can occur.[3]

The strong decline of many marine turtle populations has resulted in the listing of all species as vulnerable or endangered. These declines have generally been attributed to various anthropogenic influences, such as by-catch in prawn fishery nets and entrapment in marine debris.[4] Whether marine pollution may contribute to these trends is unknown to date, and only little information exists on marine turtle exposure to contaminants in general. Large immature and adult green turtles live and forage, except for their breeding period, in near shore environments.[5] These habitats often coincide with land-based pollution input zones. Further, their foraging areas are highly conserved and localized, and often encompass only a few km in diameter.[5] This provides a high potential for their exposure to pollutants such as POPs. Although many species feed on relatively low trophic levels, elevated levels of PCBs and/or PCDD/Fs have been reported in marine turtles from the Mediterranean,[6] and USA,[7;8] as well as Australia.[9;10]

The present study was carried out as part of a long-term project that aims to determine the risks to marine turtle populations from POP exposure. Specifically, this study was designed to determine the body burden of dioxins and PCBs in breeding adults and during the early stage of development, to evaluate the influence of parity on the contaminant load in turtle eggs, and to investigate differences in maternal transfer of these compounds between turtle species.

Materials and Methods

Approximately 25-50 ml blood (from the dorsocervial sinus) and 2 egg samples per animal were collected from five loggerhead (*Caretta caretta*) and one flatback turtle (*Natador depressus*). Anticoagulant heparinised saline (50IU in 5mls) and fixative potassium dichromate were added immediately to each blood sample. All samples were stored at -4 °C (during field work) and -20 °C until analysis. Samples were obtained from a nesting beach (Mon Repos) in South East Queensland, Australia during an annual turtle nesting observation program by the Queensland Environmental Protection Agency (Qld EPA) (Ethics Approval No ENTOX/762/04ARC Permit No WITK02868705). All turtles appeared to be healthy on visual examination. Samples were chosen based on the animals' foraging areas and/or parity (determined by laparoscopy[11]). In addition, the age of one of the loggerhead turtles (LH1; 29 years) could be established. This unique information was available from long-term efforts by Qld EPA. All loggerhead turtles were known to forage in Moreton Bay, a semi-enclosed embayment located off Queensland's capital Brisbane, Australia. Among these animals, samples were collected from individuals that have bred for the first, third, fourth and fifth time (Table 1). The flatback turtle was primiparous, however, the foraging area of this individual was unknown.

Blood and egg samples were prepared, extracted and chemical clean-up performed according to standardized procedures for human blood and egg samples, respectively, which were described previously.[12] Analytical blanks were included in each sample batch. $^{13}C_{12}$-labelled internal standards of 17 PCDD/Fs and 12 dioxin-like PCBs were spiked to each sample prior to extraction. The final sample was diluted in a known amount of $^{13}C_{12}$-

labelled 1,2,3,4-TCDD. All instrument analysis was undertaken at ERGO on a VG Autospec or Finnigan HRGC/MS on a DB5 column; 60m, 0.1µm FT, 0.25mm ID with resolution between 8000and 9000 and DB5ms; 60m, 0.25 µm FT, 0.25mm ID with resolution between 6000-7000, respectively.

Body burden (in pg TEQ per kg body weight) for adult turtles was estimated using an assumed 10% body lipid content and an average of 113 kg body weight for loggerheads and 100 kg body weight for flatback turtles. Body burden in turtle eggs was calculated based on the lipid content and weight determined for each respective egg sample. Transfer rates of TEQ were calculated as a percentage of the TEQ load in all egg samples laid per season (estimated at 150 and 445 for flatback and loggerhead turtles, respectively[5]), compared to the TEQ load in the respective mothers. TEQs were calculated using WHO-TEFs for mammals.[13]

Results and discussion

PCDD/Fs and PCBs were detected in all samples analyzed (Table 1). Sum TEQ levels ranged from 21 to 110 pg/g lipid in turtle blood (average 51 pg/g lipid) and 14 to 34 pg/g lipid in eggs (average 24 pg/g lipid). PCDD/Fs contributed the greatest proportion to the total TEQ in blood samples for most animals (61-74%), with the exception of the first time breeding loggerhead turtle LH1 (19%). In egg samples, PCDD/F contributed 96-89% to the total TEQ. Among PCDD/Fs, 1,2,3,7,8-PnCDD contributed the greatest proportion to the TEQ in blood (43 to 71 %) and egg samples (65 to 68%). Among PCBs, non-ortho substituted congeners (in particular PCB 126) contributed the greatest proportion to the TEQ in all blood (65-83%) and all egg samples (71-86%).

Table 1. Summary of PCDD, PCDF and PCB concentrations and TEQ in pg/g lipid and percentage lipid in blood and egg samples of 1 flatback and 5 loggerheads turtles with different breeding statuses. Non-ortho and mono-ortho PCBs congeners analyzed were: 77, 81, 126, 169 and 105, 114, 118, 123, 156, 157, 167, 189

Common name	Flatback		Loggerhead		Loggerhead		Loggerhead		Loggerhead		Loggerhead	
ID	FB1		LH1		LH3		LH4		LH4b		LH5	
Foraging area	unknown		Eastern Moreton Bay		Eastern Moreton Bay		Eastern Moreton Bay		Eastern Moreton Bay		Eastern Moreton Bay	
No of times bred	1st		1st		3rd		4th		4th		5th	
Sample tissue	Blood	Egg	Blood	Egg	Blood	Egg	Blood	Egg	Blood	Egg	Blood	Egg
% lipid	0,12	5,6	0,12	4,9	0,27	4,1	0,37	5,4	0,23	5,8	0,51	4,7
Sum PCDD	380	51	95	62	89	35	140	28	130	61	210	72
Sum PCDF	29	19	30	26	4,2	26	11	13	5,1	21	19	25
Sum PCDD/F	410	70	124	88	93	62	150	41	130	82	230	97
Sum n-o PCB	560	28	3200	130	152	57	180	54	170	89	340	120
Sum m-o PCB	10000	1200	110000	12000	1800	4000	15000	7200	38000	12000	43000	27000
TEQ PCDD/F	34	13	20	25	17	12	13	9,9	41	21	31	27
TEQ n-o PCB	1,4	1,4	72	7,6	7,6	3,1	6,2	3,4	9,4	4,4	8,5	8,45
TEQ m-o PCB	1,6	0,2	14	1,7	2,5	0,55	2,0	0,96	5,1	1,6	5,7	3,5
TEQ PCB	13	1,6	86	9,3	10	3,7	8,3	4,4	14	6	18	12
Total TEQ	**48**	**15**	**110**	**34**	**28**	**16**	**21**	**14**	**56**	**27**	**49**	**39**

To date, only very little information is available on PCBs in marine turtles and comparisons are limited due to inconsistent analysis of various PCB congeners or on an Arochlor basis among the few studies. This lack of information on contaminant exposure of marine turtles or reptiles in general is even more pronounced with respect to PCDD/Fs. The PCDD/F levels found in this study are, however, comparable to those found during a concurrent study on loggerhead turtle blood and adipose tissue with the same foraging area (Eastern Moreton Bay in Queensland) (TEQ 14 to 210 pg/g lipid; average 90 pg/g lipid; n=8). Green turtles from the same area which are, in contrast to loggerhead and flatback turtles, primary consumers, have been found to contain significantly lower TEQ (PCDD/F) levels in blood (0.68 to 14 pg/g lipid, average 7 pg/g lipid, n=16). However, green turtles from the western side of Moreton Bay, which is close to shore and its terrestrial runoff, were found to have higher blood TEQ (PCDD/F) levels (2.7 to 160 pg/g lipid; average 46 pg/g lipid, n=13) compared to the same species foraging within eastern Moreton Bay.[14] Similar PCB concentrations to those of the present study have been reported in adult green turtles (adipose tissue and liver) from Hawaii (same congeners as measured for this study; 12-67 ng/g lipid,; n=2). However, PCB TEQ levels were higher in Hawaiian green turtles (22-103 pg/g lipid, average 79 pg/g lipid), predominantly due to higher contributions of PCB 126.[8]

The habitat of the loggerhead turtles sampled is located approximately 30 km off the coast from an urban area (Eastern Moreton Bay, off Brisbane) with relatively low density tertiary industry, and is considered little impacted by local point sources. Detailed information on the habitat of flatback turtles in Queensland is not available, however, these species generally forage further offshore.[5] Considering this, the TEQ levels in blood tissue and eggs of both species are relatively high. For example, similar TEQ levels have been reported from wildlife within relatively polluted marine environments, such as Baikal Seals.[15]

The 2,3,7,8-PCDD/F congener profiles in blood of the animals analyzed for this study are dominated by PCDDs, in particular OCDD (43 to 63%) (with the exception of LH4b, where 1,2,3,7,8-PnCDD dominates the profile), while PCDFs are present only in low concentrations (Table 1). This profile is consistent with that reported for most marine and terrestrial biota from Queensland, including marine turtles.[9;14;16] A similar profile was also described in freshwater turtles from the Mississippi.[17] In contrast, most marine biota from elsewhere (e.g. Europe, New Zealand) are generally found to contain higher contributions of lower chlorinated PCDD/Fs.[18;19] Similarly, PCBs contributed a relatively minor proportion compared to PCDD/Fs in most animals analyzed for this and other studies in Queensland, whereas this ratio is typically considerably higher in marine biota from elsewhere. The source of the contamination in Queensland remains unknown, however, recent studies have suggested its possible origin in pesticide derived precursors to OCDD.[20]

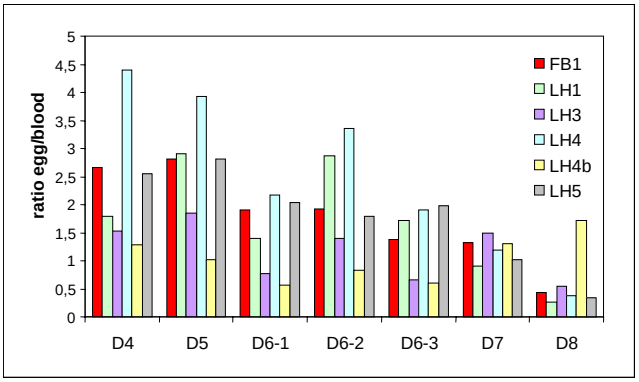

Figure 1. Ratio of % contribution of 2,3,7,8-substituted PCDD congeners to sum total PCDDs between maternal blood and respective egg samples.

A shift in 2,3,7,8-PCDD/F congener profiles was observed from turtle blood to eggs in both the loggerhead and flatback species. Comparing the relative PCDD concentrations in eggs to maternal blood, a trend of preferred transfer of the lower chlorinated PCDD congeners from mothers to eggs becomes apparent (note: blood values for D4 in FB1 and D6-3 in LH1 were below LOD) (Figure 1). Such selective transfer is due to differences in physical-chemical properties of the PCDD/F congeners, in particular their lipophilicity, and is well documented for a range of wildlife, including bird eggs and mammals.[21;22] For PCBs, such preferential transfer of lower chlorinated congeners was not found in the present study. Similarly, a lack of selective maternal transfer was reported for PCBs in penguins and the authors suggested that preferential transfer of lower chlorinated PCB congeners may only occur in animals with the barrier of a placenta.[23] In contrast, comparing the PCB concentrations in loggerhead hatchlings with those in adults animals (albeit not the respective mother), a preferential transfer of lower chlorinated PCB congeners was suggested. The authors could, however, not confirm this finding for the loggerhead egg samples analyzed.[6]

Percent transfer of PCDD/F and PCBs, on a TEQ basis, was calculated based on the estimated body burden in mother and their respective eggs. Percent TEQ transfer loads from mother to their respective eggs laid per breeding season was 1.8% in the flatback (FB1) and ranged from 2.2-5.4% in the loggerhead turtles. On a lipid basis, a transfer rate of 30-80% TEQ was obtained. Similar transfer rates have been reported from other species, e.g. grey seals or in porpoises.[21;24] Interestingly, a trend of increasing percent transfer was observed with increasing breeding cycles. This was predominantly due to a relative increase of body burden in eggs from mothers that have bread more often, however, the cause of this observation remains unknown. It has to be highlighted in this respect that, due to a lack of information on body lipid content in marine turtles, body burdens in mothers were estimated using consistent lipid weights for all animals. This may have introduced considerable uncertainties, and the transfer rates presented here should be regarded as order of magnitude estimates only.

Estimated body burdens in mother turtles and their respective eggs ranged from 3,100 to 16,000 and 800 to 1,800 TEQ pg/kg body weight, respectively. To date, no information exists on the sensitivity of reptiles to PCDD/F and PCBs and it is unknown whether these levels may have the potential to lead to adverse effects in the developing embryo or adult population. In general, sensitivity to such compounds is highest in developing organisms, and evidence for adverse effects have been reported for exposed offspring in various species.[2] A study on freshwater snapping turtles (*Chelydra serpentina serpentina*) from the St. Lawrence River has reported increased deformities by 99-1351% in eggs with 400-1356 pg/g lipid TEQ compared to a reference site.[25] Compared to the present study, these levels were at least 5-fold higher, however, adverse effect levels were not available from this study. Despite this lack of pertinent information, the cumulative data on exposure of reptiles to PCDD/Fs and PCBs highlight that these wildlife have the potential to accumulate such compounds to elevated concentrations, even in habitats that are considered relatively unimpacted by local point sources. Selective PCDD/F maternal transfer observed in this study provides further evidence for exposure of marine turtles during developing life cycle stages.

Acknowledgements
This study could not have been undertaken without support by the Queensland Turtle Conservation Programme (Queensland Environmental Protection Agency and Parks and Wildlife Service) and we would like to thank all staff and volunteers for assistance with this study. Further, we are grateful for the continuous support and expert assistance from ERGO Forschungsgesellschaft. This study was funded by Australian Research Council (LP0453669), with partner contributions by the Queensland Environment Protection Agency, ERGO Forschungssesellschaft and Queensland Health and Scientific Services (QHSS). Entox is co-funded by QHSS.

References

Borrell A, Bloch D, Desportes G. *Environ Pollut* 1995; 88:283.
Geyer HJ, Scheunert I, Rapp K, Kettrup A, Korte F, Greim H, Rozman K. *Toxicology* 1990; 65:95.
Geyer HJ, Schramm K-W, Anton Feicht E, Behechti A, Steinberg C, Bruggemann R, Poiger H, Henkelmann B, Kettrup A. *Chemosphere* 2002; 48:631.
Bjorndal K, Zug GR. In: *Biology and Conservation of Sea Turtles. Revised Edition*, Bjorndal, KA (ed.), Smithsonian Institution Press, Washington DC, 1995:599.
Limpus CJ, Chalaupka M. *Mar Ecol - Prog Ser* 1997; 149:27.
Mckenzie C, Godley BJ, Furness RW, Wells DE. *Mar Environ Res* 1999; 47:117.
Keller JM, Kucklick JR, McClellan-Green PD. *Arch Environ Con and Tox* 2004; 46:254.
Miao X-S, Balazs GH, Murakawa SKK, Li QX. *Sci Total Environ* 2001; 281:247.
Hermanussen S, Limpus C, Päpke O, Blanchard W, Connell D, Gaus C. *Organohalogen Compounds* 2004; 66:1861.
Vetter W, Scholz E, Luckas B, Gaus C, Müller JF, Haynes D. *Arch Environ Con Tox* 2001; 41:221.
Limpus CJ, Limpus DJ. In: *The biology and Conservation of Loggerhead Sea Turtles*, Bolten, AB, Witherington, BE (eds.), Smithsonian Institution Press, Washington DC, 2003:199.
Päpke O, M B, A LZ, K S. *Chemosphere* 1989; 19:941.
Van den Berg M, Birnbaum L, Bosveld ATC, Brunstroem B, Cook P, Feeley M, Giesy JP, Hanberg A, al. e. *Environ Health Persp* 1998; 106:775.
Hermanussen S, Limpus C, Paepke O, Connell DW, Gaus C. *Organohalogen Compounds* 2006; in press
Tarasova EN, Mamontov AA, Mamontova EA, Klasmeier J, McLachlan MS. *Chemosphere* 1997; 34:2419.
Gaus C, Correll R, Müller J, Holt E, Ellis D, Prange J, Shaw M, Bauer U, Symons R, Burniston D. *Organohalogen Compounds* 2005; 67:1271.
Kannan K, Ueda M, Shelby JA, Mendonca MT, Kawano M, Matsuda M, Wakimoto T, Giesy JP. *Arch Environ Con Tox* 2000; 38:362.
Buckland SJ, Hannah DJ, Taucher JA. *Chemosphere* 1990; 20:1035.
Jimenez B, Gonzalez MJ, Jimenez O, Reich S, Eljarrat E, Josep R. *Environ Sci Technol* 2000; 34:756.
Gaus C, Holt E, Weber R, Prange J, Kuch B. *Organohalogen Compounds* 2005; 67:1271.
Addison RF, Ikonomou MG, Stobo WT. *Mar Environ Res* 1999; 47:225.
Stephens RD, Petreas MX, Hayward DG. *Sci Total Environ* 1995; 175:253.
Tanabe S, Subramanian A, Hidaka H, Tatsukawa R. *Chemosphere* 1986; 15:343.
Subramanian A, Tanabe S, Tatsukawa R. *Mar Environ Res* 1988; 25:161.
Bishop CA, Ng P, Pettit KE, Kennedy SW, Stegeman JJ, Norstrom RJ, Brooks RJ. *Environ Pollut* 1998; 101:143.