Cyanobacteria from the Baltic Sea – A producer of hydroxylated polybrominated diphenyl ethers (HO-PBDEs), methoxylated polybrominated diphenyl ethers (MeO-PBDEs) and polybrominated-dibenzo-p-dioxin (PBDD)?

Anna Malmvärn[1], Ulla Eriksson[2], Lena Kautsky[3], Lillemor Asplund[2]

[1]Department Of Environmental Chemistry, Stockholm University
[2]Department of Applied Environmental Science, Stockholm University
[3]Department of Botany, Stockholm University

Introduction

Methoxylated polybrominated diphenyl ethers (MeO-PBDEs) were first detected in seal and fish[1] and were later observed in several compartments in the Baltic Sea[2-5]. In addition, a number of hydroxylated polybrominated diphenyl ethers (HO-PBDEs) have been identified in blood plasma from Baltic salmon[2,4] as well as in mussels and red algae from the Baltic Sea [6]. The origins of HO-PBDEs are likely both anthropogenic and biogenic. Brominated flame retardants (BFRs), e.g. polybrominated diphenyl ethers (PBDEs), are known to metabolize to HO-PBDEs in rats, mice and fish[7-11], but their potential for formation in the abiotic environment is unknown.

MeO-PBDEs (MeO-BDE68 and MeO-BDE47) were recently isolated from whale and have been confirmed to be of natural origin by the measurement of the ^{14}C content[12]. Naturally produced MeO- and HO-PBDEs have also been found in sponges collected in the marine environment from the Southern Hemisphere[13,14] and it has been suggested that the producer is the symbiotic filamentous cyanobacterium (*Oscillatoriaspongeliae*)[15]. In the highly eutrophicated Baltic Sea, the dominant cyanobacteria are *Nodulariaspumigena*, which often occurs together with smaller amounts of *Aphanizomenonflos-aquae*. Both are nitrogen fixing species[16]. These two species commonly produce blooms in the open water of the Baltic, which are transported by wind to the coastal areas. In the present study a sample collected during a bloom containing a mixture of cyanobacteria (mainly *Nodulariaspumigen*) and small amounts of zooplankton was investigated for its contents of HO-PBDEs, MeO-PBDEs and polybrominated dibenzo-*p*-dioxins (PBDDs).

Materials and Methods

Chemicals. All solvents were of *p.a*. quality unless otherwise stated. Diazomethane was prepared in house from *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide (Diazald) [17], Sigma-Aldrich (Steinheim, Germany). The methoxylated halogenated diphenyl ether standards were synthesized as described elsewhere [18].

Instruments. The GC-MS analyses were performed on a Thermoquest SSQ 7000 coupled to a Hewlett Packard 5890A GC, using ECNI ionization at 70 eV. A non-polar column, DB5 MS (15 m × 0.25 mm i.d. and 0.1 µm film thickness) from J&W Scientific, was programmed as follows: 80^{o}C (2 min), 20^{o}C min^{-1} to 200^{o}C, 6^{o}C min^{-1} to 315^{o}C (5 min). The instrument was scanned from 33 to 1000 m/z. The injector and transfer line temperatures were 280^{o}C and 300^{o}C, respectively. Helium was used as the carrier gas and the ion source temperature was 150^{o}C. Ammonia was used as the reaction gas.

Sample. The cyanobacteria were collected with a bag net close to Askö island (58^{o}49 –50`N, 17$^{o}$37 –38`E) in the Stockholm archipelago, which adjoins the northern Baltic Proper. It was not possible to separate cyanobacteria from zooplankton with this sampling method. However, the cyanobacteria were estimated to make up the main part of the sample. The cyanobacteria were sampled in July 2003 and were kept frozen at -20^{o}C until analysis.

***Extraction*.** The concentrated cyanobacteria sample (65 ml) was divided into 13 sub-samples. Each sub-sample was extracted according to a method normally used for extraction of blood samples [19]. Briefly, cyanobacterica (5 ml) were

transferred to a test tube, HCl (6M, 1 ml) and 2-propanol (6 ml) were added and the contents were vortex mixed. The samples were extracted with *n*-hexane:MTBE (1:1, 6 ml) and the organic phase was transferred to a new test tube. The residue was reextracted twice (3 ml, 2 ml) and the three organic phases were combined. The solvent volume was reduced almost to dryness and the sample was further dissolved in *n*-hexane (4 ml).

Cleanup procedure. Phenolic compounds were isolated from neutral compounds by partitioning with potassium hydroxide (0.5 M in 50% EtHO) as described earlier [19]. The fractions that contained phenol-type compounds were derivatized with diazomethane in diethyl ether (1 mL)[19]. Both the neutral and the phenolic fractions were further purified using two different columns; the first contained activated (300°C over night) silica gel impregnated with sulfuric acid (2:1 w.w. 1 g), while the second was packed with activated silica gel (1 g). DCM (24 ml) was used as the mobile phase. The sub-samples were pooled prior to GC-MS analysis. During the extraction and cleanup the tubes were wrapped with foil to protect from light to prevent debromination of the analytes. Solvent blanks were processed in parallel to the samples.

Result and Discussion

The tentative identification of the HO-/MeO-PBDEs was based on comparison of retention times and full-scan electron capture negative ionization (ECNI) mass spectra of the peaks in the sample with the corresponding peaks in synthesized reference standards[4]. The chromatograms of the neutral and phenolic fractions are shown in Figure 1.

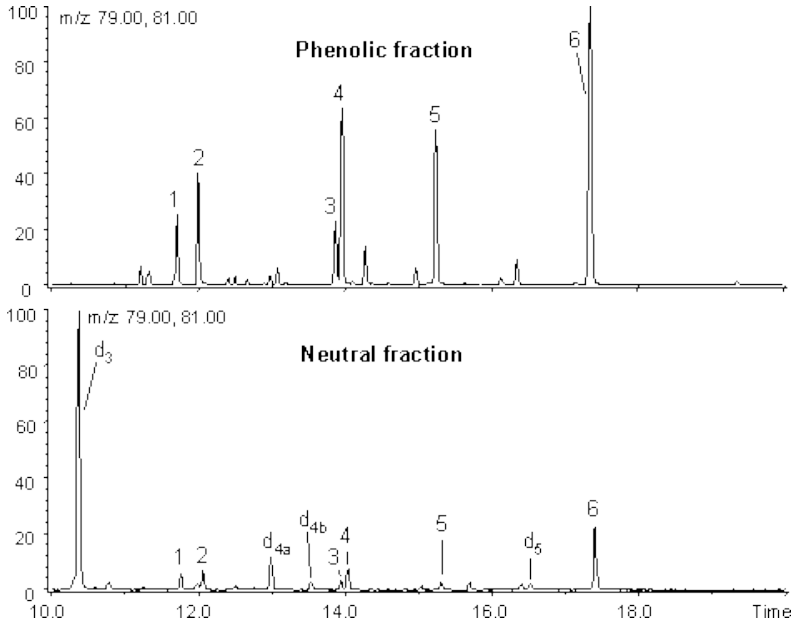

Figure 1. GC-MS (ECNI) chromatograms of bromide ions (*m/z* 79, 81). The upper chromatogram shows the methylated phenolic fraction and the lower chromatogram shows the neutral fraction of the cyanobacteria sample. The identities of the peaks marked in the chromatograms are given in Table 1.

Six HO-PBDEs (2'-HO-BDE68 (peak **1**, Figure1), 6-HO-BDE47 (**2**), 6-HO-BDE90 (**3**), 6-HO-BDE99 (**4**), 6-HO-BDE85 (**5**), 6-HO-BDE137 (**6**)) were tentatively identified in the phenolic fraction. The corresponding MeO-PBDEs were found in the neutral fraction. All these HO-/MeO-PBDE congeners have recently been identified in blue mussels and red algae in the Baltic Sea[6].

Table 1. Identities of the peaks in the GC-MS chromatogram (Figure 1). The identification numbers of the corresponding HO-/MeO-PBDEs are the same since the HO-PBDEs were methylated prior to GC-MS analysis.

	Abbreviation	Id. no
2'-Methoxy-2,3',4,5'-tetraBDE	2'-MeO-BDE68	1
6-Methoxy-2,2',4,4'-tetraBDE	6-MeO-BDE47	2
6-Methoxy-2,2',3,4',5-pentaBDE	6-MeO-BDE90	3
6-Methoxy-2,2',4,4',5-pentaBDE	6-MeO-BDE99	4
6-Methoxy-2,2',3,4,4'-pentaBDE	6-MeO-BDE85	5
6-Methoxy-2,2',3,4,4',5-hexaBDE	6-MeO-BDE137	6
Tribromo-dibenzo-*p*-dioxin	TriBDD	d_3
Tetrabromo-dibenzo-*p*-dioxin	TetraBDD	d_{4a}
Tetrabromo-dibenzo-*p*-dioxin	TetraBDD	d_{4b}
Pentabromo-dibenzo-*p*-dioxin	PentaBDD	d_5

A peak (d_3) with a molecular ion at *m/z* 418 and a full scan ECNI mass spectrum corresponding to triBDD was detected in the neutral fraction of the cyanobacteria sample. A corresponding triBDD found in blue mussels was recently identified as two coeluting triBDD congeners (1,3,7-triBDD and 1,3,8-triBDD)[20]. Furthermore, two tetraBDDs ($d_{4a,}$ d_{4b}) and one pentaBDD (d_5) were tentatively identified in the neutral fraction. The ECNI spectrum of one of the tetraBDD peaks (d_{4a}) is shown in Figure 2.

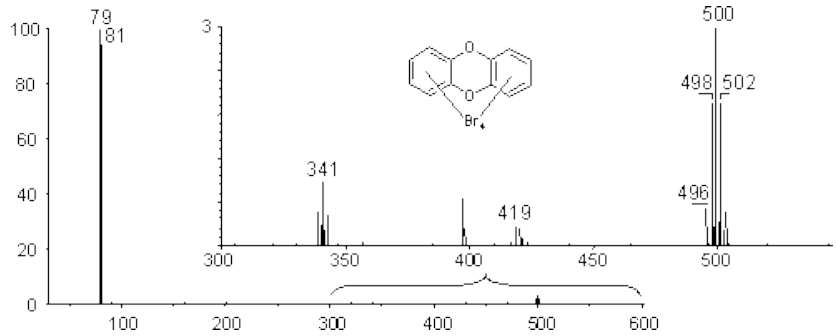

Figure 2. ECNI mass spectrum of the tetraBDD(d_{4a}), isolated from the cyanobacteria sample.

The Baltic Sea has been influenced by increased human activities during the last half century, which has lead to eutrophication[21,22] and thereby conditions favouring cyanobacteria blooms. As far as we know, this is the first time HO-/MeO-PBDEs and PBDDs have been found in a cyanobacteria sample from this area, even though cyanobacteria are known to produce other types of toxic compounds.

This study is based on samples from only one location, and hence more samples must be analyzed to confirm the presence and better characterize the concentrations of these chemicals in cyanobacteria from the Baltic Sea. The presence of PBDDs must also be verified by an analytical method that is more specific for these chemicals.

Acknowledgment

Camilla Niklasson is gratefully acknowledged for collecting the samples, Göran Marsh for synthesis of the MeO-PBDE standards, Takeshi Nakano for synthesis of the PBDD standards and Michael McLachlan for improving the language. The Swedish Research Council FORMAS financially supported the project.

References

(1) Haglund, P. S., Zook, D. R. and Hu, J. (1997) *Environ. Sci. &Technol.* 31: 3281-3287.

(2) Asplund, L. T., Athanasiadou, M., Sjödin, A., Bergman, Å. and Börjesson, H. (1999) *Ambio 28,* 67-76.

(3) Kierkegaard, A., Bignert, A., Sellström, U., Olsson, M., Asplund, L., Jansson, B. and de Wit, C. (2004) *Environmental Pollution*. 130: 187-198.

(4) Marsh, G., Athanasiadou, M., Bergman, Å. and Asplund, L. (2004) *Environ. Sci. &Technol.* 38: 10-18.

(5) Sinkkonen, S., Rantalainen, A.-L., Paasivirta, J. and Lahtiperä, M. (2004) *Chemosphere*. 56: 767-775.

(6) Malmvärn, A., Marsh, G., Kautsky, L., Athanasiadou, M., Bergman, Å. and Asplund, L. (2005) *Environ. Sci. &Technol.* 39: 2990-2997.

(7) Örn, U. and Klasson Wehler, E. (1998) *Xenobiotica*. 28: 199-211.

(8) Hakk, H., Larsen, G. and Klasson Wehler, E. (2002) *Xenobiotica*. 32: 369-382.

(9) Mörck, A., Hakk, H., Örn, U. and Klasson Wehler, E. (2003) *Drug Metab. Dispos.* 31: 900-907.

(10) Kierkegaard, A., Burreau, S., Marsh, G., Klasson-Wehler, E., de Wit, C. and Asplund, L. (2001) *OrganohalogenCompounds.* 52: 58-61.

(11) Letcher, R. J., D'Sa, I., Valters, K., Marsh, G., Li, H., Bennett, E. and Alaee, M. (2003) *Organohalogen compounds*. 61: 29-32.

(12) Teuten, E. L., Xu, L. and Reddy, C. M. (2005) *Science*. 307: 917-920.

(13) Anjaneyulu, V., NageswaraRao, K., Radhika, P. and Muralikrishna, M. (1996) *Indian Journal of Chemistry*. 35B: 89-90.

(14) Handayani, D., Edrada, R. A., Proksch, P., Wray, V., Witte, L., Van Soest, R. W. M., Kunzmann, A. and Soedarsono. (1997) *J. Nat. Prod.* 60: 1313-1316.

(15) Unson, M. D., Holland, N. D. and Faulkner, D. J. (1994) *Marine Biology*. 119: 1-11.

(16) Kautsky, L. and Kautsky, N. (2000) The Baltic Sea, including Bothnian Sea and Bothnian Bay. (Sheppard C.R.C. Ed.). Seas at the millennium: an environmental evaluation. Elsevier. 1: chapter 8.

(17) B.V., Waldron, N.M., Vogel, A.I. (orginal author) (1980) Vogel's Elementary Practical Organic Chemistry. Publisher: Longman. Part 1: Preparations. 267-268.

(18) Marsh, G., Stenutz, R. and Bergman, Å. (2003) *Eur. J. Org. Chem.* 14: 2566-2576.

(19) Hovander, L., Athanasiadou, M., Asplund, L., Jensen, S. and Klasson-Wehler, E. (2000) *J. Anal. Toxicol.* 24: 696-703.

(20) Malmvärn, A., Zebühr, Y., Jensen, S., Kautsky, L., Nakano, T., Athanassiadis, I. and Asplund, L. (2005) *Submitted to this symposium*

(21) Elmgren, R. (1989) *Ambio*. 18: 322-332.

(22) Hänninen, J., Vuorinen, I., Helminen, H., Kirkkala, T. and Lehtilä, K. (2000) *EstuarineCoastal and Shelf Science*, 50: 153-171.