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DETECTION OF A POSSIBLE TRIBROMODIBENZO-*P*-DIOXIN IN BLUE MUSSELS (*MYTILUS EDULIS*) FROM THE BALTIC SEA

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Introduction

It is known that polybrominated dibenzo-*p*-dioxins (PBDD) can be formed as by-products in the process of manufacturing brominated flame retadardants (BFR). PBDDs may also be formed during combustion of products containing BFR [1, 2]. However the occurrence of PBDD in biological samples has only been reported in a few studies. PBDDs have been detected in cow's milk and in mixed fish and mussel samples [3] and two tetraBDD and one pentaBDD were identified in Japanese human adipose tissue [4]. Hydroxylated and methoxylated polybromodibenzo-*p*-dioxins have been isolated from the Australian marine sponge *Dysidea dendyi* [5] but to our knowledge non-hydroxylated PBDDs of biogenic origin has not been reported [1, 6]. In earlier studies we reported the presences of hydroxylated polybrominated diphenyl ethers (OH-PBDE) in the phenolic fraction from salmon blood, blue mussels and red algae, collected in the Baltic Sea [7, 8]. An unknown compound, in the corresponding neutral fraction, with the same molecular ion as triBDD was detected in both the mussel and algae samples. The aim of the present study was to characterize this compound.

Materials and Methods

Chemicals: All solvents were of p.a. quality unless otherwise stated. Diazomethane, used for the derivatization was prepared from N-methyl-N-nitroso-p-toluenesulfonamide [9]. The 2,2'3,4,4',5'-hexa-bromodiphenyl ether (BDE-138), 2'-hydroxy-2,3',4,5'-tetra-brominodiphenyl ether (OH-BDE-68) and the 6- hydroxy-2,2', 4,4'-tetra-bromodiphenyl ether (OH-BDE-47) standards were synthesized [10, 11]. The 2,3,7-triBDD was a gift from Prof. Stephen Safe (Texas A& University, USA).

Instruments: The gas chromatography mass spectrometry (GC/MS) analysis was performed on a Finnigan MAT TSQ700. The ion source and transferline temperature were set at 150˚C and 270˚C, respectively. A Varian 3400 gas chromatograph was equipped with a Varian 1077 split/splitless injector operating in splitless mode at 280˚C and a DB-5HT column (30 m x 0.25 mm i.d and 0.1 μm film) from J&W Scientific. Helium was used as carrier gas at 12 psi. The GC oven was programmed as follows: 80˚C (1 min), 20˚C min^{-1} to 240˚C and then 5˚C min^{-1} to 300˚C (10 min). The MS instrument was set up for detecting negative ions formed by electron capture negative ionisation (ECNI) using methane as reagent gas (5.5 Torr). The instrument was scanned from m/z 33 to m/z 600 to obtain complete mass spectrum.

***Samples*:** Blue mussels (*Mytilus edulis*) are the biomass-dominating invertebrate species on rocky bottoms in the Baltic Sea. The mussels were collected at a 3-4 meters depth close to Askö, in the northern Baltic Proper, 70 km south of Stockholm.

Extraction, clean-up and analysis: The samples (3.5g, shell-free, dry weight) were extracted according to Jensen et al. [12]. The samples were partitioned with KOH (0.5 M KOH in 50% ethanol) and separated in a phenolic and a neutral fraction. The neutral fraction was further cleaned-up on a series of silica/sulfuric acid gel columns (1g) eluted with DCM (24 ml), as described in Hovander et al. [13], followed by a silica gel column (0.5g) eluted with DCM (14 ml). The neutral fraction was analyzed by GC/MS and the chromatogram revealed an unknown peak d_3 (Fig.1). The samples were derivatized with diazomethan, diluted and divide into two halves of which one of them was fractionated on a charcoal column (10 mg charcoal, Serva SP-1, on 90 mg Chromosorb 100/120 Mesh). The column was eluted with 2x10 ml hexane and 2x15 ml toluene. Four fractions were collected. A standard mixture of dioxins and furans (1,3,8-triBDF, 2,3,7-triBDD, 2,3,7,8-tetraBDF, 1,2,3,4-tetraBDD, 1,2,4,7,8-pentaBDD) was fractionated on a charcoal column in the same way as the sample. 2-OH-BDE-68 and 6-OH-BDE-47, were partitioned with KOH and separated in a neutral and a phenolic fraction. All fractions from both the sample and the standards were analyzed by GC/MS, ECNI. Procedure blanks were analysed in parallel with all the samples.

Results and discussion

The neutral fraction was analyzed by GC/MS, ECNI. Fig.1 shows a mass chromatogram of the bromide ion *m/z* 79 from of the mussel sample with the unknown peak marked d_3 in the chromatogram.

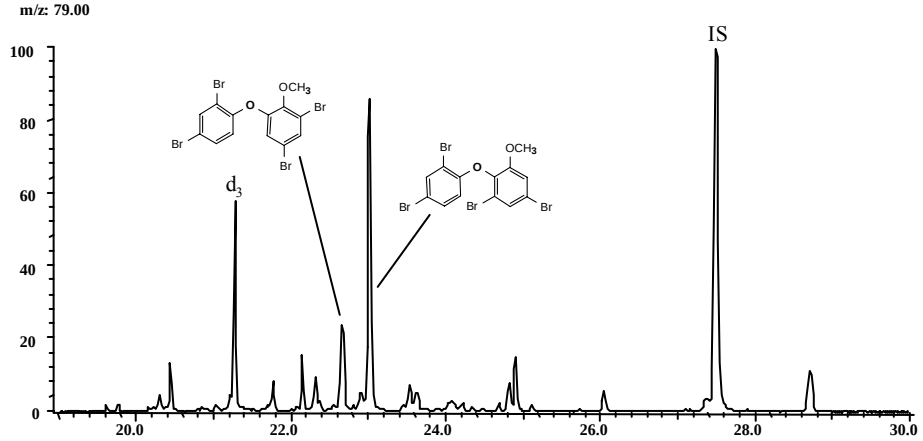

Figure 1 GC/MS (ECNI) chromatogram showing the bromide ion *m/z* 79 in the neutral fraction of the mussel sample. The unknown compound is marked d_3. IS (BDE-138).

However, the raw extract from the mussel sample contained various neutral organohalogen substances, e.g. PBDEs and PCBs. Some of these substances co-elute on the GC with the unknown compound, giving rise to interferences in the mass spectrum. Most of these substances will however elute from the charcoal column in the hexane fraction while the unknown compound was recovered in the first toluene fraction, which was also true for standards of 2,3,7-triBDD. The mass spectrum of the unknown compound, d_3 in the first toluene fraction from the charcoal column is shown in Fig. 2a. In Fig. 2b a spectrum of 2,3,7-triBDD is shown as comparison.

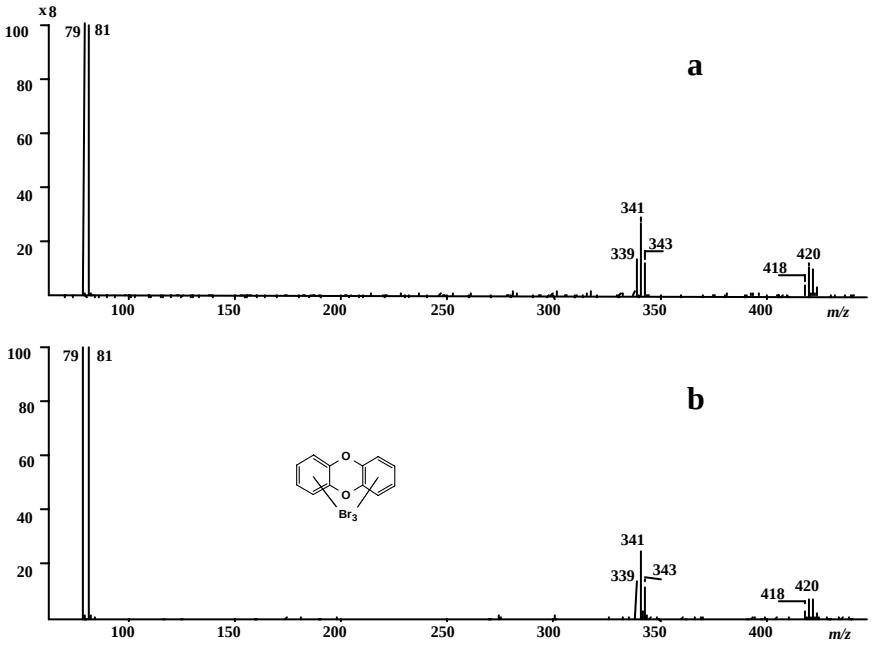

Figure 2. a) ECNI spectrum of the unknown compound in the mussel sample. b) ECNI spectrum of the 2,3,7-tribromdioxin standard.

The mass spectrum of the substance (d_3) reveals an ion at m/z 418 with an isotope cluster corresponding to three bromine atoms and intense ions at m/z 79, 81. The mass spectrum did not reveal any ions at m/z 159/161/163 typically for the ECNI spectra of brominated diphenyl ethers. As can be seen, the mass spectrum of the unknown compound d_3 and the 2,3,7-triBDD standard correspond well. In addition, compound d_3 elute just before the 2,3,7-triBDD on the GC, which strongly indicate that the unknown compound could be a triBDD.

To exclude that some OH-PBDE still remains in the neutral fraction and thereby may form brominated dioxin by ring closure in the gas chromatograph, the sample was treated with diazomethane. The extract was reanalyzed on GC/MS and the d_3 peak still remained in the chromatogram and gave an identical mass spectrum and has the same retention time as the peak in the original sample.

In order to rule out that the triBDD could be formed during the sample clean up, solutions of authentic standards of OH-BDE-47 and OH-BDE-68 were shaken with KOH. The neutral and phenolic fractions were analysed by GC/MS. No triBDD could be detected in neither of the fractions.

Altogether the results indicated the presence of a triBDD in the sample. In the analyzed mussel sample the peak (d_3) is one of the major peaks in the chromatogram besides the methoxylated-PBDEs and is also more abundant than the PBDEs present in the samples.

Analysis of the neutral fraction from the red algae (*Ceramium tenuicorne*) from the Baltic proper, previously analysed for OH-PBDEs [8], also shows an "unknown peak" in the chromatogram at the same retention time as the d_3 peak in the mussel samples.

As earlier reported the substitution pattern of OH-PBDE in the salmon, mussel and red algae samples from the Baltic, have similarities with the pattern reported from the naturally produced OH-PBDE (e.g. have the hydroxy- group in the *ortho*-position and bromine atom in *ortho* -position in the opposite ring) [6]. This indicates that the OH-PBDEs found in the samples most likely are naturally produced [8].

Most of the OH-PBDE found in the samples in the previously studies [8, 14], are pre-dioxins and it cannot be excluded that they can be transformed to PBDD under the natural environmental condition occurring in the Baltic Sea.

However, further studies have to be carried out to verify the presence and identity of the PBDD in the Baltic Sea biota. For example, analysis on HR/MS with additional authentic reference standards as well as analysis on at least two different GC columns will be needed to confirm the results.

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References

1. Polybrominated dibenzo-*p*-dioxins and dibenzofurans. Geneva, Switzerland, ISBN 94-4-1572051 WHO/IPCS (1998) Environmental Health Criteria (205)
2. Sakai S., Watanabe J., Honda Y., Takatsuki H., Aoki I., Futamatsu M. and Shiozaki K. (2001) Chemosphere. 24, 519
3. De Jong A.P.J.M., van der Heeft E., Marsman J.A. and Liem A.K.D. (1992) Chemosphere. 25, 1551
4. Choi J.W., Fujimaki S., Kitamura K., Hashimoto S., Ito H., Suzuki M., Sakai S.I. and Morita M. (2003) Environ Sci Technol. 37, 817
5. Utkina N.K., Denisenko V.A., Virovaya M.V., Scholokova O.V., and Prokoféva N.G. (2002) J Nat Prod. 65, 1213
6. Gribble G. (2000) Environ Sci & Pollut Res. 7, 37
7. Marsh G., Athenasiadou M., Bergman Å., Jakobsson E., Asplund L. (2001) Organohalogen Compounds. 52, 64
8. Asplund L., Malmvärn A., Marsh G., Athanasiadou M., Bergman Å. and Kautsky L. (2001) Organohalogen Compounds. 52, 67
9. Furniss BS., Hannaford AJ., Smith PWG., Tatchell AR. (1989) Vogel´s Textbook of Practical Organic Chemistry. Harlow: Longman Scientific & Technial, ISBN 0-582-46236-3
10. Örn U., Eriksson L., Jakobsson E., Berman Å. (1996) Acta Chem Scand. 50, 802
11. Marsh G., Stenutz R. and Bergman Å. (2003) Eur J Org Chem. accepted
12. Jensen S., Reytergårdh L., Jansson B. (1983) FAO Fish Tech Pap. 212, 21
13. Hovander L., Athanasiadou M., Asplund L., Jensen S., Klasson Wehler E. (2000) Journal of Analytical Toxicology. 24, 696
14. Mash G. (2003) Polybrominated diphenyl ethers and their hydroxy and methoxy derivatives-congener specific synthesis and analysis. (Thesis), Stockholm university, ISBN 91-7265-616-6