PBDD, MeO-PBDE, OH-PBDE and brominated phenols in blue mussels from the Swedish coast line.

Löfstrand K[1], Malmvärn A[1], Haglund P[2], Bignert A[3], Bergman Å[1] and Asplund L[4]

[1]Department of Environmental Chemistry, Stockholm University, SE-106 91 Stockholm, Sweden
[2]Environmental Chemistry, Department of Chemistry, Umeå University, SE-901 87 Umeå, Sweden
[3]Department of Contaminant Research, Swedish Museum of Natural History, SE-104 05 Stockholm, Sweden
[4]Department of Applied Environmental Science (ITM), Stockholm University, SE-106 91 Stockholm, Sweden

Abstract
Quantitative analyses of blue mussel samples from three locations along the Swedish coastline were made by GC-MS (ECNI) for distribution and in-between year variations. Polybrominated dibenzo-*p*-dioxins (PBDDs), methoxylated polybrominated diphenyl ethers (MeO-PBDEs), and hydroxylated polybrominated diphenyl ethers (OH-PBDEs) were found around the Swedish coastline with the highest abundance in the Baltic proper. Simple phenols and anisoles were also detected including 2,4-dibromophenol and 2,4,6-tribromophenol and the corresponding anisoles. The relative abundance of the simple phenols and anisoles, as compared to the other brominated analytes, is higher in the mussels from the west coast than in those from the east coast.

Introduction
Brominated substances such as PBDDs, MeO-PBDEs, and OH-PBDEs have been detected in biota from the Baltic Sea[1-6]. The origins of these substances have not yet been fully resolved. Several hydroxylated and methoxylated polybrominated diphenyl ethers have been shown to be natural products[7-10] but OH-PBDEs have also been reported as metabolites of polybrominated diphenyl ethers (PBDEs)[11,12]. Many marine species, e.g. alga and cyanobacteria, contain haloperoxide enzymes that can catalyse bromination of a suitable precursor in presence of bromine and hydrogen peroxide[13]. Hypothetically, one such precursor could be simple phenols that subsequently can be biotransformed into PBDDs[2] and OH-PBDEs[14]. The aim of this study was to analyse and quantify PBDDs, MeO-PBDEs, OH-PBDEs, polybrominated phenols (PBPs) and polybrominated anisoles (PBAs) in blue mussels from three locations along the Swedish coastline.

Materials and Methods
Samples
Blue mussels *(Mytilus edulis)* from three locations along the Swedish coast line, from Kvädöfjärden (58° 2' N, 16° 46' E) in the Baltic Proper, from Fladen (57° 14' N, 11° 50' E) in Kattegat and from Väderöarna (58° 31' N, 10° 54' E) in Skagerack (locations marked in Figure 1) were collected during autumn as a part of the National Swedish Contaminant Monitoring Programme. Mussel tissue from ten individuals from each location were homogenised and stored frozen until chemical analysis. Three homogenate samples from year 2005 (A-C) were analysed from Kvädöfjärden, as were homogenates from years (2003-2005) from Fladen and Väderöarna.

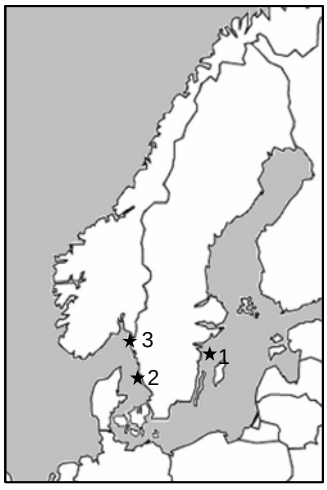

Figure 1. Sample site;
[1]Kvädöfjärden, [2]Fladen, [3]Väderöarna.

Chemicals
Acetone, *n*-hexane and dicloromethane (DCM) were of pesticide grade (Merck, Darmstadt, Germany), methyl *tert*-butyl ether of HPLC grade, distilled before use (Rathburn Chemicals, Walkerburn, Scotland,UK), water of HPLC grade (Scharlau Chemie, Barcelona, Spain), sulfuric acid, phosphoric acid, potassium hydroxide (KOH) and sodium chloride of pro analysis quality and silica gel (0.063-0.2 mm, Merck, Darmstadt, Germany) were purchased from indicated sources. Diazomethane was prepared in house from *N*-methyl-*N*-nitroso-*p*-toluene sulfonamide

(Diazald)[15] obtained from Sigma-Aldrich (Steinheim, Germany). The OH- and MeO-PBDE reference compounds were synthesised in house [16].

Analytical method
Two sets of 10 g (fresh weight) mussel homogenate were extracted, one for PBDD analysis and one for MeO- and OH-PBDE analysis. The mussels were extracted according to Jensen *et al.* 1983[17] with acetone, methyl-*tert*-butyl ether (MTBE) and *n*-hexane. Separation of neutral and phenolic compounds was carried out with potassium hydroxide (KOH) partitioning (0.5 M KOH in 50% ethanol) [18]. The phenolic fraction was derivatized with diazomethane. Both fractions were cleaned-up with concentrated sulfuric acid and sulfuric acid/silica gel (33% w/w) columns (1g) eluted with 28 ml DCM, followed by one silica gel column (0.5 g) eluted with 14 ml DCM. Analysis was carried out by gas chromatography-mass spectrometry (GC-MS).

The samples for PBDD analysis were cleaned up with concentrated sulfuric acid treatment, charcoal column fractionation into three fractions, containing the bulk of PCBs (*n*-hexane), *mono-ortho* PCBs (*n*-hexane:DCM, 1:1) and *non-ortho* PCBs, PCDD/F and PBDD/Fs (toluene, back-flush) and finally, a small multilayer silica gel column eluted with *n*-hexane.[2] Analysis was carried out by GC high resolution MS (SIM).

Instrumental analysis
GC-LRMS
Chromatographic separation was carried out on a DB-5 MS capillary column (30 m x 0.25 mm i.d., 0.25 µm particle size; J&W Scientific, Palo Alto, CA, USA) in a Varian 3400 GC equipped with an AS200S CTC autosampler and a spilt/splitless injector running in splitless mode for 1 minute. The helium carrier gas was at head pressure 12 psi and the injector temperature was held at 260°C. The GC-oven temperature program was: 80°C (2 min), 10°C min^{-1} to 300°C (10 min). Analyses were preformed with a Finnigan TSQ 700 (ThermoFinnigan, Bremen, Germany) quadrupole MS, operated in the electron capture negative ionization (ECNI) mode using selected ion monitoring (SIM) and scanning for the negative bromide ion (isotopes m/z 79 and 81). The ion source and the transfer line were set at 150°C and 270°C respectively.

GC-HRMS
Analyses were preformed using an Agilent HP6890 GC directly interfaced to a Micromass Ultima high-resolution MS (Waters Corp., Milford, MA, USA), operated in electron ionization (EI) mode (34 eV) at 10000 resolution, and using SIM. The chromatographic separation was carried out on a polar GC column (60 m, 0.25 mm i.d., 0.20 µm particle size; Supelco SP-2331, Bellefonte, Pennsylvania, USA) using a constant flow of helium at 1.0 ml min^{-1} and a GC oven temperature program of 190°C (2 min), 3°C min^{-1} to 280°C (10 min).

Results and Discussion
The highest concentrations of ΣPBDD, ΣOH-PBDE and ΣMeO-PBDE were found in Kvädöfjärden in the Baltic Proper (Figure 2). The concentrations were at least 10 times higher than in the samples from the west coast of Sweden (Fladen and Väderöarna).

The ΣPBDD consists mostly of triBDDs, primarily 1,3,7- and 1,3,8-triBDD, but also some diBDDs and tetraBDDs. The ΣOH-PBDE is calculated from the following congeners: 6-OH-BDE47, 2´-OH-BDE68, 2-OH-BDE85, 6-OH-BDE90, 6-OH-BDE99, 2-OH-BDE123 and 6-OH-BDE137. The ΣMeO-PBDE is calculated from the corresponding methoxylated congeners.

The three samples from Kvädöfjärden show similar concentrations in the three homogenates analysed (Figure 2), but, still, the in-year variation for ΣMeO-PBDEs in Kvädöfjärden is greater than the in-between year comparison for Fladen and Väderöarna. The levels of ΣPBDDs, ΣMeO-PBDEs and ΣOH-PBDEs in the west coast locations seem fairly constant and indicate no trends.

Interestingly, the relative abundance of the three groups of contaminants (Figure 2) differs. In the Kvädöfjärden samples, MeO-PBDEs dominates; in the Fladen samples the OH-PBDEs occurs at twice the levels of the MeO-PBDEs; and in the Väderöarna samples, the MeO- and the OH-PBDEs both occur at approximately the same levels. The samples at the two locations on the west coast have almost the same concentrations of ΣMeO-PBDE, but the Fladen samples contain additional ΣOH-PBDE compared to Väderöarna.

The brominated contaminants have a decreasing spatial trend from the Baltic Proper to the locations on the west coast. The total PBDD concentration is highest in Kvädöfjärden mussels followed by Fladen and Väderöarna mussels. Almost the same trend is seen for ΣMeO-PBDE and ΣOH-PBDE. The PBPs and PBAs (Figure 3) on the other hand exhibit an opposite trend, with higher abundances in the west coast samples. On the west coast, especially in Fladen, there is also a higher relative abundance of the PBAs compared to PBPs than in the Kvädöfjärden samples. These substances also seem to have a greater between-year variation. Some indications of an increase in PBA concentrations (Figure 3), over time, can be seen in the west coast samples but further investigations are needed before any conclusions can be drawn.

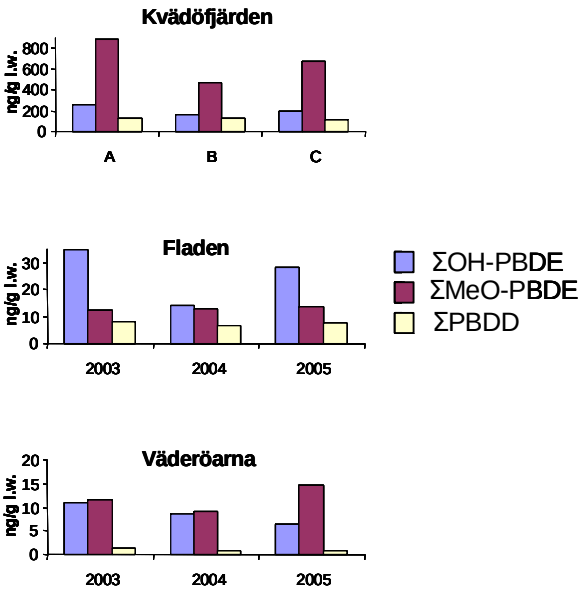

Figure 2: Spatial trend of MeO-PBDE, OH-PBDE and PBDD in blue mussels from Kvädöfjärden, Fladen and Väderöarna. Concentrations are given in ng/g lipids.

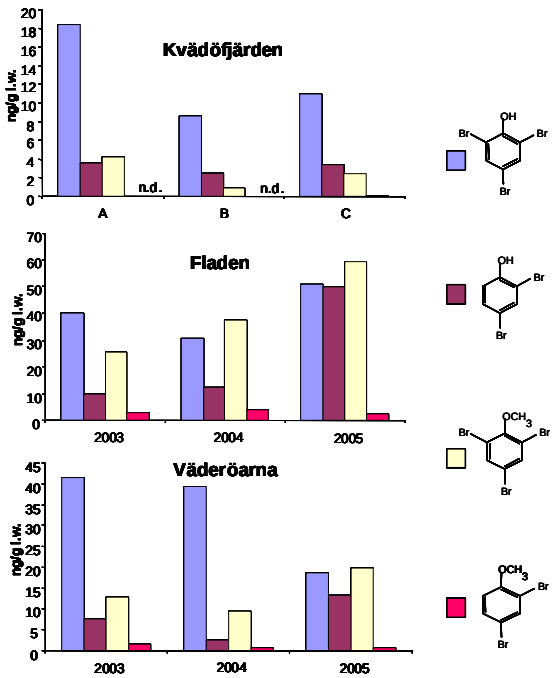

Figure 3: Spatial trend of PBPs and PBAs in blue mussels from Kvädöfjärden, Fladen and Väderöarna. Concentrations are given in ng g^{-1} lipids, n.d. stands for not detected.

The levels of ΣPBDD and ΣMeO-PBDE in Kvädöfjärden mussels, 100 ng g^{-1} lipids and 800 ng g^{-1} lipids, respectively, are remarkably high. They are comparable to, or higher than, the levels of PCBs in blue mussels from the same location[19]. The high concentrations of PBDD can not be explained by the relatively small amounts released into the environment by combustion and by-product formation during BFR production, but indicate a natural origin. Moreover, there are no known anthropogenic sources of MeO-PBDEs but there are of OH-PBDEs, being metabolites of the anthropogenic PBDEs. Several methoxylated and hydroxylated PBDEs have been shown to be natural products[7-10]. The major part of the brominated substances found in biota from the Baltic Sea is most likely naturally produced by primary producers such as algae and cyanobacteria. Blue mussels accumulate the substances through filtering of particles or through direct absorption of exudates. The levels in biota may vary with the occurrence and seasonal variations of algae/cyanobacteria.

From an ecological, as well as from a human consumption point of view these high concentrations of PBDDs can be of concern. The toxicity of PBDDs is generally similar to the chlorinated dioxins, but the levels of PBDDs are much higher than the chlorinated dioxins in the mussels investigated. The toxicity of the most abundant triBDDs (1,3,7- and 1,3,8-triBDD) are however unknown, which currently makes a risk assessment very difficult. Thus, there is a need for biological effect data for these compounds.

Recently a notably high toxicity was reported for OH-PBDEs. van Boxtel *et al*.[20] have shown, through *in vivo* studies, that 6-OH-BDE47 is acutely toxic to zebrafish embryos and also gives toxic effects *in vitro* on human cells. The blue mussel concentration of this specific congener is approximately half the total level of OH-PBDEs in Kvädöfjärden, and approximately 25% of the level in the west coast mussels.

Acknowledgement
Ionnis Athanassiadis is gratefully acknowledged for GC/MS analyses, Göran March for synthesis of MeO- and OH-PBDE standards, Takeshi Nakano for synthesis of the PBDD standards, and the Aquatic Environment Section at the Swedish Environmental Protection Agency for financial support.

References

1. Asplund L, Malmvärn A, Marsh G, Athanasiadou M, Bergman Å, and Kautsky L. 2001; 52:67.
2. Haglund P, Malmvärn A, Bergek S, Bignert A, Kautsky L, Nakano T, Wiberg K, and Asplund L. *Environ. Sci. Technol.* 2007.
3. Haglund PS, Zook DR, and Hu J. *Environ. Sci. Technol.* 1997; 31:3281.
4. Malmvärn A, Marsh G, Kautsky L, Athanasiadou M, Bergman Å, and Asplund L. *Environ. Sci. &Technol.* 2005; 39:2990.
5. Malmvärn A, Zebühr Y, Jensen S, Kautsky L, Greyerz E, Nakano T, and Asplund L. *Environ. Sci. &Technol.* 2005; 39:8235.
6. Marsh G, Athanasiadou M, Bergman Å, and Asplund L. *Environ. Sci. Technol.* 2004; 38:10.
7. Anjaneyulu V, Nageswara Rao K, Radhika P, and Muralikrishna M. *Indian J. Chem.* 1996; 35B:89.
8. Cameron GM, Stapleton BL, Simonsen SM, Brecknell DJ, and Garson MJ. *Tetrahedron* 2000; 56:5247.
9. Kitamura M, Koyama T, Nakano Y, and Uemura D. *Chem. Lett.* 2005; 34:1272.
10. Teuten EL, Xu L, and Reddy CM. *Science* 2005; 307:917.
11. Hakk H and Letcher RJ. *Environ. Int.* 2003; 29:801.
12. Marsh G, Athanasiadou M, Athanassiadis I, and Sandholm A. *Chemosphere* 2006; 63:690.
13. Neidleman, S.L. and Geigert, J. *Ellis Horwood series in organic chemistry*, Wiely,1986.
14. Malmvärn A, Zebühr Y, Kautsky L, Bergman Å, and Asplund L. 2007.
15. Vogel.,Eds.: Smith, B.V. and Waldron, N.M., Longman Inc.,1980.
16. Marsh G, Stenutz R, and Bergman Å. *Eur. J. Org. Chem.* 2003;2566.
17. Jensen S, Reutergårdh L, and Jansson B. *FAO Fish. Tech. Pap.* 1983; 212:21.
18. Hovander L, Athanasiadou M, Asplund L, Jensen S, and Klasson Wehler E. *J. Anal. Toxicol.* 2000; 24:696.
19. Asplund L, Nylund K, Eriksson U, and Bignert A. Dioxin2007, Tokyo, Japan, 2007, Sept. 2-7.
20. van Boxtel T, Kamstra J, Cenijn P, Marsh G, Brouwer A, Bergman Å, and Legler J. BFR2007, Amsterdam, The Netherlands, 2007, April 25-27.