DIOXINS AND DIOXIN-LIKE PCBs IN EDIBLE MARINE SPECIES FROM MORETON BAY, QUEENSLAND, AUSTRALIA

Matthews V[1], Papke O[2], Matthews C[3] ,Gaus C[1]

[1]EnTox, University of Queensland, 39 Kessels Road, Coopers Plains, Queensland, 4108, Australia; [2]Eurofins-ERGO Forschungsgesellschaft mbH · Geierstrasse 1, D-22305, Hamburg, Germany; [3]Centre for Environmental Systems Research, Griffith University, 170 Kessels Road, Nathan, Queensland, 4111, Australia.

Introduction

For the general population, food ingestion contributes approximately 90% of total exposure to persistent organic pollutants (POPs), such as dioxins, and the majority of this exposure results from lipid rich products such as seafood[1]. In Australia, limited data exists on POP levels in seafood. A national supermarket food survey in 2004 concluded that the exposure risk from dioxins and dioxin-like contaminants in food (including seafood) was very low for the general population.[2]

Despite the low density of POP point sources within south-east Queensland, recent studies have shown elevated PCDD/F levels in marine sediments in Moreton Bay.[4] Further biomagnification processes resulting in elevated TEQ levels within animals such as dugong and turtle were reported from Queensland.[3,4] To date, no information exists on whether these processes may result in elevated TEQ levels in commonly consumed seafood species from the bay.

This paper reports the outcome of a pilot study undertaken in 2005. The aim of the study was to gain a preliminary indication of PCDD/PCDF and dioxin-like PCB levels in a range of seafood sources from Moreton Bay and also to investigate contamination variability due to size, habitat location and trophic level. It is part of a broader investigation into the pathways and processes of POP contamination in a marine tropical environment and the effect on Moreton Bay edible marine species. This longer term study aims to provide information for exposure assessments of coastal communities in local contaminated areas.

Materials and Methods

A total of 11 seafood species (7 fish, 3 shellfish, 1 crustacean) were sampled in July and August 2005 from Moreton Bay, a semi-enclosed embayment in south-east Queensland, Australia, off the capital Brisbane. Fish and crustacean species were obtained from commercial fishermen in Western Moreton Bay (WMB) close to the mainland shore. In addition, 4 of the 11 fish species were sourced from the ocean side of North Stradbroke Island (NSI). Two to three size classes were obtained for each fish species. The shellfish were collected with assistance from community members at typical harvesting locations in Eastern Moreton Bay (EMB) and Main Beach on the ocean side (OS) (Figure 1). Seafood samples were analysed for 2,3,7,8-substituted PCDD/F and non-ortho PCB profiles at Eurofins-ERGO Forschungsgesellschaft mbH in Hamburg, Germany. Approximately 70-90g of shellfish tissue (pooled) and 50g of individual fish and mudcrab tissue were homogenised by blender and then ground in mortar and pestle with anhydrous sodium sulphate. The homogenates were spiked with $^{13}C_{12}$-labelled PCDD/F standard containing all 17 2,3,7,8-substituted PCDD/Fs and 12 dioxin-like PCBs. Lipids were extracted using several rinses of dichloromethane/cyclohexane (1:1), with the first rinse left soaking for 2 hours, and lipid content determined gravimetrically. Clean-up included a silver nitrate column, followed by fractionation on a carbon cartridge (Supelco). The PCDD/F fraction was further subjected to

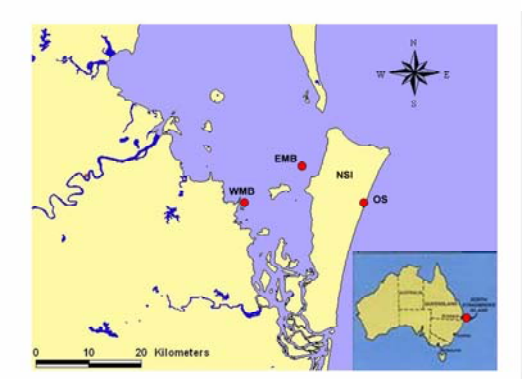

Figure 1 Sampling locations in Moreton Bay.

clean-up using a tandem acid/base alumina column. Extracts were then transferred to a vial, evaporated to near dryness and 10µl of injection standard (1,2,3,4 TCDD and $^{13}C_6$ 1,2,3,4,6,7,8 HpCDF) was added for recovery calculations. For quality assurance, blanks and reference material were processed with each batch. Instrument analysis was performed on an Autospec HRGC/MS (DB-5 column, 60m x 0.25mm i.d., film thickness 0.1µm) operating on a resolution of 9,000 throughout the sample sequence. Individual congeners were identified using retention times of the labelled standards and ion abundance ratios at M^+ and $M+2^+$ or $M+4^+$. The toxic equivalent concentrations for PCDD/Fs and PCBs were calculated using 1998 WHO TEFs (mammalian). For TEQ calculations, concentrations lower than the limit of detection or quantification were regarded as zero.

Results and Discussion

Results obtained from this pilot study are presented on a lipid basis to facilitate comparisons between fish species, size class and locations. It was noted that the lipid yields obtained from the present study were often below (approximately 1.5 - 7 fold) those reported for the same species in earlier studies.[5,6] The cause of this is unclear, but may be related to intraspecies variability due to differing size class and/or breeding status. Alternatively, it has been shown that various commonly used lipid extraction methods can produce yields that differ by up to 3.5 fold.[7]

PCDD/Fs and non-ortho PCBs were detected in all samples analysed. TEQ (PCDD/Fs and non-ortho PCBs) levels ranged from 14 to 160 pg/g lipid in fish, 100 pg/g lipid in crab and 10 to 70 pg/g lipid in shellfish samples. PCDD/Fs contributed the major proportion (28 to 100%, average 68%) to the total TEQ among most samples analysed (Figure 2). Among PCDD/Fs, 1,2,3,7,8 PeCDD contributed the major proportion to TEQ levels in most samples (22 to 75%). Among non-ortho PCBs, PCB 126 contributed the greatest percentage to total TEQ (21 to 99%). PCDD/F and PCB TEQ levels were generally variable within each species (17 to 95%). Total concentrations of ΣPCDD/PCDFs in fish ranged from 66 to 1,400 pg/g lipid. The muscle tissue of the crustacean and shellfish species contained consistently higher PCDD/F concentrations, ranging from 4,300 to 37,000 pg/g lipid. Non-ortho PCB concentrations ranged from 28 to 3,000 pg/g lipid in fish and 9.1 to 100 pg/g lipid in shellfish, whereas 1,400 pg/g lipid was present in the single mudcrab sample.

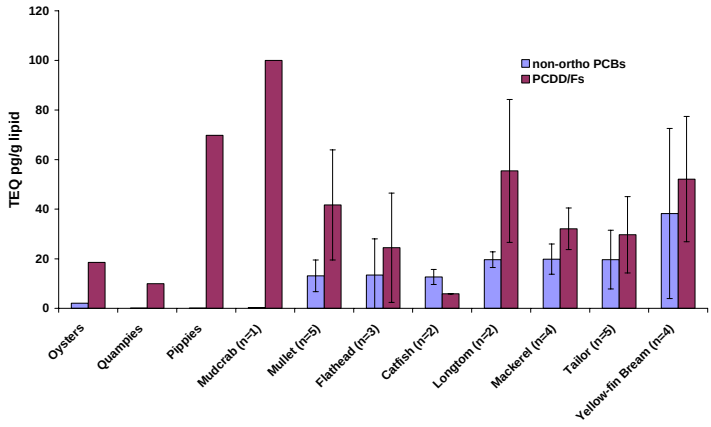

Figure 2 TEQ levels and variability (% Standard Deviation) for PCDD/Fs and non-ortho PCBs in seafood from Moreton Bay, Queensland, Australia.

It is difficult to compare the TEQ levels obtained from this study with those reported from other seafood, since a multitude of factors (e.g. trophic level position, species, seasonality, size class, time-trends, catchment area, sampling methodology) have been shown to influence TEQ levels in seafood. In general, however, it can be observed that despite the relatively low density of typical PCDD/F and PCB point sources around south east Queensland, the average TEQ level of fish (54 pg/g lipid) from this study is elevated by 9 to 65 fold compared to recent Australian national data on marine/estuarine fishes and retail food respectively. Relatively low PCB and PCDD/F TEQ levels (0 to 12 pg/g lipid, average 0.82 pg/g lipid; n=19 composite) were reported in fish fillets and portions from supermarkets around Australia, however, it is unknown where these samples originated from.[2] Fish samples collected from Australian marine and estuarine waters ranged from 0.72 to 68 pg/g lipid (average 6 pg/g lipid; n=20).[5] Similarly, TEQ levels in seafood from Moreton Bay is generally elevated compared to those reported from national studies in New Zealand, Spain and Korea.[8,9,10] Compared to seafood from highly polluted sites, however,

TEQ levels in seafood from this study are considerably lower or within the lower range of reported levels. For example, recent findings from Sydney Harbour and its tributaries, which were impacted via long-term operations of a chemical manufacturing plant, reported elevated TEQ levels in bream ranging from 6.6 to 141 pg/g fw (average 29.1) and 3.1 to 22.9 pg/g fw (average 11.3) in prawns (no lipid yields are available). Median (and range) $TEQ_{D/F}$ levels in Baltic Sea herring have been reported to vary from 57 (35-59) to 340 (160-970) pg/g lipid, depending on size class and catchment area.[11]

TEQ levels in seafood from Moreton Bay are unusually elevated considering the bay's (and Queensland's in general) sediment PCDD/F contamination pattern that predominantly consists of OCDD (66 to 98% of sum PCDD/F).[4] Non-2,3,7,8-substituted PCDDs are another major contributor to sum PCDD/F concentrations in sediments, in particular 1,4,6,9-substituted isomers.[12] The lower chlorinated 2,3,7,8-substituted, more toxicologically potent, congeners (and most PCDFs), are typically below the limit of detection in most sediment samples analysed from Moreton Bay and Queensland to date.[4] PCDD/F congener profiles in seafood from Moreton Bay reflect this sediment pattern and were dominated by PCDDs, in particular OCDD (8.5 to 63%, average 30%), while PCDFs were present only in relatively low concentrations (D/F ratio 3.4-540, average 105). Similarly, non-2,3,7,8-substituted PCDDs were found to be present in most seafood species (average 23% in fish), in particular in lower trophic specimens (average 62% in bivalves and crustaceans). However, in contrast to sediments, bioaccumulation and biomagnification processes result in increased concentrations of 2,3,7,8-TCDD and 1,2,3,7,8-PnCDD in Moreton Bay seafood species across all trophic levels (Figure 3). This is consistent with PCDD/F profiles reported for other biota from Queensland and in Australia in general.[4,5] It has previously been shown that bioaccumulation and biomagnification processes in this environment can result in elevated TEQ levels even in Queensland's low herbivorous species such as green turtles and dugongs (e.g. TEQ up to 140 pg/g lipid), due to a high biomagnification potential of the lower chlorinated PCDDs in these animals.[4] This highlights the inappropriateness of applying TEQs to abiotic environmental matrices for assessment of potential exposure or even risks for wildlife and humans (i.e. TEQ in sediments from Moreton Bay would be comparable to typical "background" contamination, 0.21 to 4.9 pg/g dw).[4]

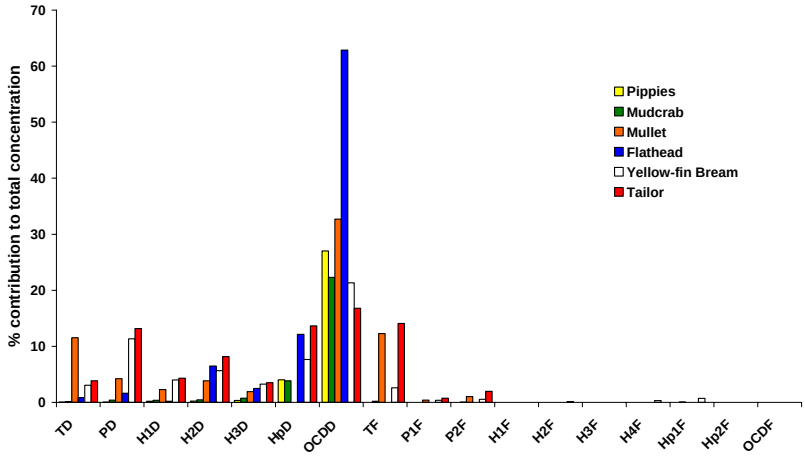

Figure 3 % contribution of 2,3,7,8-substituted PCDD/F congeners to sum total PCDD/F concentrations in different trophic level seafood from Moreton Bay

Between the various trophic level seafood species analysed, the contribution of lower chlorinated PCDD/F to TEQ was generally observed to increase from sediment feeders to predators (Figure 3). This biomagnification of toxicologically more potent congeners is well documented in many species including fish,[13,14] and reflects a) the greater capacity of these congeners to be absorbed and retained within biological tissues due to physical-chemical properties and b) species specific metabolic transformation rates.[15] One exception to this general trend was mullet, a herbivorous bottom dwelling fish species, which feeds on predominantly diatoms. Despite its low trophic position, the congener profiles of all mullet samples analysed (n=5) displayed an unusually elevated contribution of 2,3,7,8-TCDD, 1,2,3,7,8-PnCDD and 2,3,7,8-TCDF (Figure 3), similar to that observed in higher trophic fish species (eg. tailor). The cause of this biomagnification is currently unclear but may reflect a) the contamination of mullet food

via other point sources, e.g. mullet may have migrated from point source influenced areas into Moreton Bay and/or b) a slow metabolic capacity in mullet fish for these compounds.

As indicated above, TEQ level variability within seafood species was relatively high (17 to 95%). To investigate potential variables, different size classes were collected for all fish species and four species were collected from different locations. Seasonal influences can be excluded due to the collection of all samples during the same winter season. Various studies have reported clear trends of increasing TEQ levels with increasing size and age of fish.[11,16] Among the seven fish species collected, an increase in TEQ with size class was only observed for mullet (Moreton Bay sampling sites; n=2) and tailor (both oceanic (n= 2) and Moreton Bay sampling sites (n= 3)).

With respect to seafood harvesting location (i.e. oceanic versus within bay sampling sites), no clear trend could be observed in TEQ levels or PCDD/F and PCB concentrations. While sample numbers available for detailed evaluations on such influencing parameters are low to date, it is noteworthy that shellfish were exclusively sampled from "clean water" sites in Eastern Moreton Bay, distant from terrestrially sourced pollution inputs. Despite this, TEQ levels in these species were elevated, ranging from 10 to 70 pg/g lipid. Overall, the present study highlights that seafood contaminant levels in local marine environments may differ considerably from the national averages. However, this pilot study does not allow conclusions on human exposure. This will require information on seafood consumption and more detailed investigations on seafood contaminant levels, which will be the subject of future work.

Acknowledgements
The authors thank Darren Burns, the Quandamooka community and John Page for provision of seafood samples and Siobhan Hermanussen and Sharon Grant for laboratory assistance. Continuous support and professional advice from staff at Eurofins-ERGO Forschungsgesellschaft is gratefully acknowledged. This study is funded by the National Health and Medical Research Council (grant no: 351572). The National Research Centre for Environmental Toxicology is co-funded by Queensland Health Scientific Services (QHSS).

References

1. Fuerst P, Beck H, Theelen R. *Toxic Substances Journal*. 1992;12:133.
2. Food Standards Australia New Zealand. *Dioxins in Food.* 2004.
3. Gaus, C. PhD Thesis. School of Public Health, Griffith University, Queensland, Australia. 2002.
4. Hermanussen S, Limpus CJ, Päpke O, Blanshard W, Connell D, Gaus C. *Organohalogen Compounds.* 2004; 66:1837.
5. Müeller J, Muller R, Goudkamp, K, Mortimer M. *National Dioxins Program – Dioxins in Aquatic Environments in Australia.* Department of the Environment and Heritage, Canberra. 2004.
6. NSW Food Authority. *Dioxins in Seafood in Port Jackson and its Tributaries – Report of the Expert Panel*. 2006.
7. Randall RC, Lee II HL, Ozretch RJ, Lake JL, Preull RJ. Environmental Toxicology and Chemistry 1991;10:1431.
8. Buckland SJ, Scobie S, Heslop V. *Organochlorines in New Zealand: Concentrations of PCDDs, PCDFs and PCBs in retail foods and an assessment of dietary intake for New Zealanders.* Ministry for the Environment, Wellington, 1998.
9. Bordajandi LR, Martín I, Abad E, Rivera J, González, MJ. *Chemosphere*.(article in press) 2006.
10. Moon HB, Ok G. *Chemosphere*. 2006;62:1142.
11. Kiviranta H, Vartiainen T, Parmanne R, Hallikainen A. Koistinen J. *Chemosphere.* 2003;50:1201.
12. Gaus C, Brunskill GJ, Connell DW, Prange J,Muller JF, Papke O, Weber R. *Environmental Science & Technology*. 2002;36:3542.
13. Opperhuizen A, Sijm DTHM. *Environmental Toxicology and Chemistry.* 1990:9:175.
14. Okumura Y, Yamashita Y, Isagawa S. *Journal of Environmental Monitoring.* 2003;5:610.
15. Wan Y, Hu J, Yang M, An L, An W, Jin X, Hattori T, Itoh M. *Environmental Science & Technology* 2005;39:2417.
16. Parmanne R, Hallikainen A, Isosaari P, Kiviranta H, Koistinen J, Laine O, Rantakokko P, Vuorinen PJ, Vartiainen T. *Marine Pollution Bulletin*. 2006;52:149.