

## IDENTIFICATION OF A SOURCE FOR BIOACCUMULATIVE NATURAL BROMINATED HYDROCARBONS

Walter Vetter<sup>1</sup>, Mary J. Garson<sup>2</sup>, Shireen J. Fahey<sup>2</sup>, George M. Cameron<sup>3</sup>, Caroline Gaus<sup>3</sup>,  
Jochen F. Müller<sup>3</sup>

<sup>1</sup> Friedrich-Schiller-University Jena, Department of Food Chemistry, Dornburger Str. 25, D-07743 Jena, Germany

<sup>2</sup> Department of Chemistry, University of Queensland, St Lucia Queensland, Australia

<sup>3</sup> Nat. Res. Cent. Environ. Toxicol., 39 Kessels Rd., Coopers Plains, Qld 4108, Australia

### Introduction

Anthropogenic persistent organohalogen pollutants are one of the most hazardous risks to human health and the environment. A number of acute and chronic diseases have been traced back to compounds such as DDT, PCBs, dioxins, chlordane, dieldrin, and toxaphene throughout the last 40 years [1]. A major concern is that some persistent anthropogenic pollutants have no natural analogues and it has been suggested that the enzyme systems of higher organisms may thus not be prepared to metabolize certain chemicals to non-toxic metabolites.

In recent years, a series of new organohalogen compounds have been found in the environment. These include the mixed halogenated N,N'-dimethyl bipyroles [2], the heptachloro methylbipyrrole Q1 [3], and a dibromotrichloro-monoterpene (MHC-1) [4] all of which were found to be natural products.

Similarly it has been suggested that five major (BC-1, BC-2, BC-3, BC-10, and BC-11) and six minor (BC-4 to BC-8) brominated compounds (BC's) detected in the blubber of several species of marine mammals from Queensland, Australia were of non-anthropogenic origin [5][6].

In this study we investigated whether some of the BC's identified in the marine mammals are identical with brominated secondary metabolites recently isolated from sponges (*Dysidea sp.*) [7] from nearby regions.

### Materials and Methods

**Sample materials.** Blubber of bottlenose and common dolphins (*Turisops truncatus* and *Delphinus delphis*), and melon-headed and pygmy sperm whales (*Kogia breviceps* and *Peponocelphala electra*) were collected from stranded animals in Queensland, North-Eastern Australia (**Figure 1**). Sponges (*Dysidea sp.*) were collected from the same geographic region as the marine mammals [7].

**Figure 1:** Map of Australia with marked (dotted) sampling area



# FORMATION AND SOURCES II

**Sample clean-up procedures.** Blubber samples were purified using combined focused-open vessel, microwave-assisted extraction and gel-permeation chromatography followed by adsorption chromatography on 3 g deactivated silica [8]. Aliquots were fractionated on 8 g activated silica [9].

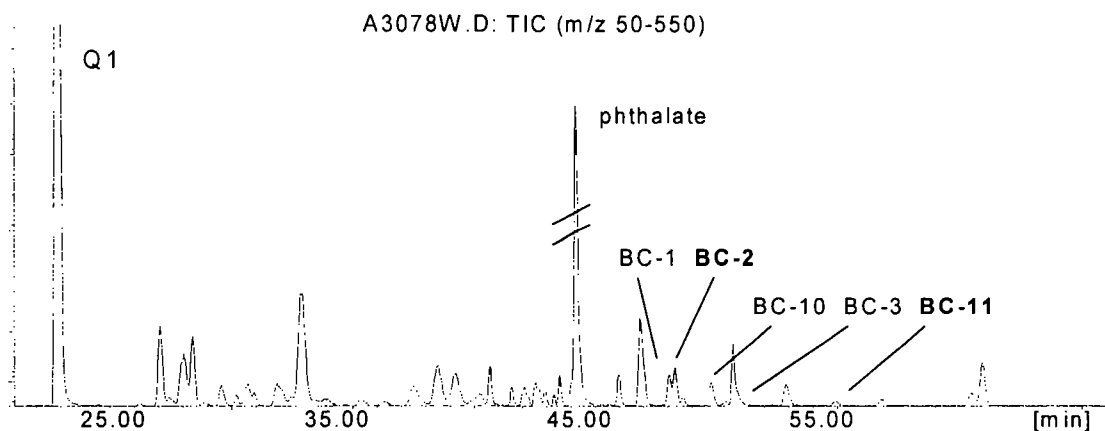
Isolation and structure elucidation of brominated secondary metabolites from sponges were recently described in detail [7]. In this study we used 0.7 mg of the pure isolate of BC-2 from *Dysidea sp.* that has been used for elucidation of the chemical structure of BC-2 (sample 1). This isolate served as a quantitative standard of BC-2 using a stepwise dilution. In addition a *Dysidea sp.* extract with unknown amounts of BC-2 and BC-11 (sample 2) was purified on 3 g deactivated silica.

## GC/MS parameters.

GC/ECNI-MS SIM and full scan, as well as GC/EI-MS full scan experiments were performed using a Hewlett-Packard 5890 gas chromatograph interfaced to a 5989 MS engine. A  $\beta$ -BSCD column was installed in the GC. Parameters were identical with those previously described in detail [4][5][6].

## Results and Discussion

GC/ECNI-MS full scan chromatograms of the investigated samples confirmed high levels of halogenated compounds (**Figure 1**).



**Figure 1:** GC/ECNI-MS full scan chromatogram (m/z 50-550) of cleaned blubber extract of a melon-headed whale (*Peponocephala electra*)

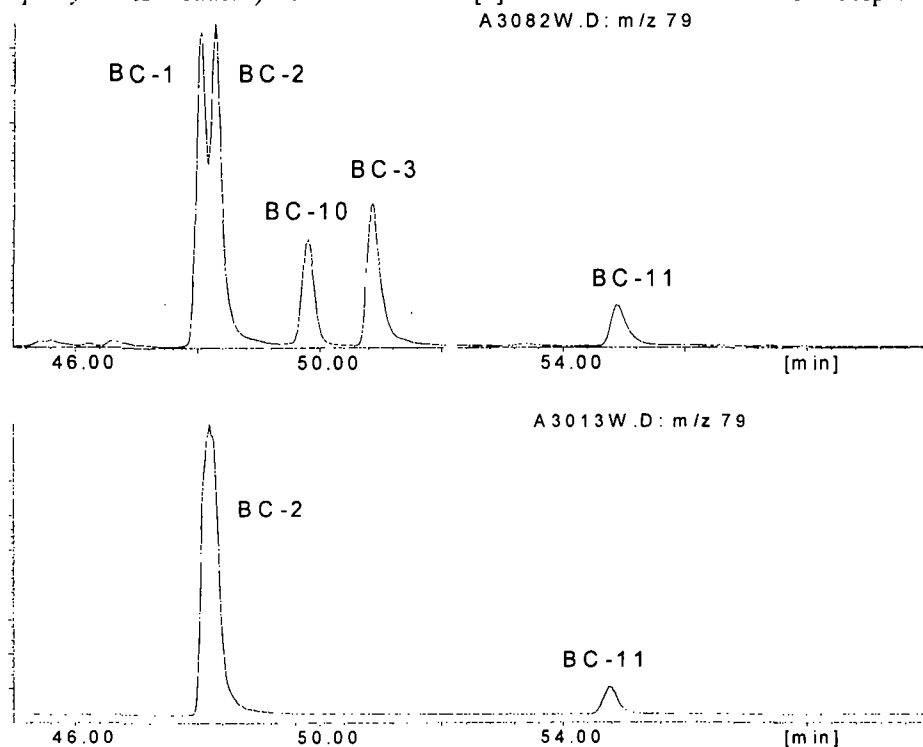
The major compound in the sample, Q1, was recently identified as a natural heptachlorobipyrrole derivative that is a major organohalogen compound in marine mammals from Australia, Africa, the Antarctic, and other locations around the globe [10][11][12]. The BC's were detected at high retention times. BC-1, BC-2, and BC-3 were determined in the blubber of dolphins from Australia [5], and BC-10 and BC-11 were identified only in samples of melon-headed and pygmy sperm whales [6]. Previous work suggested that the BC's are natural brominated compounds. BC-2 and BC-3 were interpreted as tetrabromo phenoxyanisoles (aka tetrabromo methoxy-diphenyl ethers)

## FORMATION AND SOURCES II

[5][6], whereas BC-1 carried 14 u more or an additional “-CH<sub>2</sub>-“ unit [5]. Identity of BC-10 and BC-11 remained unclear [5][6].

Brominated phenoxyanisoles were recently isolated from sponges (*Dysidea sp.*) and nudibranchs that feed on them, all collected in the same geographic area as the mammals [7][15]. Investigation of isomers isolated from sponges enabled structural assignment to BC-2 (Figure 2). Both retention times and EI- and ECNI-MS spectra of BC-2 in sponges and melon-headed whale and other marine mammals from Australia were identical. Therefore, the chemical name of BC-2 is 4,6-dibromo-2-(2',4'-dibromo)phenoxyanisole (Figure 3). Structural assignment of BC-11 is in progress. To our knowledge, this is the first time that a producer of bioaccumulative natural halogenated compounds is elucidated. There is some indication that halogenated compounds serve in a chemical protection role [13]. E. g., BC-2 exhibited antibacterial activity against *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus* [14].

Recent screening of samples for brominated compounds resulted in the detection of BC-2 in samples of monk seals (*Monachus monachus*) from Mauretania/West-Sahara (Africa) and Weddell seals (*Leptonychotes Weddelli*) from the Antarctic [6]. This confirms that BC-2 is widespread.



**Figure 2: (a) GC/ECNI-MS-SIM chromatogram of melon-headed whale and (b) GC/ECNI-MS full scan chromatogram of an isolate from the marine sponge *Dysidea sp.* (sample 2) confirm the identity of BC-2 and B-11 in marine mammals and sponge**

Sample 1 (see above) was used to prepare a quantitative standard of BC-2. Unexpectedly, a 1:1 (concentration to concentration) mixture of BC-2 and PCB 153 resulted in a roughly 10-fold lower peak area for BC-2 as compared with PCB 153.

## FORMATION AND SOURCES II

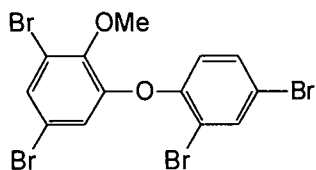


Figure 3: Structure of BC-2

Therefore, full scan screening of samples as in **Figure 2** significantly underscores the relevance of BC-2, which was present at higher concentrations than any anthropogenic contaminant analysed in the sample. In ECD analysis, the response factors of PCBs and BC-2 was lower, and BC-2 was the most abundant compound in the sample, next to Q1.

Owing to the availability of a quantitative external standard we can present for the first time quantitative levels of BC-2. In dolphins, BC-2 was found in the range 0.2 – 4 mg/kg. This clearly demonstrates that natural halogenated compounds can reach concentrations in biological samples that are in the range or higher than residue limits of anthropogenic contaminants in food. We therefore submit, that a thorough investigation and toxicological evaluation of natural bioaccumulative organohalogen is an urgent task.

### Acknowledgement

The authors like to thank David Haynes (GBRMPA) and Wendy Blanchard (Seaworld) for providing the samples for this study.

### References

- [1] Bernes, C. *Persistent Organic Pollutants. A Swedish view of an international problem.* Swedish Environmental Agency, Monitor 16, 1998.
- [2] Tittlemier, S.A., Simon, M., Jarman, W.M., Elliott, J.E., Norstrom, R.J. *Environ. Sci. Technol.* **33**, 26-33 (1999).
- [3] Vetter, W., Alder, L., Palavinskas, R. *Rapid Comm. Mass Spectr.* **13**, 2118-2124 (1999).
- [4] Vetter, W., Hiebl, J., Oldham, N. J. *Environ. Sci. Technol.* 2001, submitted.
- [5] Vetter, W., Scholz, E., Gaus, C., Müller, J. F., Haynes, D. *Arch. Environ. Toxicol. Chem.* 2001, in press.
- [6] Vetter, W., Müller, J., Gaus, C., Hiebl, J. *Abstracts of "The Second International Workshop on Brominated Flame Retardants"*, May 14-16, 2001, Stockholm, Sweden. Vetter, W. *Anal. Chem.* 2001, submitted.
- [7] Cameron, G.M., Stapleton, B.L., Simonsen, S.M., Brecknell, D. J., Garson, M.J. *Tetrahedron* **56**, 5247-5252 (2000).
- [8] Vetter, W.; Weichbrodt, M.; Hummert, K.; Glotz, D.; Luckas, B. *Chemosphere* **37**, 2439-2449 (1998).
- [9] Klobes, U., Vetter, W., Luckas, B., Hottinger, G. *Organohalogen Compd.* **35**, 359-362 (1998).
- [10] Vetter, W., Alder, L., Kallenborn, R., Schlabach, M., *Environ. Poll.* **110**, 401-409 (2000).
- [11] Vetter, W. *ACS Symp. Ser.* **773**, 243-259 (2000).
- [12] Vetter, W. *Chemosphere*, 2001, in press.
- [13] Gribble, G. *Chem. Soc. Rev.* **28**, 335-346 (1999).
- [14] Kuniyoshi, M., Yamada, K., Higa, T. A. *Experientia* **41**, 523-524 (1985).
- [15] Fahey, S.J. Garson, M.J. *J. Chem. Ecol.* submitted.