AQUATIC SPONGE – A PRODUCER OF BROMINATED DIOXINS IN THE BALTIC?

Unger Maria[1,2], Malmvärn Anna[1], Gustafsson Örjan[1], Asplund Lillemor[1]

[1]Department of Applied Environmental Science, Stockholm University, 106 91 Stockholm, Sweden
[2]Department of Environmental Chemistry, Stockholm University, 106 91 Stockholm, Sweden

Introduction

Polybrominated dibenzo-*p*-dioxins (PBDDs) are known to be formed during combustion of brominated flame retardants (BFRs) such as polybrominated diphenyl ethers (PBDEs)[1,2]. Recent reports also indicate a natural production of PBDDs in the marine environment[3,4]. In the Baltic, PBDDs have been reported in several biological matrices such as red algae[4], blue mussels[3,5] and cyanobacteria[4]. Reported concentrations in blue mussels are similar to those of methoxylated PBDEs (MeO-PBDEs)[5]. A natural origin of MeO-PBDEs has been proven for one North Atlantic whale blubber sample by radiocarbon measurement of two congeners[6] and a possible path of formation of PBDDs from the also abundant hydroxylated PBDEs (OH-PBDEs) or brominated phenols has been suggested[3].

Aquatic sponges, or their associated microorganisms, are well-known producers of brominated organic compounds[7,8,9]. In some instances the amount of brominated metabolites represents as much as 12% of the dry weight of the sponge[9]. Among the brominated compounds believed to be produced by sponges are both MeO-PBDEs and OH-PBDEs[9,10].

In this study we analyse and compare the brominated dioxin pattern in the freshwater sponge *Ephydatia fluviatilis* and the marine blue mussel *Mytilus edulis* that coexist in the brackish Baltic Sea.

Materials and Methods

Samples. Sponge (*Ephydatia fluviatilis*) and blue mussels (*Mytilus edulis*) were collected in September 2007 in the Borgholm harbour on the island Öland off the Swedish east coast (N56°52'50'', E16°38'48''). Although the *E. fluviatilis* is a freshwater species and *M. edulis* is a marine species, they coexist in the brackish Baltic Sea. Both species were found under the floating bridges in the harbour. The samples were transported to Stockholm where they were kept frozen at –18°C until extraction.

Extraction. Both mussels and sponge were extracted using the method developed by Jensen and co-workers[11]. Briefly, 10 samples of 10 g sponge tissue, 5 samples of 5 g mussel tissue and 5 samples of 10 g whole mussel were homogenized in acetone:*n*-hexane (25:10) and thereafter extracted twice with *n*-hexane:diethyl ether (9:1). The organic solvent phase was then washed with a water phase containing 0.1M H_3PO_4 and 0.9% NaCl. The organic phase was transferred to a pre-weighed beaker where the solvent was allowed to evaporate and the lipid weights of the samples were determined.

Clean-up. The lipid samples were then redissolved in *n*-hexane (1 mL) and transferred to a test tube. Concentrated sulfuric acid (10 mL) was added. The samples were turned upside down 20 times and centrifuged to separate the phases. The *n*-hexane phases were pooled with 5 samples in each pool and reduced to 0.2 mL using a gentle N_2 flow. A column with 1 g activated (300°C over night) SiO_2 in a Pasteur pipette was prepared and pre-washed with 10 mL *n*-hexane. The samples were eluted with (i) 5 mL *n*-hexane, (ii) 13 mL *n*-hexane and (iii) 12 mL dichloromethane. Fraction 2 was pooled for each sample. The sponge sample was reduced to 0.2 mL and further cleaned up by eluting with 15 mL *n*-hexane on a similar column with 0.5 g SiO_2:H_2SO_4 (60:40 w/w) and 0.1 g activated (300°C over night) SiO_2 pre-washed with 5 mL *n*-hexane.

Instruments. Analysis of fraction 2 was performed on a Themoquest SSQ 7000 scanning from 33 to 1000 *m/z* using ECNI ionisation at 70eV and an ion source temperature of 180°C. Ammonia was used as reaction gas. A Hewlett Packard 5890A gas chromatograph with a non-polar DB5 MS (15 m, i.d. 0.25 mm, film thickness 0.1

µm) column was used for separation. The GC program was injection at 80°C (2 min), 20°C min^{-1} to 200°C, 5.5°C min^{-1} to 315°C (5 min).

Results and Discussion

Analysis of fraction 2 from the silica column gave the chromatograms shown in Figure 1. Four PBDDs and three Br/Cl-dioxins were tentatively identified by their ECNI full scan mass spectra in comparison to a reference standard. Names of the identified peaks, the abbreviations used in the text and the peak number in the chromatograms shown in Figure 1 can be found in Table 1.

The dominating PBDD congener (peak 1) in both sponge and mussel was identified as a triBDD. A corresponding peak in blue mussels has previously been shown to consist of two coeluting triBDD congeners (1,3,7-triBDD and 1,3,8-triBDD)[5]. Two tetraBDDs (peak 5 and 6) were also identified in both samples. A pentaBDD (peak 7) was tentatively identified in both samples as well as three Br/Cl-dioxins (peak 3-5) in the sponge but not in the blue mussels. Tri- and tetraBDD have been found in several biological matrices in the Baltic[3] and recently a pentaBDD was identified in cyanobacteria[4]. Br/Cl-dioxins have not been reported as a potential natural product although other mixed halogenated compounds have been identified[12,13].

ECNI spectra of tri- and tetraBDD from sponge are shown in Figure 2. The molecular ions of *m/z* 418 and 496 respectively are clearly seen as well as the bromine isotope pattern indicating three and four bromine atoms respectively. The ion cluster with *m/z* 392 in the triBDD spectrum is caused by a coeluting heptachloro biphenyl.

Although the dioxin pattern is similar in both species with triBDD as the most abundant followed by tetraBDD and then pentaBDD the relation between the congeners appears to differ. When set in comparison to triBDD the largest tetraBDD peak is about twice as large in sponge compared to in mussel. However, quantification needs to be done before any conclusions can be drawn regarding the concentrations found in either sample. Also, synthesized authentic reference standards are needed to identify the structure of the tetraBDDs and pentaBDD. To our knowledge, this is the first time brominated dioxins have been reported in sponge and the first time mixed Br/Cl dioxins is reported for any environmental media.

The environmental importance of PBDDs is yet unclear. Reports indicate that the toxicological effect of PBDDs is similar to that of their well-studied chlorinated analogues and that Br/Cl-dioxins might have even larger impact[2,14]. However, the effect varies greatly for the different congeners and until the specific PBDD and Br/Cl-dioxin congeners found here have been identified we are unable to draw any firm conclusions as to the environmental importance of our results.

Acknowledgements

Ulla Eriksson is gratefully acknowledged for technical assistance. Henrik Petersson at Stenninge Bygg & Dyk is acknowledged for the sampling. Laura Sanchez-Garcia also assisted with collecting the samples. Roland Engkvist at the University of Kalmar identified the Ephydatia fluviatilis. We thank Takeshi Nakano for the reference standard. This project was financially supported by the Swedish Research Council (FORMAS).

References

1. Buser H. R. *Environ. Sci. Technol.* 1986; 20: 404.
2. World Health Organization *Environ. Health Crit. 205* 1998.
3. Haglund P., Malmvärn A., Bergek S., Bignert A., Kautsky L., Nakano T., Wiberg K. and Asplund L. *Environ. Sci. Technol.* 2007; 41: 3069.
4. Malmvärn A., Zebuhr Y., Kautsky L., Bergman Å. and Asplund L. *Chemosphere* 2008; doi:10.1016/j.chemosphere.2008.03.036:
5. Malmvärn A., Zebuhr Y., Jensen S., Kautsky L., Greyerz E., Nakano T. and Asplund L. *Environ. Sci. Technol.* 2005; 39: 8235.
6. Teuten E. L., Xu L. and Reddy C. M. *Science* 2005; 307: 917.
7. Utkina N. K., Denisenko V. A., Scholokova O. V., Virovaya M. V., Gerasimenko A. V., Popov D. Y., Krasokhin V. B. and Popov A. M. *J. Nat. Prod.* 2001; 64: 151.

8. Melcher J., Janussen D., Garson M. J., Hiebl J. and Vetter W. *Arch. Environ. Con. Tox.* 2007; 52: 512.
9. Unson M. D., Holland N. D. and Faulkner D. J. *Mar. Biol.* 1994; 119: 1.
10. Cameron G. M., Stapleton B. L., Simonsen S. M., Brecknell D. J. and Garson M. J. *Tetrahedron* 2000; 56: 5247.
11. Jensen S., Reutergårdh L. and Jansson B. *FAO Fish Tech. Pap.* 1983; 212: 21.
12. Vetter W. and Jun W. *Chemosphere* 2003; 52: 423.
13. Malmvärn A., Marsh G., Kautsky L., Athanasiadou M., Bergman Å. and Asplund L. *Environ. Sci. Technol.* 2005; 39: 2990.
14. Birnbaum L. S., Staskal D. F. and Diliberto J. J. *Environ. Int.* 2003; 29: 855.

Table 1: Name of tentatively identified compounds in mussel and sponge samples, the abbreviations used in the text and number used in chromatograms (Figure 1).

Name	Abbreviation	Number in chromatogram
Tribromo-dibenzo-*p*-dioxin	TriBDD	1
Tribromo-monochloro-dibenzo-*p*-dioxin	Br/Cl-dioxin	2
Tribromo-monochloro-dibenzo-*p*-dioxin	Br/Cl-dioxin	3
Tribromo-monochloro-dibenzo-*p*-dioxin	Br/Cl-dioxin	4
Tetrabromo-dibenzo-*p*-dioxin	TeBDD	5
Tetrabromo-dibenzo-*p*-dioxin	TeBDD	6
Pentabromo-dibenzo-*p*-dioxin	PeBDD	7

Figure 1: GC-ECNI chromatogram of A) sponge and B) mussel fraction 2. Identities of marked peaks are given in table 1.

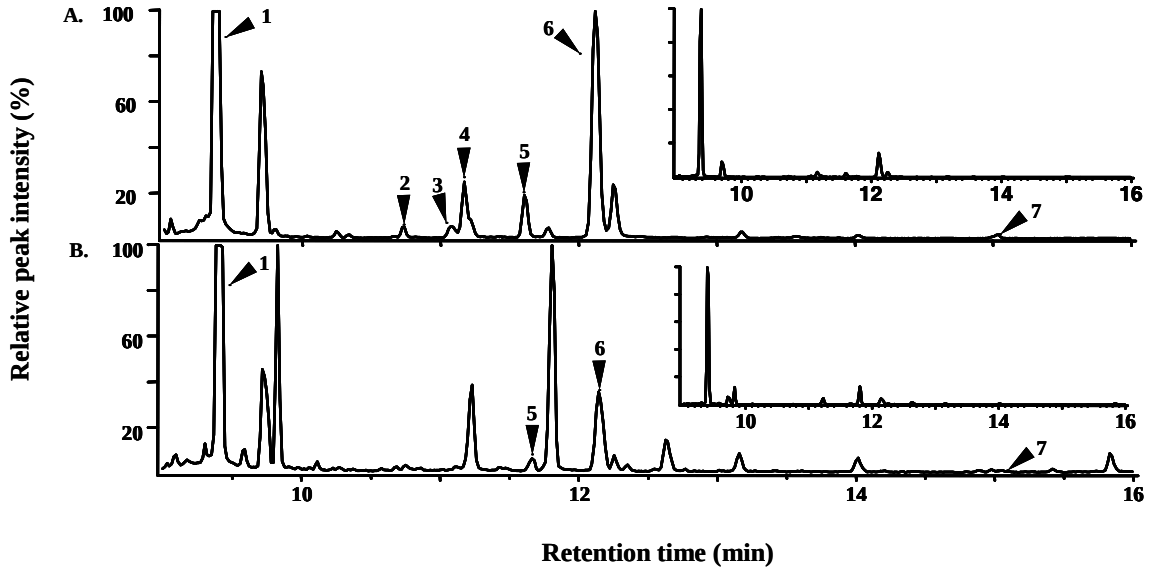

Figure 2: ECNI spectra of A) TriBDD and B) TeBDD