# LEVELS OF HALOGENATED NATURAL PRODUCTS ON THE GREAT BARRIER REEF, AUSTRALIA FROM 2007-2013

Kaserzon S.[1]*, Gallen C.[1], Knoll S.[2], Gallen, M.[1], Hauler C.[2], Vetter W.[2], Mueller J.[1]

[1]The University of Queensland, and National Research Centre for Environmental Toxicology (EnTox), 39 Kessels Road, Coopers Plains 4108, Australia;
[2]University of Hohenheim, Institute of Food Chemistry, Garbenstrasse 28, D-70599 Stuttgart, Germany

## Introduction

An increasing number of halogenated natural products (HNPs) previously assumed to be remnants of anthropogenic pollutants are now being identified as natural biosynthesized products. Reports of HNPs occurrence in marine wildlife from all oceans are increasing[1] with recent studies showing biomagnification factors (BMFs) for some HNPs can be similar or higher than those obtained for anthropogenic sources of PBDEs.[2] The risks associated with exposure to HNPs are not elucidated, though studies suggest that some species have similar toxic properties to PBDEs.[3] Although progress has been made on the isolation of some HNPs from a number of marine biota (i.e. blue mussels, sponges and red algae), the ubiquity of these chemicals in the marine environment and the potential risk they may cause to wildlife and humans remains poorly understood.

The difficulty with detecting and quantifying HNPs in marine systems stem from their often trace level presence in the environment and lipophilic affinity. Thus the enrichment of large quantities of water would typically be required as well as sensitive analytical methods. Passive sampling (PS) tools have proven an effective technique for the *in-situ* enrichment of lipophilic organohalogen compounds in marine environments. Where calibration data is available, PS provides time-weighed concentration estimates of the analytes instead of a snapshot at the point of sample collection. The polydimethylsiloxane (PDMS) and semipermeable membrane device (SPMD) samplers are among the most frequently used for organic contaminants with log $K_{ow}$ > 3. To date studies have shown that accumulation of HNPs in SPMDs is successful,[1, 4] though no calibration data for these chemicals with passive sampling tools are currently available to achieve quantitative concentration estimates.

The Great Barrier Reef (GBR) in Australia stretches for about 2500 km from the northern coastline of eastern Australia and is home to a rich and diverse ecosystem. The presence of species on the GBR that produce HNPs has previously been verified, although the type and extent of this production and what may influence it are still largely unknown.[1] As part of a protection plan outlined for the GBR, routine multi-year sampling campaigns have been carried out with the aim of monitoring the health of the reef's ecosystem and the inputs of organic contaminants. For the purpose of this study we were able to access archived PDMS samplers from this unique sample-set deployed at one site on the GBR (Normanby Island), over a period of 6 years. With these samples we attempted to verify the presence and type of HNPs typical for this marine environment. Furthermore, we conducted a PDMS field calibration study off the coast of Heron Island on the GBR with the purpose of obtaining calibration data to quantify HNPs in the marine system. This site was chosen since HNPs were previously identified in marine biota samples collected from around the Island and the area was recognized as a prime site for HNP biosynthesis.

Thus, the aims of this study were twofold; (i) to determine the occurrence and profile of HNPs at a well monitored site on the GBR, over a period of 6 years. (ii) Quantify the levels of HNPs in the GBR marine system through data obtained from a field calibration study as well as modelled data.

## Materials and methods

### *PDMS calibration study*

PDMS samplers (92 cm x 2.5 cm x 0.5 cm; *n*=2) were pre-cleaned with acetone and *n*-hexane, loaded into stainless steel cages and were deployed off the coast of Heron Island, GBR, in a staggered deployment design for

2,3,4,5,10 and 19 days. Blanks accompanied field samples during transport extraction and analysis. Passive flow monitors (PFMs) were co-deployed to estimate the average water velocity during the deployment. Prior to extraction each PDMS strip was cleaned by scrubbing with water and dried. Each PDMS strip was extracted in 2 x 200 mL of *n*-hexane on a shaker at room temperature (21 °C) for two 24 h periods. The combined extracts from each sampler were then reduced to about 1 mL using rotary evaporators. Extract were then passed through a column with about 2 g of sodium sulfate to remove moisture. The extracts were reduced in volume and filtered (0.45 µm PTFE) into 10 mL of dichloromethane (DCM) and subjected to clean up using size exclusion gel permeation chromatography (GPC). The collected fraction was reduced to a final volume of 100 µL (PDMS extracts) and 40 µL (water samples) in DCM.
Grab samples (1 L) were collected in pre-rinsed glass amber jars on days 1,2,3,4,5,10 and 19 from the site in which PDMS were deployed. Grab samples were immediately stored in the fridge on the Island until extraction. Grab samples (1 L) were extracted by liquid-liquid extraction with 2 x 150 mL DCM. 20 µL of nonane was added before samples were reduced on a rotary evaporator to ~ 10 mL. Samples were then filtered and evaporated under gentle stream of nitrogen to 1 mL. PDMS and grab sample extracts were analysed by gas chromatography with electron capture detection (GC/ECD). A full description of the analytical procedure as well as the HNP recovery and quantification standards used are provided in[4]

***Great Barrier Reef temporal study***
PDMS sampler (*n*=2) along with PFMs were routinely deployed off the coast of Normanby Island, on the GBR for periods of between 21 -100 days, from May 2007 – February 2013. Archived samples that were analysed were stored at -20 °C. The handling, extraction and analysis of PDMS samplers was performed as described above. However, PDMS replicate extracts were combined for analysis.

***Data modelling***
*Experimental data*: For HNPs that showed linear accumulation in PDMS, Sampling rates ($R_s$) were determined via [$R_s = k_oA$] where $k_o$ is the chemical mass transfer coefficient and A = surface area of the sampler.[5]
*Modelled Data*: Where calibration data was not available, log sampler water partition coefficient (log $K_{sw}$) values were estimated as a function of log $K_{ow}$ [$K_{sw} = 0.82 \log K_{ow} + 0.24$].[6]
$R_s$ were predicted via a quadratic equation [$(Y=B_0 + B_1*X + B_2*X^2)$], in which X = log $K_{ow}$, from a second order polynominal plot of log $K_{ow}$ vs $R_s$ that was previously derived under a number of flow velocity conditions for model chemicals with log $K_{ow}$ of 4.8 – 8.15.[7] Water concentrations for HNPs that were in linear accumulation in PDMS were estimated from [$C_w = N_s/R_st$] where $C_w$ = water concentration and $N_s$ = amount of chemical accumulated in the sampler. Water concentrations for HNPs that had reached water-PDMS equilibrium were calculated based on the differential equation that governs the chemical uptake process [$Ak_o/V_s$ ($C_w$-($C_s/K_{sw}$)], where $V_s$ = volume of the sampler, $C_s$ = concentration of chemical accumulated in the sampler.

***HNPs included in the study***: 2,4-dibromophenol (2,4-DBP), 2,6-dibromophenol (2,6-DBP), 2,4-dibromoanisole (2,4-DBA), 2,4,6-tribromoanisole (2,4,6-TBA), 2,4,6,-tribromphenol (2,4,6-TBP), 2,3,4,5-tetrabromomethylpyrrole (TBMP), 2,3,3',4,4',5,5'-heptachloro-1'-methyl-1,2'-bipyrrole (Q1), 1,1'-dimethyl-3,3',4,4'-5,5'-hexachloro-2,2'-bipyrrole ($Cl_6$-DBP), 2,2'-dimethoxy-3,3',5,5'-tetrabromobiphenyl (2,2'-diMeO-BB 80 or BC-1), 4,6-dibromo-2-(2',4'-dibromo)phenoxyanisole (2'-MeO-BDE 68 or BC-2), 3,5-dibromo-2-(2',4'-dibromo)phenoxyanisole (6-MeO-BDE 47 or BC-3), 5,5'-dichloro-1,1'-dimethyl-3,3',4,4'-tetrabromo-2,2'-bipyrrole (BC-10) and 3,5-dibromo-2-(3',5'-dibromo-2'-methoxy)phenoxyanisole) (2',6-diMeO-BDE 68 or BC-11).

## Results and discussion

### Calibration study
Linear accumulation in PDMS was observed for 2,6-DBP, 2,4-DBA, 2,4,6-TBA, TBMP, Q1, BC-1, BC-2, BC-3 and BC-11 over the 19 day field calibration study on Heron Island (Figure 1). Experimental $R_s$ ranged from 14 – 28 L $d^{-1}$ (Table 1). $R_s$ for TBMP, BC-1, BC-2 and BC-11 could not be derived as these chemicals could not be quantified in grab water samples. 2,4-DBP and 2,4,6-TBP showed inconsistent accumulation in PDMS, possibly due to low levels at the sampling site. BC-10 was not detected at Heron Island in PDMS or grab samples. The linear accumulation observed for HNPs in PDMS supports the utility of this technology for the monitoring of these chemicals in aquatic systems, in particular for HNPs with log $K_{ow} > 5$, especially given the challenges in

obtaining reliable quantification from grab sample data for these compounds. Where available, a good correlation was observed between modelled and experimental $R_s$ values (i.e. Q1 and BC-3). This suggest that the current modelling methods[8] used to predict log $K_{sw}$ for hydrophobic chemicals as a function of log $K_{ow}$ values based on good correlation of physicochemical properties are valid for these HNPs. HNPs with log $K_{ow}$ < 5 are likely to reach equilibrium during extended deployment periods. In such cases log $K_{sw}$ values are typically used and provide point in time rather than time-weighted average estimations of water concentrations.

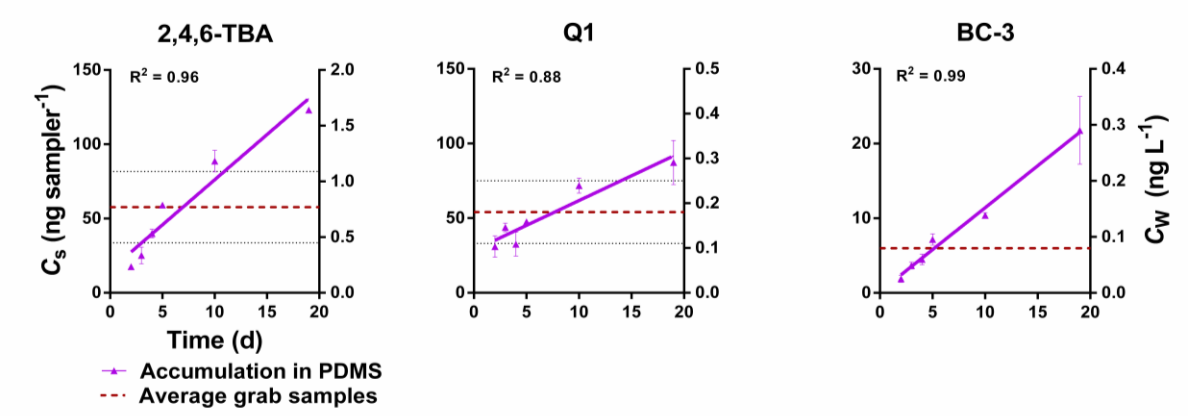


**Figure 1 Uptake profiles of 2,4,6-TBA, Q1 and BC-3 (ng sampler$^{-1}$) in PDMS samplers deployed on Heron Island, GBR, and their concentrations in water (ng L$^{-1}$) obtained from grab samples**

**Table 1 Modelled and experimental calibration parameters for HNP with PDMS and frequency of detection and estimated water concentrations of HNPs from Normanby Island, GBR**

| NHPs | PDMS Calibration parameters | | | | | HNPs detected at Normanby, GBR | |
|---|---|---|---|---|---|---|---|
| | log $K_{ow}$ . | Sampling phase | Modelled parameters* log $K_{sw}$ (L kg$^{-1}$) | Modelled parameters* $R_s$ (L d$^{-1}$) | Experimental parameters $R_s$ (L d$^{-1}$) | Frequency of detection (%) ($n$ = 40) | Estimated water concentration range (Mean) (pg L$^{-1}$) |
| 2,4-DBP | 3.3 | Equilibrium | 2.9 | | | 100 | 23 - >1000 (4000) |
| 2,6-DBP | 3.3 | Equilibrium | 2.9 | | 14 ± 5 | 30 | 0 - >1000 (78) |
| 2,4-DBA | 3.8 | Equilibrium | 3.3 | | 14 ± 4.8 | 100 | 21 - >1000 (470) |
| 2,4,6-TBA | 4.5 | Equilibrium | 3.9 | | 15 ± 2 | 100 | 6 - >1000 (175) |
| 2,4,6-TBP | 3.9 | Equilibrium | 3.4 | | 11 ± 0 | 50 | 0 - 318 (22) |
| TBMP | 4.0 | Equilibrium | 3.5 | | | 90 | 0 - >1000 (226) |
| Q1 | 6.6 | Linear | 5.6 | 26 | 21 ± 4 | 100 | 9 - 230 (68) |
| $Cl_6$-DBP | 5.7 | Linear | 4.9 | 24 | | 45 | 0 - 12 (0.7) |
| BC-1 | 6.1 | Linear | 5.2 | 26 | | 98 | 0 - 22 (4) |
| BC-2 | 6.3 | Linear | 5.4 | 26 | | 100 | 4.4 - 60 (15) |
| BC-3 | 6.3 | Linear | 5.4 | 26 | 28 ± 2 | 98 | 0 - 108 (31) |
| BC-10 | 6.5 | Linear | 5.6 | 26 | | 100 | 2.8 - 69 (19) |
| BC-11 | 5.3 | Linear | 4.6 | 20 | | 98 | 0 - 42 (10) |

*See *Data modeling* section for a description of how $R_s$ and log $K_{sw}$ model parameters were derived.

**Great Barrier Reef temporal study**

HNPs were consistently detected in PDMS over a 6 year period at Normanby Island, GBR (Figure 2) with detection frequencies ranging from 30% - 100% (Table 1). No correlation was observed between mass of HNP detected and rainfall during the dry (May – November) and wet (December – April) seasons. Modelled and experimental calibration parameters have allowed estimation of water concentrations of these chemicals in the marine system and mean water concentrations ranged from 0.7 pg L$^{-1}$ ($Cl_6$-DBP) – 4000 (2,4-DBP) pg L$^{-1}$ over the sampling period (Table 1). Although water concentration estimates suggests that aqueous levels of HNP are very low, their constant presence and persistence coupled with the high biomagnifications factors of some compounds may indicate the potential of risk to species through the food chain. For example, high levels of Q1,

BC-1, BC-2, and BC-3 have previously been reported in dolphin blubber from Northeast Queensland, Australia.[9] While this research targets a number of known HNPs, it is very likely that additional yet undiscovered chemicals are present in the marine system. Further, it is possible that events such as warming water temperatures, changing climate and nutrient inputs may affect the biosynthesis of these chemicals. Therefore further research into the species and triggers for the production of HNPs and the continued monitoring of these chemicals in the environment are warranted. The PDMS may have application in this regard.

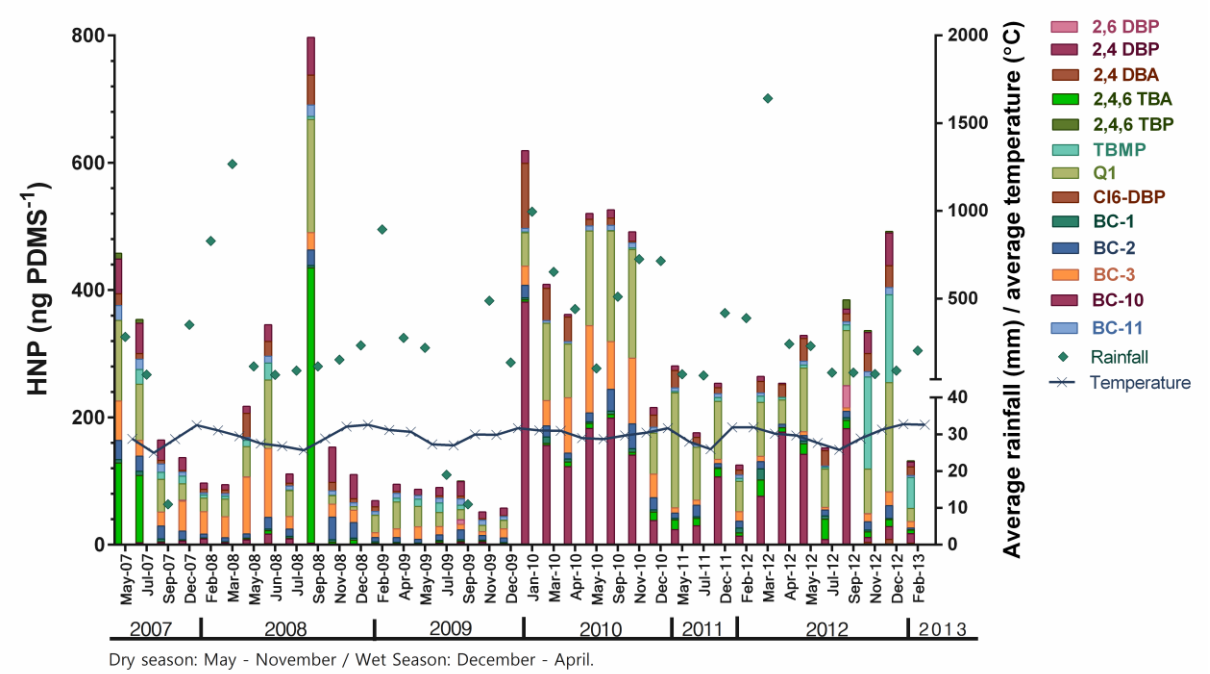


**Figure 2 Amount (ng sampler$^{-1}$) of individual NHPs detected in PDMS (standardized to 30 days) from May 2007 – February 2013 at Normanby Island on the Great Barrier Reef**


**Acknowledgements**

This work was possible through scholarship and travel support from GO8-DAAD (German Academic Exchange Service). We thank the group at the National Research Centre for Environmental Toxicology, The University of Queensland and HIRS staff for assistance with field deployments and GBRMPA for access to archive samples.


**References**


1. Gaul, S.; Bendig, P.; Olbrich, D.; Rosenfelder, N.; Ruff, P.; Gaus, C.; Mueller, J.; Vetter, W.(2011); *Mar Poll Bull. 62* (11), 2463-2468.
2. Stapleton, H.M.; Dodder, N.G.; Kucklick, J.R.; Reddy,C. M.; Schantz, M.M.; Becker,P.R.; Gulland,F.; Porter,B.J.; Wise,S.A. (2006); *Mar Poll Bull. 52*, 522–531.
3. Wiseman, S.; Wan, Y.; Chang, H.; Zhang, X.; Hecker, M.; Jones, P.D.; Giesy, J.P. (2011); *Mar Poll Bull. 63*, 179-188.
4. Vetter,W.; Haase-Aschoff, P.; Rosenfelder, N.; Komarova, T.; Mueller, J.(2009); *Environ Sci Technol. 43* (16), 6131-6137.
5. Booij, K.; Vrana, B.; Huckins, J. N., Chapter 7.In *Comprehensive Analytical Chemistry*, Greenwood, R.; Mills, G. A.; Vrana, B., Eds. Elsevier: 2007; Vol. 48, pp 141-169.
6. Rusina, T. P.; Smedes, F.; Koblizkova, M.; Klanova, J. (2010); *Environ Sci Technol. 44*, 362-367.
7. O'Brien, D. S.; Komarova, T.; Mueller, J. (2012); *Mar Poll Bul. 64* 1005–1011.
8. Booij, K.; Smedes, F. (2010); *Environ Sci Technol. 44* (17), 6789-6794.
9. Vetter, W.;Scholz, E.;Gaus, C.;Mueller, J.;Haynes, D. (2011); *Environ Contam Toxicol. 41*, 221-231.