# Identification of brominated dibenzo-p-dioxins in blue mussels (Mytilus edulis) from the Baltic Sea

Anna Malmvärn[1], Yngve Zebuhr[2], Jensen Sören[1], Lena Kautsky[3], Takeshi Nakano[4], Ioannis Athanassiadis[1], Lillemor Asplund[5]

[1]Department of Environmental Chemistry, Stockholm University
[2]Department of Applied Environmental Science, Stockholm University
[3]Department of Botany, Stockholm University
[4]Hyogo Prefectural Institute of Public Health and Environmental Science
[5]Department of Applied Environmental Science (ITM), Stockholm University

## Introduction

Polybrominateddibenzo-*p*-dioxins (PBDDs) are known to be formed as by-products in the processes of manufacturing brominated flame retardants (BFRs) and may also be formed during combustion of products containing BFRs.[1,2] However, the presence of PBDDs in biological samples has only been reported in a few studies, for example, PBDD have been detected in a mixed fish /mussel sample[3] and also in human samples from Japan.[4] To our knowledge, PBDDs of biogenic origin have never been reported even though substituted PBDDs such as, hydroxylated and methoxylated PBDDs have been isolated from the Australian marine sponge *Dysidea dendyi.*[5,6] The knowledge about exposure and health effects of PBDDs are limited compared to their chlorinated homologs.[7] The toxicity varies for different PBDD congeners, however, the binding affinity to the Ah receptor for certain triBDD congeners was determined to be as high as 0.8 compared to 2,3,7,8-TCDD.[7]

In an earlier preliminary study, the presence of a triBDD was indicated in blue mussels (*Mytilus edulis*) collected in the Baltic Sea.[8] The aim of the present study was to confirm these results and to estimate the PBDD concentrations, by use of an analytical method adapted for dioxin analyses including HR-MS.

## Materials and Methods

***Chemicals.*** All solvents were of *p.a.* quality unless otherwise stated. The dioxin standards were gifts from Prof. Takeshi Nakano[9] and Prof. Stephen Safe.

***Instruments.*** GC-HRMS-analyses were performed on a Micromass Autospec Ultima magnetic sector mass spectrometer operating at 10 000 resolution using EI ionization at 32 eV. The detection was carried out in SIM mode on the two most abundant ions of the molecular bromine cluster. Three different GC columns were used: a non-polar PTE 5 capillary column, (15 m x 0.25 mm i.d. with 0.25 µm film); a polar SP-2331 column (30 m x 0.25 mm i.d. and 0.25 µm film thickness) and a capillary column with a mixture of OV1701 and heptakis ((2,3-di-*O*-methyl-6-*O*-*t*-hexyl)-*β*-cyclodextrin (1:1)) as the stationary phase (10 m x 0.25 mm i.d).

***Samples.*** The mussels *(Mytilus edulis*) were collected at 1-2.5 meters depth at the coast close to Oxlesund in the northern Baltic proper.

***Extraction and cleanup.*** The mussel tissue (40 g fresh weight, without shells) was extracted according to Jensen et al.[10] After gravimetric determination of the lipids the sample was treated with sulfuric acid and fractionated on a silica gel column. Further cleanup was done by the use of an amino column and a PYE column for isolation of PBDDs.[11]

## Results and Discussion

The GC-MS chromatogram of the mussel sample was dominated by a peak (Figure 1, peak C), which showed a mass spectrum with amolecular ion at *m/z* 418, corresponding to triBDD. The peak was shown to consist of two co-

eluting triBDD congeners. The identification of these two congeners (1,3,7-BDD and 1,3,8-BDD) was done by comparison of retention times on three GC columns as well as comparison of MS fragmentation pattern (EI and ECNI) between samples and standards. The identity was also confirmed by accurate mass determination by a method suggested by Grange et.al.[12] The mean value of three runs was 417.7840 (resolution 20 000), which is 0.04 mDa from the theoretical value 417.78396.

The analytical method includes several steps aimed to isolate PBDDs from other substances. The silica gel column was used for retention of OH-PBDE and the amino column was used to separate compounds with two aromatic rings from non-diaromatic compounds while the PYE column was used to separate dioxins from non-planar compounds. PBDD standards were used to determine the fractionation borders. The PBDDs detected in the mussel sample behaved in the same way as PBDD standards in the analytical procedure used and was recovered in the back flush fraction from the PYE column.

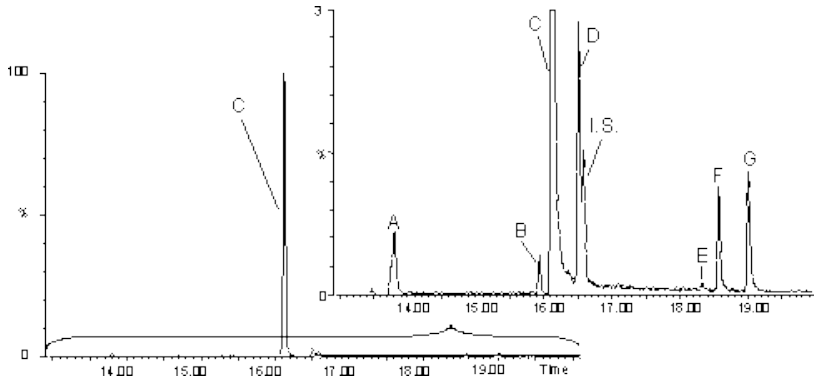


**Figure 1.** GC-HRMS chromatogram (EI, SIM) of tri- to tetraBDDs detected in PYE fraction of the blue mussel sample. The identities of the peaks marked in the chromatogram are given in Table 1.

The non-polar PTE 5 column was used for identification and quantification of the PBDD congeners present in the mussel sample (Table 1). However, the PBDD standard used was a mixture of several triBDD congeners (1,3,7-BDD, 1,3,8-BDD, 1,3,6-BDD), which co-elute on the PTE 5 column. Therefore, two additional GC columns were tested for their capability to separate these substances. First the polar SP 2331 column was used in which the retention times of the triBDDs were known.[9] Since the separation on the SP 2331 column was not complete, a third column (OV1701/heptakis), normally used for separation of chiral compounds, was tested. All triBDD congeners in the standard mixture were completely separated on the OV1701/heptakis column and the sample was shown to contain two of these, in a one to one ratio. The result shows that the OV1701/heptakis column possesses a full separation capacity for these triBDDs. The other PBDD/PBDF congeners present in the mussel sample were only analyzed on the PTE 5 column (Figure1). Only a preliminary identification was possible due to the lack of standards and the possibility of co-elutions (Table 1).

The concentrations of the two coeluting triBDD congeners were estimated to be as high as 160 ng/g l.w., while the concentrations of the other congeners were between 20 to 1600 times lower (Table 1). The concentrations were calculated by response comparisons with the authentic PBDD standards analyzed in parallel with the sample. To the best of our knowledge, PBDDs have not been reported in biological samples from the Baltic Sea. However, 2,3,7,8-tetraBDD has been found in human adipose tissue from Japan at levels of 0.1–4.2 pg/g.[4] The $\sum$PCDD concentrations in blue mussels from the Baltic Sea have been reported to be 310 pg/g d.w.[13] Compared to these data, the concentrations of $\sum$PBDD in the blue mussels analyzed here were about 70 times higher.

The knowledge on PBDD toxicity is summarized in a recent review.[7] As far as we know, the toxicity of the triBDD congeners found in the mussel samples (1,3,7- and 1,3,8-triBDD) has not so far been tested.

**Table 1.** Concentrations of the compounds identified in blue mussels. The identification numbers of the PBDDs are the same as in Figure 2.

| | Abbreviation | Id. no | (ng/g, l.w.) |
|---|---|---|---|
| Dibromo-dibenzo-*p*-dioxin | DiBDD | A | — |
| Tribromo-dibenzo-*p*-furan | 1,3,8-TriBDF | B | 1 |
| 1,3,7-Tribromo-dibenzo-*p*-dioxin / 1,3,8-Tribromo-dibenzo-*p*-dioxin | 1,3,7-TriBDD 1,3,8-TriBDD | C | 160 |
| 2,3,7-Tribromo-dibenzo-*p*-dioxin | 2,3,7-TriBDD | D | 7 |
| Tetrabromo-dibenzo-*p*-dioxin | TetraBDD | E | 0,1 |
| Tetrabromo-dibenzo-*p*-dioxin | TetraBDD | F | — |
| 2,3,7,8-Tetrabromo-dibenzo-*p*-dioxin/ 1,2,3,4-Tetrabromo-dibenzo-*p*-dioxin | 2,3,7,8-TetraBDD/ 1,2,3,4-TetraBDD | G | 2 |

The origin of the triBDDs isolated from the blue mussels is unknown but a plausible hypothesis could be biogenic formation in the marine environment. This could either be caused by natural formation of PBDD or by biotransformation of naturally produced precursor molecules such as OH-PBDEs. OH-PBDEs have been shown to be present in red algae and blue mussel from the Baltic Sea.[14] A possible approach to establish if the origin of these PBDDs is anthropogenic or natural could be to investigate $^{14}C$ content of the compounds. A high $^{14}C$ content indicates natural origin of the substances.[15,16]

As far as we know, this is the first time PBDDs have been identified in biota from the Baltic Sea. Since the concentrations are high, further work is required to unambiguously determine levels, distribution, source(s) and toxicity of these substances in Baltic Sea biota.

**Acknowledgements**

Erik Greyerz and Kerstin Grunder are gratefully acknowledged for technical assistance, Michael McLachlan for improving the language, and the Swedish Research Council FORMAS for having financially supported the project.

**References**

1. WHO/IPCS. Polybrominated dibenzo-*p*-dioxins and dibenzofurans (1998) *Environmental Health Criteria* (205) ISBN 94-4-1572051, Geneva, Switzerland.

2. Sakai, S., Watanabe, J., Honda, Y., Takatsuki, H., Aoki, I., Futamatsu, M. and Shiozaki, K. (2001) *Chemosphere 42:* 519-531.

3. de Jong, A. P. J. M., van der Heeft, E., Marsman, J. A. and Liem, A. K. D. (1992) *Chemosphere 25:* 1551-1557.

4. Choi, J., Fujimaki, S., Kitamura, K., Hashimoto, S., Ito, H., Suzuki, N., Sakai, S. and Morita, M. (2003) *Environ. Sci. Technol.* 35*:* 817-821.

5. Utkina, N. K., Denisenko, V. A., Scholokova, O. V., Virovaya, M. V., Gerasimenko, A. V., Povov, D. Y., Krasokhin, V. B. and Popov, A. M. (2001) *J. Nat. Prod.* 64: 151-153.

6. Utkina, N. K., Denisenko, V. A., Virovaya, M. V., Scholokova, O. V. and Prokofeva, N. G. (2002) *J. Nat. Prod. 65:* 1213-1215.

7. Birnbaum, L. S., Staskal, D. F. and Diliberto, J. J. (2003) *Environ. Int.* 29*:* 855-860.

8. Malmvärn, A., Kautsky, L., Jensen, S., Athanassiadis, I. and Asplund, L. (2003) *Organohalogen Compounds* 61: 143-146.

9. Nakano, T., Matsumura, C. and Weber, R. (2003) *Organohalogen Compounds* 60: 379-382.

10. Jensen, S., Reutergårdh, L. and Jansson, B. (1983) *FAO Fish. Tech. Pap.* 212: 21-33.

11. Bandh, C., Ishaq, R., Broman, D., Näf, C., Rönquist-Nii, Y. and Zebühr, Y. U. (1996) *Environ.Sci.&Technol*. 30: 214-219.

12. Grange, A. H., Donnelly, J. R., Brumley, W. C., Billets, S. and Sovocool, G. W. (1994) *Analytical Chemistry* 66: 4416-4421.

13. Broman, D., Näf, C., Rolff, C., Zebühr, Y., Fry, B. and Hobbie, J. (1992) *Environ. Toxicol. Chem.* 11*:* 331-345.

14. Malmvärn, A., Marsh, G., Kautsky, L., Athanasiadou, M., Bergman, Å. and Asplund, L. (2005) *Environ. Sci. Technol.*

15. Reddy, C. M., Xu, L., Eglinton, T. I., Boon, J. P. and Faulkner, D. J. (2002) *Environmental Pollution* 120: 163-168.

16. Reddy, C. M., Xu, L., O'Neil, G. W., Nelson, R. K., Eglinton, T. I., Faulkner, D. J., Norstrom, R., Ross, P. S. and Tittlemier, S. A. (2004) *Environ. Sci. Technol.* 38*:* 1992-1997.