IDENTIFICATION OF Q1 AND OTHER HALOGENATED NATURAL PRODUCTS IN SPMDS DEPLOYED AT THE GREAT BARRIER REEF (AUSTRALIA)

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Abstract

Halogenated natural products (HNPs) such as heptachlorobipyrrole (Q1) and 4,6-dibromo-2-(2',4'-dibromo)phenoxyanisole (2'-MeO-BDE 68 or BC-2) have been detected in marine wildlife worldwide. Among the highest concentrations of some HNPs, were detected in marine mammals including dolphins from Queensland/Australia. In this study we evaluate the presence of potentially bioavailable HNPs using semipermeable membrane devices (SPMDs) passive samplers. SPMDs, deployed at 12 marine and two non-marine sites (i.e. rivers) along the Great Barrier Reef as part of a routine monitoring program during November 2007 and May 2008 were analysed. Q1 and 2'-MeO-BDE 68 were detected in about two thirds of samples collected from marine sites with up to 44 ng/SPMD (mean 12 ng/SPMD) for Q1 and up to 115 ng/SPMD (mean 20 ng/SPMD for BC-2) (values normalised to 30 days deployment). Neither of these compounds was detected in the samples from the two rivers. Other HNPs including 2,4,6 tribromoanisol (TBA) and 2,4,6 tribromophenol (TBP) were detected as well. No polybrominated flame retardants were detected in any of the samples. Highest amount of any HNP that was detectable was for TBP with 2.3 µg/SPMD. Furthermore the study demonstrated that passive sampling tools may be useful for evaluation sources and exposure of HNPs.

Introduction

During the last decade, a few halogented natural products (HNPs) were repeatedly detected as residues in higher marine organisms.[1,2] The structures and concentrations of the HNPs found in the marine environment were comparable with anthropogenic organohalogen compounds. Frequently, HNPs have been detected in the mg/kg range in the lipids of selected higher animals which do not synthesize the HNPs but have received them as a result of bioaccumulation including biomagnification along the food chain, similar to anthropogenic POPs.[2] Yet, the natural producer of Q1 has not been identified. Particularly high concentrations of Q1 were detemined in the blubber of marine mammals from Queensland/Australia.[3] These marine biota samples also contained a range of polybrominated natural products which was evident by the identification of one producer in the same habitat.[4] This suggested that Q1 might also be bio-synthesized in marine waters of the Northeastern coast of Australia. In this study we focussed on the identification of Q1 and further HNPs in the seawater from the Great Barrier Reef by means of semipermeable membrane device (SPMD) samplers. SPMDs are particularly suitable for the enrichment of lipophilic analytes with log $K_{OW} > 5.5$.[5] Standard SPMDs consist of thin low density polyethylene tubes filled with triolein. In this study we analysed samples that were deployed as part of a long term study that aims at the evaluation of pesticides along the Great Barrier Reef, Australia. We attempted to assess if HNPs can be detected in passive samplers, in order to verify the presence of Q1 and other HNPs in this unique kingdom rich in biodiversity.

Material and methods

Chemicals. Acetone and dichloromethane (both LiChrosolv) were from Merck (Darmstadt, Germany). *n*-Hexane for gas chromatography and residue analysis was re-distilled prior to use. Isooctane (for residue analysis) was from EM Science (Gibbstown/USA). Boron tribromide solution, sodium chloride and triolein (≥99%) were from Sigma-Aldrich (Steinheim, Germany). Sodium sulphate (p.a. ≥99%) and pyridine (≥99.8%) were from Fluka (Buchs/Switzerland).

Standards. 2,3,3´,4,4´,5,5´-Heptachloro-1´-methyl-1,2´-bipyrrole (Q1) was synthesized according to Wu *et al*.[6] 4,6-Dibromo-2-(2',4'-dibromo)phenoxyanisole (2'-MeO-BDE 68, BC-2) was synthesized by Vetter and Jun.[7] 3,5-Dibromo-2-(2',4'-dibromo)phenoxyanisole (6-MeO-BDE 47, BC-3), 3,5-dibromo-2-(3`,5`-dibromo,2´-methoxy)phenoxyanisole (2´,6-diMeO-BDE 68 or BC-11) and 2'-dimethoxy-3,3'5,5'-tetrabromobiphenyl (2,2'-diMeO-BB 80 or BC-1) were synthesized by Marsh *et al*. [8,9] 2,4,6-Tribromoanisole (TBA) was from Aldrich (Milwaukee, USA) and 2,4,6-tribromophenol was from Sigma-Aldrich (Steinheim, Germany).

SPMD sampler. SPMDs (2.5 cm wide and 92 cm long) were rolled and purified by accelerated solvent extraction (ASE 300 system, Dionex, Sunnyvale/USA) with extraction cells of 5 cm length and 3 cm diameter (volume 34 mL). The conditions were as follows: pressure 500 psi N_2, temperature 40 °C, static time 20 min, flush volume 60%. Purge time 70 s, static cycles 5, solvent *n*-hexane/acetone (9:1, v/v). The LDPE tubes were dried under a gentle stream of nitrogen and sealed on one side using an ME-200 HI impulse sealer (MeC, Taipeh/Taiwan). One milliliter triolein (purity 99%, Sigma-Aldrich) was injected and the tube was sealed on the other side. Two obtained SPMD devices (active length 92 cm) were mounted inside a stainless steel cage[10] in the laboratory. The cages were enclosed in metal cans rinsed with acetone which were then transported to the sites on ice. SPMDs were deployed at twelve marine sites along the Great Barrier Reef, as well as in two rivers. Samplers were deployed for about one to two months (for details see below) at a water depth of ~1m below floats in the water column.[11] For each deployed SPMD, series SPMD blanks (replicates) were prepared, stored in the freezer, and analyzed together with the respective SPMDs after deployment.

Cleanup procedure for SPMDs after deployment. After retrieval, sampling cages were enclosed in the corresponding metal cans and transported on ice to the laboratory where they were refrigerated. At the laboratory each SPMD was taken from the cage, individually wrapped in acetone rinsed aluminium foil and stored at -17 °C. The samplers (including blanks) were cleaned by scrubbing with cold water and carefully dried with paper tissue. Then, the sampler was submerged into *n*-hexane (30 s) followed by 0.5 M HCl (20 s), and finally rinsed with acetone and 2-propanol.[10] After that, the SPMDs were placed in 34 mL extraction cells and ASE extracted using the same method as for the LDPE tube pre-cleaning (see above). The ASE extracts were concentrated to 1 mL, filtered through a Millex 0.45 µm syringe driven filter unit and made up to 7 mL with dichloromethane, five of which were subjected to gel permeation chromatography (GPC) used for the separation of lipids co-extracted by ASE.[11] Prior to GC/ECD measurements and subsequent GC/MS analysis, the solvent dichloromethane was replaced with isooctane. This procedure optimized with standards provided recoveries between 87% (2,6-dibromophenol) and 101% (MeO-BDEs). The volume of the sample extracts was adjusted to 0.22 mL and analyzed by GC/ECD and GC/MS. Since two SPMDs were combined, the amounts determined were divided by a factor of two.

GC/ECD analyses (HP 5890 series II GC/ECD with HP 7673A autosampler, Hewlett-Packard). The GC column was a 27 m length x 0.25 mm internal diameter fused silica capillary coated with 0.25 µm HP-5 MS (Agilent J&W Scientific). The detector temperature was set at 300 ºC. Nitrogen (purity 5.0, BOC Gases, Sydney/Australien) was used as make-up gas at a velocity of 60 mL/min. One microliter of sample extract was injected splitless (275 °C) using a constant flow rate of 2.0 mL/min hydrogen (purity 5.0; BOC Gases, Sydney/Australia) as the carrier gas. The GC oven was programmed as follows: start 100 ºC (1.5 min), then at 8 ºC/min to 190 ºC (0 min), at 3.5 ºC/min to 275 ºC (0 min), and finally at 50 ºC/min to 315 ºC (2.16 min); total run time 40 min.

GC/ECNI-MS analyses (CP-3800/1200, Varian, Darmstadt, Germany) were performed according to Vetter and Rosenfelder[12] except for using ammonia (pressure 3.6 Torr) as moderation gas instead of methane. In the full scan mode, *m/z* 30-800 was screened throughout the run.

Quality control. SPMD blanks did not contain peaks in the ECD chromatograms at the retention time of the analytes except 2,4-dibromoanisole. The later was thus excluded from the evaluation. Detection limits were assigned to approximately 2 ng/SPMD (based on a 30-days deployment time). All analytes determined in this

study by GC/ECD were verified by GC/ECNI-MS. Selected samples were analyzed by GC/ECNI-MS in order to assess whether anthropogenic brominated organic chemicals including >100 individual polybrominated flame retardants (polybrominated diphenyl ethers, hexabromocyclododecane, polybrominated biphenyls[12]) were also present in the samples.

Results and discussion

Initial verification of the HNPs. GC/ECD chromatograms of the SPMD extracts cleaned with GPC were of sufficient purity and could be analyzed with ease (**Figure 1**).

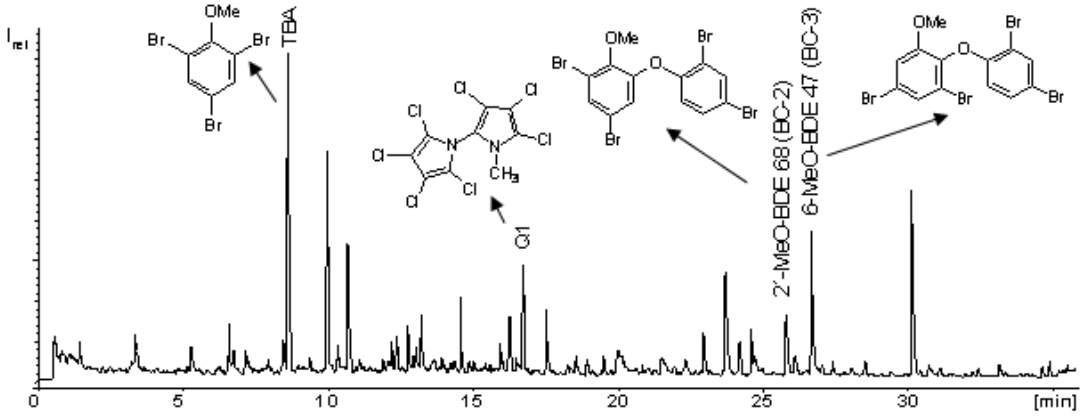

Figure 1: GC/ECD chromatogram of a purified SPMD sampler from Day Island (December 2007)

GC/ECNI-MS full scan analyses confirmed the presence of Q1 in the SPMDs (**Figure 2**). Owing to the non-destructive sample preparation procedure, this is clear evidence that Q1 occurs in water in the form it subsequently enters the marine food chain. Along with Q1, at least two abundant $BrCl_6$ congeners of Q1, traces of Br_2Cl_5-congeners, and ultratraces of Br_3Cl_4-congeners were qualitatively detected in SPMDs and thus we may assume that they occur in the water. Presence of 2´-MeO-BDE 68 (BC-2), 2,2´-diMeO-BB 80 (BC-1), 6-MeO-BDE 47 (BC-3), 2´,6-diMeO-BDE 68 (BC-11), TBA, and TBP was verified as well by GC/ECNI-MS.

Amounts of HNPs in the SPMD samplers. Seven HNPs (TBA, TBP, Q1, 6-MeO-BDE 47, 2´-MeO-BDE 68, 2´,6-diMeO-BDE 68, and 2,2´-diMeO-BB 80) were identified and all but TBP and TBA were quantified in 39 SPMD samplers from 12 marine sites from coastal Queensland. Additionally, six SPMD samplers from two rivers were analyzed as well, but these did not contain measureable amounts of the HNPs.

The accumulation of analytes in passive samplers was treated as a first order process and the SPMD data was evaluated by assuming a linear enrichment of analytes thoughout the deployment period(s). Deployment times of 30 days were chosen for normalization of the samplers. Q1 was identified for the first time by passive sampling in the water phase. In addition, the frequency of detection (64%) was high and was present along the whole coastline of the Great Barrier Reef (**Table 1**). The most remarkable occurrence of Q1 was observed in samplers deployed at the Outer Whitsundays and at High and Normanby Islands with more than 30 ng/SPMD, respectively. However, the amounts targeted in these samples were only two fold higher than at many other sites with detectable amounts of Q1. The mean amount in all marine SPMD samplers was 12 ng (**Table 1**). Thus, it is most likely that the natural producer of Q1 is found widely distributed in water of the Great Barrier Reef.

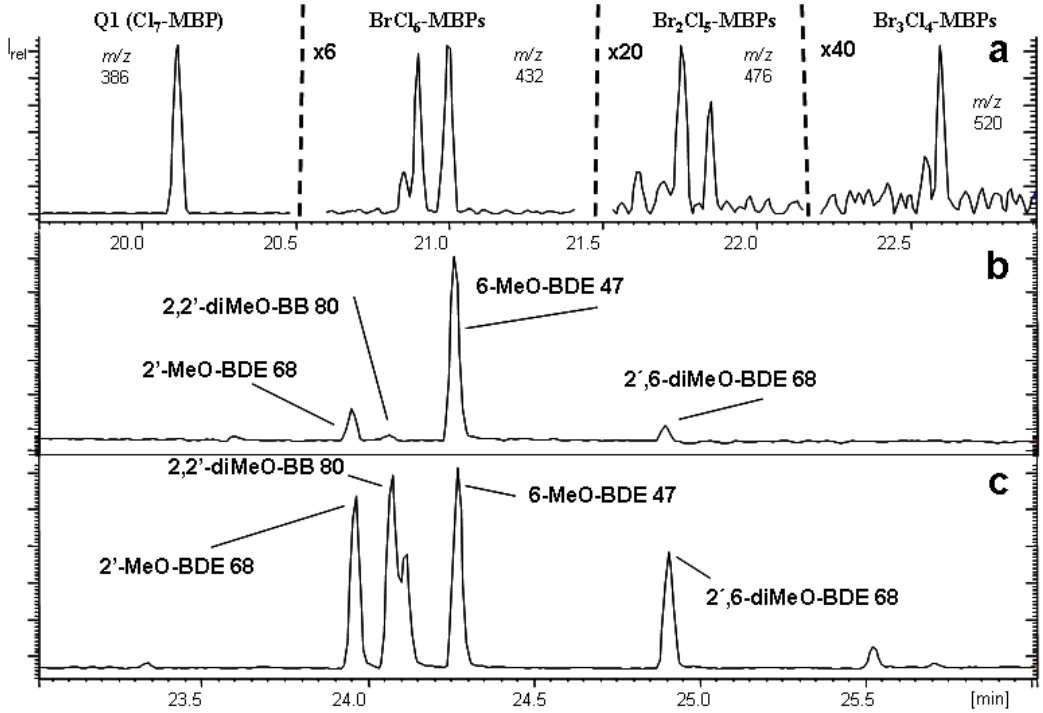

Figure 2: GC/ECNI-MS verification of HNPs in the SPMDs. (a) The heptachloro-1´-methyl-1,2´-bipyrrole Q1 (Cl_7-MBP) (segment to the left), three $BrCl_6$-MBPs (second segment, *m/z* 432, 6-fold enlarged), four Br_2Cl_5-MBPs (third segment, *m/z* 476, 20-fold enlarged), and traces of two Br_3Cl_4-MBPs (forth segment, *m/z* 520, 40-fold enlarged) in an SPMD deployed at Hamilton Island (March 2008); (b) full scan (*m/z* 30-800) chromatogram of a sample from Lizard Island (March 2008) and (c) a standard solution of HNPs.

Table 1: Frequency of detection (n=39), amounts (ng) in SPMD samplers from marine sites, and physico-chemical parameters of HNPs

Compound	Q1	2'-MeO-BDE 68	2,2´-diMeO-BB-80	6-MeO-BDE 47	2',6-diMeO-BDE 68
Sites detected	25	27	7	22	17
Frequency of detection	64%	69%	18%	56%	44%
Highest (mean)* amounts [ng]	44 (12)	115 (20)	84 (4)	270 (28)	27 (5)
log K_{OW}**	5.65	5.60	5.51	5.60	5.13

* non-detected was taken into account as 1 ng; values based on a 30-day deployment time
** calculated using Advanced Chemistry Development (ACD/Labs) Software V9.04 for Solaris (via SciFinder)

The data for Q1 do not offer much information about seasonal trends. This again pointed to a rather equal distribution of Q1 along the Great Barrier Reef sampling sites. It is evident that the still-unknown natural producer(s) of Q1 are widespread in the Great Barrier Reef.

The frequency at which 2′-MeO-BDE 68 (69%) was detected in the SPMDs was even higher than for Q1, as was the mean 2′-MeO-BDE 68 concentration (20 ng/SPMD, **Table 1**). The fluctuation in the 2′-MeO-BDE 68 concentrations at Low Island from November to May was in full contrast to the behavior of Q1. It is therefore more than likely that both compounds are produced by organisms with very different ecology. The sponge-derived 2′,6-diMeO-BDE 68 (BC-11) was also frequently detected (44%) but the amounts in the SPMDs were lower than for 2′-MeO-BDE 68. Typically the level of 2′,6-diMeO-BDE 68 amounted to ~1/6 of 2′-MeO-BDE 68. Both compounds were previously detected in the same sponge and dolphins at about the same ratio.[12] 6-MeO-BDE 47 was detected less frequently than 2′-MeO-BDE 68 (**Table 1**). Moreover, the amounts did not correlate. In many samples, 6-MeO-BDE 47 was less concentrated than 2′-MeO-BDE 68 but the highest amount detected in any sample for MeO- and diMeO-BDEs was determined for 6-MeO-BDE 47 at Magnetic Island. The 2,2′-diMeO-BB 80 was primarily detected in samples from the northern part of the Great Barrier Reef with a total frequency of 18% (**Table 1**).

Acknowledgment. The study relied on support from many staff, colleagues and volunteers that assisted with all the passive sampling. Financial support for the sampling component of this program is based on funding from the Great Barrier Reef Marine Park Authority. EnTox is co-funded by Queensland Health Scientific Services.

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