Trends and exposure of naturally produced brominated substances in Baltic biota
- with focus on OH-PBDEs, MeO-PBDEs and PBDDs

Karin Löfstrand

Department of Materials and Environmental Chemistry
Stockholm University

Stockholm 2011
Abstract

The semi-enclosed and brackish Baltic Sea has become heavily polluted by nutrients, anthropogenic organic and inorganic chemicals via human activities. Persistent organic pollutants (POPs) have been thoroughly investigated due to their linkage to toxic effects observed in Baltic biota. There has been far less focus on semi-persistent pollutants e.g. naturally produced organohalogen compounds (NOCs) and their disturbances in the environment. This thesis is aimed on assessment of levels and trends of naturally produced brominated compounds in Baltic biota; more specifically on hydroxylated polybrominated diphenyl ethers (OH-PBDEs), methoxylated PBDEs (MeO-PBDEs) and polybrominated dibenzo-p-dioxins (PBDDs). These, NOCs, may originate from production in algae and cyanobacteria. OH-PBDEs and MeO-PBDEs may also be formed as metabolites of polybrominated diphenyl ethers (PBDEs), i.e. well-known commercial flame retardants.

High levels of OH-PBDEs, MeO-PBDEs and PBDDs are shown within Baltic biota (cyanobacteria, algae, mussels, fish), often in much higher concentrations than PBDEs which are possible anthropogenic precursors of OH- and MeO-PBDEs. The levels of OH-PBDEs, MeO-PBDEs and PBDDs are higher in the Baltic Sea than on the west coast of Sweden. Temporal and seasonal variations show fluctuations in concentrations of OH-PBDEs, MeO-PBDEs and PBDDs, possibly related with macroalgal life-cycles. OH-PBDEs, MeO-PBDEs and PBDDs are present in several filamentous macroalgae species, but considering the levels quantified, the time of peak exposure and the species life-cycle the macroalgae, Pilayella, Ceramium and Cladophora are suggested as major natural producers of OH-PBDEs and PBDDs.

The high levels of OH-PBDEs, MeO-PBDEs and PBDDs in the Baltic Sea may affect numerous organisms in the ecosystem. The toxic effects of OH-PBDEs and PBDDs are of particular concern. This thesis stress the importance of assessing and monitoring these substances, since the exposure to OH-PBDEs and PBDDs, during summer, may cause acute effects in Baltic fish and wildlife.
Till min älskade familj
i väntans tider
List of papers

This thesis is based on the following papers, which are referred to in the text by their roman numerals, I-IV. Paper I, II and III are reproduced with the kind permissions of the publishers. Some unpublished results are also included in the thesis.


Table of contents

Abstract .......................................................................................................................................................... ii

List of papers............................................................................................................................................... iv

Table of contents........................................................................................................................................ v

Abbreviations............................................................................................................................................... vii

1. Introduction ........................................................................................................................................... 9
   1.1 Aim...................................................................................................................................................... 9

2. Background ............................................................................................................................................ 11
   2.1 Baltic Sea ........................................................................................................................................... 11
   2.2 Biogenic production of natural organohalogen compounds......................................................... 11
   2.3 Hydroxylated polybrominated diphenyl ethers .............................................................................. 15
   2.4 Methoxylated polybrominated diphenyl ethers .............................................................................. 17
   2.5 Polybrominated dibenzo-\(p\)-dioxins and dibenzofurans ............................................................... 18
   2.6 Polybrominated phenols and anisoles ............................................................................................... 19
   2.7 Biological description of studied species ......................................................................................... 20
      2.7.1 Algae .......................................................................................................................................... 20
      2.7.2 Cyanobacteria ............................................................................................................................ 21
      2.7.3 Blue mussels .............................................................................................................................. 21
      2.7.4 Baltic clam ................................................................................................................................. 22
      2.7.5 Perch .......................................................................................................................................... 22
      2.7.6 Flounder ..................................................................................................................................... 22
      2.7.7 Grey seal .................................................................................................................................... 23

3. Analytical methods .................................................................................................................................. 24
   3.1 Samples and sampling ...................................................................................................................... 24
      3.1.1 Algae .......................................................................................................................................... 24
      3.1.2 Cyanobacteria ............................................................................................................................ 25
      3.1.3 Mussels ....................................................................................................................................... 25
      3.1.4 Fish ............................................................................................................................................ 25
      3.1.5 Seal ............................................................................................................................................ 26
      3.1.6 Sediment ................................................................................................................................... 26
   3.2 Extraction methods ............................................................................................................................. 26
   3.3 Determination of extractable material and carbon content ............................................................. 27
   3.4 Lipid removal ..................................................................................................................................... 28
   3.5 Separation of substance groups ....................................................................................................... 31
      3.5.1 Separation of neutral and phenolic compounds ...................................................................... 31
      3.5.1 Separation of non-planar and planar compounds ................................................................... 32
   3.6 Derivatisation .................................................................................................................................. 32
   3.7 Instrumental analysis ......................................................................................................................... 33
3.8 Quality Assurance/Quality Control ................................................................. 33

4. Additional results .................................................................................................................. 35
   4.1 Flounders ......................................................................................................................... 35
   4.2 PBDD concentration in Askö samples taken at different trophic levels .... 37
   4.3 Samples from New Zealand .......................................................................................... 37
   4.4 Herring and seal blood concentrations ........................................................................ 39

5. Discussion ............................................................................................................................. 41
   5.1 Data normalization ........................................................................................................... 41
   5.2 Trends ............................................................................................................................ 41
      5.2.1 Temporal variations ................................................................................................. 41
      5.2.2 Seasonal variations .................................................................................................. 42
      5.2.3 Geographical distribution ........................................................................................ 45
   5.3 Food web distribution ..................................................................................................... 47
   5.4 Exposure and uptake ........................................................................................................ 49
   5.5 Origin ............................................................................................................................. 50
   5.6 Ecological perspective .................................................................................................... 51

6. Future perspectives .............................................................................................................. 52

7. Acknowledgements ............................................................................................................. 53

8. References ............................................................................................................................ 55
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADP</td>
<td>Adenosine diphosphate</td>
</tr>
<tr>
<td>ASE</td>
<td>Accelerated solvent extraction</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>BCF</td>
<td>Bioconcentration factor</td>
</tr>
<tr>
<td>BFRs</td>
<td>Brominated flame retardants</td>
</tr>
<tr>
<td>BMF</td>
<td>Biomagnification factor</td>
</tr>
<tr>
<td>CYP</td>
<td>Cytochrome P-450</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>ECD</td>
<td>Electron capture detector</td>
</tr>
<tr>
<td>ECNI</td>
<td>Electron capture negative ionisation</td>
</tr>
<tr>
<td>EI</td>
<td>Electron ionization</td>
</tr>
<tr>
<td>EOM</td>
<td>Extractable organic matter</td>
</tr>
<tr>
<td>GC</td>
<td>Gas chromatography</td>
</tr>
<tr>
<td>GPC</td>
<td>Gel permeation chromatography</td>
</tr>
<tr>
<td>HRMS</td>
<td>High resolution mass spectrometry</td>
</tr>
<tr>
<td>l.w.</td>
<td>Lipid weight</td>
</tr>
<tr>
<td>LOD</td>
<td>Limit of detection</td>
</tr>
<tr>
<td>Log $K_{ow}$</td>
<td>Octanol-water partition coefficient</td>
</tr>
<tr>
<td>LOQ</td>
<td>Limit of quantification</td>
</tr>
<tr>
<td>LRMS</td>
<td>Low resolution mass spectrometry</td>
</tr>
<tr>
<td>MeO-PBDEs</td>
<td>Methoxylated polybrominated diphenyl ethers</td>
</tr>
<tr>
<td>MS</td>
<td>Mass spectrometry</td>
</tr>
<tr>
<td>n.a.</td>
<td>Not analysed</td>
</tr>
<tr>
<td>NOCs</td>
<td>Natural organohalogen compounds</td>
</tr>
<tr>
<td>OC</td>
<td>Organic carbon</td>
</tr>
<tr>
<td>OHCs</td>
<td>Organohalogen compounds</td>
</tr>
<tr>
<td>OH-PBDEs</td>
<td>Hydroxylated polybrominated diphenyl ethers</td>
</tr>
<tr>
<td>OH-PCBs</td>
<td>Hydroxylated polychlorinated biphenyls</td>
</tr>
<tr>
<td>PBAs</td>
<td>Polybrominated anisoles</td>
</tr>
<tr>
<td>PBDDs</td>
<td>Polybrominated dibenzo-p-dioxins</td>
</tr>
<tr>
<td>PBDEs</td>
<td>Polybrominated diphenyl ethers</td>
</tr>
<tr>
<td>PBDFs</td>
<td>Polybrominated dibenzofurans</td>
</tr>
<tr>
<td>PBPs</td>
<td>Polybrominated phenols</td>
</tr>
<tr>
<td>PCBs</td>
<td>Polychlorinated biphenyls</td>
</tr>
<tr>
<td>PCDDs</td>
<td>Polychlorinated dibenzo-p-dioxins</td>
</tr>
<tr>
<td>PCDFs</td>
<td>Polychlorinated dibenzofurans</td>
</tr>
<tr>
<td>$pK_{a}$</td>
<td>Acid dissociation constant</td>
</tr>
<tr>
<td>PLE</td>
<td>Pressurized liquid extraction</td>
</tr>
<tr>
<td>POPs</td>
<td>Persistent organic pollutants</td>
</tr>
<tr>
<td>psu</td>
<td>Practical salinity units</td>
</tr>
<tr>
<td>S/N</td>
<td>Signal to noise</td>
</tr>
<tr>
<td>-----</td>
<td>----------------</td>
</tr>
<tr>
<td>SIM</td>
<td>Selected ion monitoring</td>
</tr>
<tr>
<td>TMF</td>
<td>Trophic magnification factor</td>
</tr>
<tr>
<td>TTR</td>
<td>Transthyretin</td>
</tr>
<tr>
<td>w.w.</td>
<td>Wet weight</td>
</tr>
</tbody>
</table>
1. Introduction

In the second phase of the industrial revolution, i.e. in the first half of the 20th century, organohalogen compounds (OHCs) started to be produced on a commercial basis, to aid in everyday life. Several of these chemicals were designed to be stable to enable long lasting use in their applications. Unfortunately, the chemical stability soon proved to have its drawbacks, also being stable in the environment, i.e. persistent. The persistency of these compounds resulted in increasing concentrations in the environment and they were soon shown to cause adverse effects in wildlife, in particular at high trophic levels.

During the last Century, the Baltic area was subjected to major discharges of anthropogenic chemicals and became heavily contaminated. Monitoring programs were initiated in the Baltic region, including several marine and terrestrial wildlife species. The research within the monitoring programs was focused to what we today may call traditional anthropogenic contaminants, persistent organic pollutants (POPs). In addition to the POPs there are several thousand of substances, with chemical and structural similarities, but formed through natural processes [1]. Such chemicals, natural products, are either formed via biogenic synthesis or metabolic transformations. It is reasonable to believe that natural products and anthropogenic chemicals may act through similar mechanisms leading to potentially adverse effects in wildlife. Far less is known about semi-persistent pollutants, including anthropogenic contaminants and those being natural products.

1.1 Aim

This thesis focuses on polybrominated compounds in Baltic biota, more specifically, in biota collected in areas along the Swedish coastline. The aim was to evaluate origin, assess concentrations, geographical distribution and indicate ecological relevance of mainly three groups of chemicals; hydroxylated polybrominated diphenyl ethers (OH-PBDEs), sometimes referred to as polybrominated phenoxyphenols; methoxylated PBDEs (MeO-PBDEs), also known as polybrominated phenoxyanisols; and polybrominated dibenzo-\(\text{p}\)-dioxins (PBDDs). The thesis objectives include studies of time trends and geographic and inter-species distribution of these chemicals. The specific objectives of the individual papers are described hereunder.

Paper I: The exposure of brominated compounds, in particular OH-PBDEs, MeO-PBDEs, PBDDs and simple brominated phenols and anisoles were investigated in a filtrating species, the blue mussel. The study was aimed to
assess differences and similarities in the chemical composition in blue mussels collected at the west coast of Sweden and in the Baltic proper.

**Paper II:** The original aim was to investigate whether the levels of PBDDs and MeO-PBDEs in Perch from Kvädfjärden in the Baltic Sea have increased during the last two decades and if there were any correlation between the two substance groups within the studied species. During the data evaluation the focus was shifted to discuss the individual congener retention and metabolic stability in perch versus their molecular structures.

**Paper III:** The goal of this study was to determine any seasonal variation, within the summer season (May-September), of OH-PBDEs and MeO-PBDEs, and to discuss possible correlations with the lifecycle of some primary producers, i.e. algae and cyanobacteria.

**Paper IV:** This article objective was to study the seasonal and geographic distribution of OH-PBDEs, MeO-PBDEs and PBDDs in several algae species and blue mussels as well as inter-species differences between the algae. The paper also aims to investigate possible relations between the mussel concentrations and the algae species to try to identify major producers of OH-PBDEs, MeO-PBDEs and PBDDs.
2. Background

2.1 Baltic Sea

The Baltic Sea including its large bays, the Gulf of Bothnia, Gulf of Finland and Gulf of Riga, provides a coastal zone for nine countries; Sweden, Finland, Russia, Estonia, Latvia, Lithuania, Poland, Germany and Denmark. The drainage area is even greater, also including Belarus, Czech Republic, Norway, Slovakia and Ukraine. The surrounding lands have approximately two-hundred rivers giving a yearly fresh water runoff of about 500 km³, a large contribution of water to this semi-enclosed sea, holding a total water volume of 21 760 km³. The only inlet of saline waters to the Baltic Sea comes from the North Sea via the small sounds in Denmark and between Denmark and Sweden. This results in a low salinity in the Baltic Sea in general, but also gives the sea a gradient from the southwest to the north and the east of eight to one practical salinity units (psu). In addition, a large part of the Baltic Sea is vertically stratified into two parts, with the saltier, heavier and oxygenated water from the North Sea at the bottom. This barrier prevents the mixing of oxygen and nutrients in the Sea, leading, in the long run, to dead zones. The salt gradient together with the parallel temperature gradient affects the flora and fauna, limiting the biodiversity in the Baltic Sea [2].

Eutrophication is a large problem in most parts of the Baltic Sea [3]. The nutrient load, mainly originating from municipal and rural human sources and agriculture, has significantly increased during the 20th century [3]. A slight decrease in nutrient load in the open Baltic proper has been observed in the beginning of the 21th century [4]. Regional differences in eutrophication occur, especially in the coastal waters [5]. The more obvious effects seen in the Baltic Sea, due to the nutrient enrichment are, the large-scale cyanobacteria blooms that occur during summer months [6,7], large amounts of macroalgae ending up on the shores [8], reduced habitat of some species in favour of other more adaptable species [2] and oxygen depletion [2].

2.2 Biogenic production of natural organohalogen compounds

Formation of natural organohalogen compounds (NOCs) is a common phenomenon in both the terrestrial and marine environment, with over 3800 identified NOCs produced by either abiotic processes or by biota [9]. The marine environment is by far the most important source of biogenic NOCs, with producers such as algae, sponges, corals, tunicates and bacteria as summarized by Gribble [9]. In the terrestrial environment NOCs may be produced by plants, fungi, lichen, bacteria, insects, and even in some higher animals including humans [9].
NOCs may include any of the four halogen elements but over 95% of all NOCs contain either bromine and/or chlorine [9]. Structures of four common NOCs are presented, as examples thereof, in Figure 2.1. Bromomethane (1, Figure 2.1) is a good example of the simple haloalkanes found in both marine and in terrestrial plants [9]. Indoles (2), bipyroles (3) and MeO-PBDEs (4) are commonly detected in the marine biota. These compounds are according to present knowledge produced by marine sponges, bacteria and fungi, [9].

Figure 2.1. Examples of some brominated NOCs. 1) bromomethane; 2) 3,6-dibromoindole, 3) 1,1'-dimethyl-3,3',4,4',5,5'-hexabromo-2,2'-bipyrole; 4) 6-methoxy-2,2',4,4'-tetrabromodiphenyl ether

2.2.1 Biosynthesis
In general, the hydrocarbon skeleton of the NOC is formed first, followed by halogenation. More complex structures can also be formed through fusion of smaller NOCs. The hydrocarbon skeleton of aromatic organic compounds can be biosynthesized by three main pathways; the acetate pathway forming acetogenins, the mevalonate pathway forming terpenoids and sterols or the shikimate pathway forming aromatic compounds e.g. phenols [10]. Most brominated simple phenols are produced by the shikimate pathway (starting with D-glucose), either via 4-hydroxybenzoate or phenol. Since all NOCs included in this thesis probably are derived via phenol, only the shikimate pathway is further discussed. This pathway is schematically shown in Figure 2.2 [10,11]. The reaction is enzyme driven and energy consuming, however the exact mechanism of which the enzyme participates is not known.
The bromination mechanism of phenolic compounds seems to be a bromoperoxidase catalysed cationic reaction in the presence of bromine and hydrogen peroxide [10]. This synthesis begins with an enzymatically catalysed reaction of hydrogen peroxide and bromine to form the reagents, hypobromous acid as shown in Figure 2.3. The reagents then undergo an electrophilic reaction with high electron density centres (Figure 2.3), e.g. an electrophilic aromatic substitution reaction. The halogen atom is usually introduced in the ortho- or para-position to the phenolic group in naturally occurring brominated phenolic compounds. It is also plausible for substrates with low electron density to go through an anionic bromination by direct insertion of bromide, e.g. brominated hydroquinones (with hydroxyl groups in the para-positions) [10].
After halogenation, dimerization is achieved through peroxidase catalysed radical reactions of single ringed, aromatic compounds, forming products such as biphenyls, diphenyl ethers, *bis*-indoles, dibenzofurans and dibenzo-*p*-dioxins (examples in Figure 2.4) [10]. The peroxidase initiate the coupling reaction of phenolic dimerization products by a one-electron oxidation in the presence of hydrogen peroxide, giving a phenoxy radical in the *ortho*- or *para*- position (Figure 2.4). Generally, the oxidation is catalysed by peroxidase, but coupling may also be catalysed by the cytochrome P-450 system in vascular plants as shown for the formation of diphenyl ether alkaloids by *Berberis stolonifera* [12].

![Figure 2.4. Example of dimerization reactions of phenolic radicals, forming an ortho-hydroxylated diphenyl ether (left) and an ortho-dihydroxylated biphenyl (right). The figure is modified from a figure presented by Nielson [10].](image-url)
Among all possible NOCs in the environment, this thesis concentrates on a few groups thereof.

2.3 Hydroxylated polybrominated diphenyl ethers

OH-PBDEs can be formed as metabolites of PBDEs as reviewed by Haak and Letcher [13] and as presented in more recent articles [14-16]. However, OH-PBDEs are also known to be natural products [1]. The naturally formed OH-PBDEs identified so far, have the hydroxyl group in an ortho-position while PBDE metabolites seem to preferentially have the hydroxyl group in either a meta- or para-position [13,16]. The ortho-substituted 2’-OH-BDE28, 6-OH-BDE47 and 2’-OH-BDE66, identified as minor PBDE congener metabolites, are exceptions [16].

Naturally formed OH-PBDEs are widespread and have been identified in several species as exemplified in Table 2.1. They are believed to be synthesised by primary producers such as algae, marine sponges and cyanobacteria [1,17]. Additional suggested sources are the formation of OH-PBDEs from the corresponding naturally produced MeO-PBDE congener via biogenic demethylation [18], or by abiotic oxidation in the atmosphere via reaction of PBDEs with hydroxyl radicals [19].

Table 2.1. Examples of the worldwide distribution of detected OH-PBDEs in marine species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Detected in</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyanobacteria</td>
<td>Baltic Sea [20]</td>
</tr>
<tr>
<td>Algae</td>
<td>Baltic Sea [21], Philippines [22]</td>
</tr>
<tr>
<td>Marine Sponge</td>
<td>Indo-pacific ocean [23], Indonesia [24], Palau [25,26], Mozambique [27]</td>
</tr>
<tr>
<td>Mussels</td>
<td>Baltic Sea [21]</td>
</tr>
<tr>
<td>Fish</td>
<td>Baltic Sea [28,29], Detroit River [30]</td>
</tr>
<tr>
<td>Seal</td>
<td>Baltic Sea [31], Svalbard [31], East Greenland [32]</td>
</tr>
<tr>
<td>Polar bear</td>
<td>Norway [33], East Greenland [32]</td>
</tr>
</tbody>
</table>
The OH-PBDEs have log $K_{ow}$ values varying between 5 and 9 (Table 2.2) depending on congener structure but also on method of calculation or software applied. Although the log $K_{ow}$ values are high and indicates a high hydrophobicity, the OH-PBDEs do not bioaccumulate in lipid tissue. OH-PBDEs behaves like many other halogenated phenolic compounds, such as halophenols and hydroxylated PCBs (OH-PCBs), by association to blood proteins, e.g. transthyretin [34]. Further, the water solubility of OH-PBDEs may increase due to the inverse relationship between water solubility and pK$_a$ (Table 2.2) of the phenolic compounds. At natural pH in the marine waters, at least half of the OH-PBDE concentrations may be present in its ionic form. The range in pK$_a$ values are from approx. 5 to 7 which may result in different uptake of OH-PBDE congeners and leading to congener specific exposure. In addition to being a factor in exposure via direct uptake from water, the pK$_a$ value is also relevant for uptake via the diet.

The OH-PBDEs are linked to several toxicological effects. For example, 6-OH-BDE47 is confirmed to be acutely toxic in developing and adult zebrafish at concentrations in the nanomolar range [35]. The effects are contributed to disruption of oxidative phosphorylation [35], i.e. inhibiting the phosphorylation of adenosine diphosphate (ADP) to adenosine triphosphate (ATP), eventually leading to energy depletion. OH-PBDEs also have a potential to disrupt the endocrine system [36]. 6-OH-BDE47 and 4′-OH-BDE49 are shown to have competitive binding to transthyretin (TTR) [37]. In vitro studies in human cells suggest that meta- and para-OH-substituted PBDEs have 160-1600 higher relative binding potencies to TTR than BDE-47 [38]. OH-PBDEs are shown to have both estrogenic effects, through interactions with the estrogen receptor [39], and anti-estrogenic effects by inhibition of estradiol sulfotransferase [38]. Several OH-PBDEs are also found to inhibit CYP17 and CYP19 (aromatase) activity in human adrenocortical carcinoma (H295R) cells in micromolar concentrations [40] and aromatase activity in the human placenta [41]. Further, 6-OH-BDE47 and 6-OH-BDE85 are shown to be cytotoxic in micromolar concentrations in H295R cells, but do not generate DNA-damage [42]. Dingemans et al. found 6-OH-BDE47 to be neurotoxic, by disrupting the calcium ion homeostasis in pheochromocytoma cells [43].
Table 2.2. Calculated Log $K_{ow}$ and $pK_a$ values [17,44] for some of the most common naturally formed OH-PBDEs and log $K_{ow}$ values [45] of naturally occurring MeO-PBDEs.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Log $K_{ow}$ ACD</th>
<th>Experimental</th>
<th>pK$_a$ ACD</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-OH-BDE47</td>
<td>6.8 ± 0.2</td>
<td>5.82 ± 0.03</td>
<td>6.8 ± 0.2</td>
</tr>
<tr>
<td>2'-OH-BDE68</td>
<td>7.2 ± 0.6</td>
<td>5.36 ± 0.04</td>
<td>6.6 ± 0.2</td>
</tr>
<tr>
<td>6-OH-BDE85</td>
<td>8.3 ± 0.7</td>
<td>5.83 ± 0.02</td>
<td>6.2 ± 0.2</td>
</tr>
<tr>
<td>6-OH-BDE90</td>
<td>8.2 ± 0.7</td>
<td>5.83 ± 0.03</td>
<td>5.7 ± 0.2</td>
</tr>
<tr>
<td>6-OH-BDE99</td>
<td>8.4 ± 0.7</td>
<td></td>
<td>5.2 ± 0.2</td>
</tr>
<tr>
<td>2-OH-BDE123</td>
<td>8.3 ± 0.7</td>
<td>5.82 ± 0.03</td>
<td>5.7 ± 0.2</td>
</tr>
<tr>
<td>6-OH-BDE137</td>
<td>9.3 ± 0.8</td>
<td>6.45 ± 0.03</td>
<td>4.7 ± 0.2</td>
</tr>
<tr>
<td>6-MeO-BDE47</td>
<td>6.44 ± 0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2'-MeO-BDE68</td>
<td>6.16 ± 0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-MeO-BDE85</td>
<td>6.26 ± 0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-MeO-BDE90</td>
<td>6.65 ± 0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-MeO-BDE123</td>
<td>6.62 ± 0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-MeO-BDE137</td>
<td>6.98 ± 0.03</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ACD/LABs TM software

2.4 Methoxylated polybrominated diphenyl ethers

MeO-PBDEs have been identified as natural products by determination of radiocarbon ($^{14}$C) content of two MeO-PBDEs (6-MeO-BDE47 and 2'-MeO-BDE68) isolated from a True's beaked whale (*Mesoplodon mirus*) [46]. MeO-PBDEs have also been identified in a whale oil sample from 1921, sampled before any industrial production of OHCs started [47]. For many years no MeO-PBDEs metabolites have been indicated due to PBDE exposure, and accordingly considered to be solely of natural origin. Lately however, Feng et al. reported MeO-PBDEs in rainbow trout after exposure to decabromodiphenyl ether [48]. This is to my knowledge the only study supporting the metabolic formation of MeO-PBDEs from PBDEs. Further, microbial methylation of OH-PBDEs may occur in e.g. sediments by microorganisms [49-54]. MeO-PBDEs are known to bioaccumulate in tissue as indicated by their log $K_{ow}$ (Table 2.2) and have been identified in many species worldwide, some of which are summarized in Table 2.3. The log $K_{ow}$ values, varying from 6-7 (Table 2.2.), are higher for the MeO-PBDEs than the OH-PBDEs.
Table 2.3. Examples of the worldwide distribution of detected MeO-PBDEs in marine species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Detected in</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyanobacteria</td>
<td>Baltic Sea [20]</td>
</tr>
<tr>
<td>Algae</td>
<td>Baltic Sea [21], Australia [55-57], China [58], Philippines [22]</td>
</tr>
<tr>
<td>Mussels</td>
<td>Baltic Sea [21], China [59], Canadian Arctic [60]</td>
</tr>
<tr>
<td>Fish</td>
<td>Baltic Sea [28,29,61,62], Canadian Arctic [60], Mediterranean Sea [63], Detroit River [30]</td>
</tr>
<tr>
<td>Seal</td>
<td>Baltic Sea [61], Canadian Arctic [60], East Greenland [32], Southern North Sea [64]</td>
</tr>
<tr>
<td>Polar bear</td>
<td>Norway [33], East Greenland [32]</td>
</tr>
</tbody>
</table>

The toxicity of MeO-PBDEs is low, but some studies have reported effects in cell cultures [40,41,65-67] with very high but not environmentally relevant levels. For example, 6-MeO-BDE47 inhibits the CYP17 [65] and the aromatase (CYP19) [40,67] activity but does not affect the sex hormone production [67] or show any cytotoxicity [40,65]. However, the possibility of demethylation [18,68-70], forming the corresponding OH-PBDE, may have toxicological implications.

2.5 Polybrominated dibenzo-p-dioxins and dibenzofurans

PBDDs and polybrominated dibenzofurans (PBDFs) are coplanar compounds, formed as by-products in brominated flame retardant (BFR) production [71] and combustion of BFR containing products [71-74]. PBDFs can also be formed though photolytic transformation of decaBDE [75,76]. PBDDs/Fs undergo photolysis more rapidly than polychlorinated dibenzo-p-dioxins (PCDDs) in sun and indoor light [77]. In addition, PBDDs may be formed via photolysis of OH-PBDEs [78].

The PBDDs formed in combustion are dominated by tetra- and pentaBDDs, but the congener composition may differ with material and combustion temperature [72]. High levels of lower brominated dibenzo-p-dioxins (Br1-Br4), in Baltic Sea biota have led to a discussion of natural formation of these compounds [17,20,79-81] (Paper I, II and IV). Non-halogenated dibenzofurans are common among identified and reported natural products [10,82], but halogenated dibenzofurans and dibenzo-p-dioxins are not [10]. One example of a natural PBDF is the 2-bromodibenzofuran found in the sponge
Further, derivatives of PBDDs have been isolated from marine sponges [83,84] and derivatives of PBDFs from red algae [85]. Naturally produced PBDDs and PBDFs may be formed through diaryl coupling of phenolic radicals, as presented in chapter 2.2.1 and Figure 2.4.

PBDDs/Fs have e.g. been found in cyanobacteria [20], algae [20], mussel [79,81], marine sponge [86] and fish [79] from the Baltic Sea, in shellfish and fish from the west coast of Sweden [79], in marine shellfish from United Kingdom [87], as well as, in human adipose tissue from Japan [88] and in human breast milk [89]. PBDDs/Fs have also been detected in sediments [90,91].

Information on biological effects of PBDDs/Fs is limited and often deduced from the knowledge obtained from studies of PCDDs and polychlorinated dibenzo-furans (PCDFs). In vivo toxicity studies of PBDDs show biological effects associated with PCDDs/Fs, i.e. lethality, wasting, thymic atrophy, tetratogenicity, reproductive effects, chloracne, immunotoxicity, enzyme induction, decrease in T4 and vitamin A and increased hepatic porphyrins [71]. In addition, in vitro enzyme induction and anti-estrogenic activity are linked to PBDDs/Fs [71]. PBDDs/Fs are also potent inducers of microsomal monoxygenase activity, aryl hydrocarbon hydrolase and ethoxyresorufin-o-deethylase (EROD) both in vitro and in vivo [92]. PBDDs/Fs can bind to the aryl hydrocarbon receptor (AhR) [93,94], but the binding affinity is generally half compared to the chlorinated analogues as reviewed by Birnbaum et al. [95]. The toxic equivalent system used to compare PCDD toxicity is not yet developed to include the PBDDs.

2.6 Polybrominated phenols and anisoles

PBPs are produced in several anthropogenic processes. 2,4,6-TriBP is produced in large scale and is by far the most common PBP in the world. In 2001 the worldwide annual production was 9500 tonnes. 2,4,6-triBP is used as a wood preservative and, both 2,4-diBP and 2,4,6-triBP are employed as reactive flame retardant intermediates [96-99]. PentaBP has been used as a mulluscicide [100] as well as an intermediate in the production of pentabromophenoxy compounds. 2-monoBP, 2,4-diBP, 2,6-diBP, 2,4,6-triBP has been identified in vehicle emission of leaded petrol [101].

PBPs are also produced naturally in large quantities in marine biota [1]. For example, the acorn worm Balanoglossus biminiensis produces up to 15 mg 2,6-diBP per animal as a defensive secretion [102]. Several species of marine algae are known to contain [56,57] and biosynthesis [55,103] brominated phenols. The abundance of PBPs is both spatially and temporally correlated with the
abundance of infauna that produces these metabolites [104]. Although there is a worldwide anthropogenic production and use of 2,4,6-triP, the amounts released into the environment from natural sources is proposed to be more abundant.

PBPs can also be formed via biodegradation of other pollutants, such as brominated benzenes and some brominated diphenyl ethers [1,18,105]. Further, PBPs can be formed from demethylation of polybrominated anisoles (PBAs) under anaerobic conditions [100].

The estimated bioaccumulation potential of PBPs increases with the degree of bromination, as indicated by the log $K_{ow}$ values presented in Table 2.4. Predicted bioconcentration factors (BCFs) (Table 2.4) suggest some potential for bioaccumulation [100]. However, at natural marine pH both the 2,4,6-triBP and the pentaBP are mostly dissociated (Table 2.4), and accordingly the route of uptake differs between congeners.

**Table 2.4.** Reported log $K_{ow}$ and pK$_a$ [106] and BCF [100] values for some PBPs.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Log $K_{ow}$</th>
<th>pK$_a$</th>
<th>BCF</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-monoBP</td>
<td>2.62</td>
<td>9.17</td>
<td>20</td>
</tr>
<tr>
<td>2,4-diBP</td>
<td>3.48</td>
<td>7.79</td>
<td>24</td>
</tr>
<tr>
<td>2,4,6-triBP</td>
<td>4.24</td>
<td>6.08</td>
<td>120</td>
</tr>
<tr>
<td>Penta-BP</td>
<td>5.30</td>
<td>4.40</td>
<td>3100</td>
</tr>
</tbody>
</table>

$^1$at 25ºC [106]  
$^2$Calculated using Bcfwin [100]

PBAs are formed in methylation processes in the marine environment, e.g. 2,4,6-triBA can be formed as a fungal metabolite of 2,4,6-triBP [10] or by O-methylation in bacteria [53].

**2.7 Biological description of studied species**

A short introduction to the biology of the species analysed and discussed within this thesis is given here. More detailed information of the samples and sampling can be found in chapter 3.1 and the Papers I-IV.

**2.7.1 Algae**

The family of algae is vast, including marine autotrophic and eukaryotic organisms, ranging from unicellular to multicellular organisms. This thesis is
concentrated on macroscopic, multicellular, benthic marine algae, summarized from now on as filamentous macroalgae. The term includes members of red, brown and green algae. The difference in colour of the algae is attributed to their pigments being optimized to absorb light at their habitat sea depth. The green algae grow closest to the surface followed by the brown algae and the red algae.

The red algae are one of the oldest and the largest of the groups of eukaryotic algae, with somewhere between 6000 and 10000 species. Most of which are filamentous macroalgae with sexual reproduction. The red colour is given by the accessory pigments, phycobiliproteins. The brown algae is a large group of mostly marine multicellular algae with about 1500-2000 species. They play an important role in marine environments both as food, and for the habitats they form. Most brown algae contain the pigment fucoxantin (brown pigment) and chlorophyll (green pigment), giving them their characteristic greenish-brown colour. Brown algae reproduce by means of both flagellate spores and gametes. The green algae are usually single cell organisms, while others form colonies, long filaments or macroscopic seaweeds. There are about 8000 species of both fresh water (7000) and marine green algae (1000). The green colour is given from chlorophyll \( a \) and \( b \) and the reproduction is commonly sexual.

2.7.2 Cyanobacteria

Cyanobacteria constitute a large and diverse group of bacteria capable of oxygen photosynthesis and are found in most waters worldwide. Cyanobacteria are unicellular or filamentous and can form colonies or aggregates.

In the Baltic Sea there are three main species of cyanobacteria; *Nodularia spumigena*, *Aphanizomenon flos-aquae* and *Anabaena spp*. The cyanobacteria blooms are initiated by calm and sunny weather, elevated surface water temperature and thermal stratification. Nitrogen fixing cyanobacteria such as *Aphanizomenon* and *Nodularia* are also depending on phosphate availability. The *Aphanizomenon flos-aquae* or hepatoxin containing *Nodularia spumigena* usually dominated the large cyanobacteria blooms formed during the summer in the Baltic.

2.7.3 Blue mussels

The blue mussel (*Mytilus edulis*) is a suspension-feeding marine bivalve mollusc found worldwide in temperate and cold oceans. The blue mussels attach themselves to hard surfaces such as cliffs, rocks or tongs with their byssus threads. They are very robust and can stand large variations in temperature and salt content. The blue mussel will reach reproductive age at one year. Reproduction occurs from early spring into the autumn by releasing their
gametes into the surrounding waters. The larvae are pelagic and swim for 2-3 weeks before attaching themselves to any surface.

In the Baltic Sea the blue mussels have adapted to the brackish water, but they are much smaller than in areas like the North Sea where the salinity is higher. The differences are genetic [107], morphologic [108] and physiologic [109]. Some even argue that the Baltic blue mussel belong to a different sub-species and has been named *Mytilus edulis trossulus* [110]. In this thesis, however, the sub-species of the blue mussels have not been considered as a factor since the species are only used to monitor exposure of contaminants.

2.7.4 Baltic clam

The Baltic clam (*Macoma baltica*) is a bivalve living in sandy and clayey Sea bottoms. It is so named since its habitat is the entire Baltic Sea. The Baltic clam lives buried in the sediments eating small plant and animal parts from the Sea bottom. The Baltic clam is a popular diet for *Saduria entomon*, an isopod crustacean without a common name, and flounders.

2.7.5 Perch

The Perch (*Perca fluviatilis*) is a relative stationary fish species, only migrating to reach their spawning location. The sexual maturity is reached at the age of 2-4 years for the males and 3-5 years for the females. The spawning takes place during April to June in the Baltic Sea. During the first life year the Perch feeds on zooplankton and then moves on to insect larvae, crustaceans and small fish.

2.7.6 Flounder

The European flounder (*Platichthys flesus*) is an ocean-dwelling flatfish of European coastal waters, feeding on invertebrates, especially crustaceans, worms, molluscs, as well as small fish. The flounder used within this thesis were all from the Swedish coast, both from the west coast of Sweden (Skagerrak and Kattegat) and from the Baltic Sea. The feeding habits are somewhat different between the two coasts, e.g. flounders from the Baltic feed on blue mussels which is not possible for the flounders on the west coast. The time of reproduction is different as well; in Skagerrak and Kattegat it takes place in January to April and in the Baltic Sea from May to June. This has lead to some debate whether the flounders from these locations are of the same sub-species or not. Reproductive age for males and females are reached at two and three years of age, respectively.
2.7.7 Grey seal

The Grey seals (*Halichoerus grypus*) in the Baltic Sea are an isolated population and thus called (*Halichoerus grypus balticus*). This mammal feeds on a wide variety of fish, e.g. sand eels, cod, flatfish but mainly herring. Grey seals are feeding at a high trophic level and are a well-studied species [111]. It is suffering from health effects like decreased body weight and/or blubber thickness [112,113] and colonic ulcers [114].
3. Analytical methods

The analytical methods used within this thesis are well established methods for environmental contaminant analysis. However, in some cases these methods were modified to meet the requirements of the objectives in this thesis. Detailed information of the methods used, are given in the separate publications (Paper I-IV).

3.1 Samples and sampling

Samples from a number of sampling locations along the Swedish coastline were used in this thesis. The locations are indicated on the map shown in Figure 3.1 and each of them is further presented in Paper I-IV. Additional samples were collected to study food web distribution of OH-PBDEs, MeO-PBDEs and PBDDs (see Chapter 4, below).

![Figure 3.1 Map over the Swedish coastline with the sampling locations marked 1-9. They are: 1.) Hornslandet, 2.) Arholma, 3.) Askö, 4.) Kvädöfjärden, 5.) Öland, 6.) Abbekås, 7.) Fladen, 8.) Väderöarna, 9.) Tjärnö.](image)

3.1.1 Algae

Brown algae, *Dictyosiphon foenicolaceus* (Paper I), *Fucus vesiculosus* (Paper IV) from Kvädöfjärden (4 in Figure 3.1) and *Pilayella littoralis* from Askö (3) and Hornslandet (1) (Paper IV) were collected in the autumn of 2006. Red algae, *Ceramium tenuicorne* from Askö (3), Hornslandet (1), Arholma (2) and Öland (Byxelkrok) (5) and *Ceranium rubrum* from Tjärnö (9), *Polysiphonia fucoids* from Askö (3) and Abbekås (6), *Polysiphonia brodari* from Tjärnö (9) and *Furcellaria lumbricalis* from Askö (3) and Abbekås (6) (Paper IV) were collected between 2006 and 2009. Green algae, *Cladophora glomerata* from Askö (3), Hornslandet (1) and Öland (5), *Cladophora albida* from Tjärnö (9).
and *Enteromorpha intestinalis* from Öland (5) (*Paper IV*) were collected between 2006 and 2009. The algae were collected by hand, extensive water was wrung out, and the samples were homogenized (*Paper I and IV*).

### 3.1.2 Cyanobacteria

Cyanobacteria (*Nodularia spumigena*) from Landsort Deep (*Paper I*) were collected in the autumn of 2005 *Aphanizomenon sp.* (Chapter 4) was sampled from Askö (3) during 2006.

### 3.1.3 Mussels

The blue mussels, presented in *Paper I*, were sampled from the background location, Kvädöfjärden (4), as well as, from two background locations along the west coast of Sweden, Fladen (7) and Väderöarna (8) by hand or by nets or using scapers. Blue mussels were also collected from Askö (*Paper III and Paper IV*), Kvädöfjärden, Arholma, and Abbekås (*Paper IV*) (Figure 3.1). Sampling was either done with a scraper dragged along the bottom behind a small boat, or collected by divers.

Baltic clams were sampled from the Askö area (3) (Chapter 4.2) using a scoop that was lowered down to the sea bottom to collect sediment also containing the Baltic clams. The sediment was removed by running water over a sieve and the mussels handpicked.

Each mussel locations and time points were considered as one sample. The samples were homogenised to reduce the effect of individual variations.

### 3.1.4 Fish

The perch and flounder, presented in *Paper I and II*, were sampled within the Swedish Environmental Monitoring Program on Contaminants in Biota (SEMPC). Perch and flounder were collected using gill nets from Kvädöfjärden (4) located close to Swedish Baltic coastline. Flounders were also collected from the west coast locations, Fladen (7) and Väderöarna (8). The perch were selected by age (2-years) and all samples were collected during late summer or autumn to ensure that the sampled individuals were well nourished and were not reproducing. Fish muscle from the middle dorsal muscle layer (without skin and subcutaneous fat) was used for analysis. Composite samples of >10 fishes were prepared in order to reduce the effect of individual variations. Perch, flounder and herring (*Clupea harengus*) were all collected from Askö (3) using gill nets (Chapter 4). Blood were sampled from herrings directly after the fish had been detangled from the gill nets. Blood from 21 herrings were drawn with a small syringe from the blood vessel at the backbone. The fishes were numbed before sampling and were immediately put to death by a crushing blow to the head.
afterwards. The appropriate permit for animal experiments was obtained (No: N 147/06 and N 170/09). Heparin was added to the blood samples and the plasma was separated from the blood cells.

3.1.5 Seal

Blood coagulate samples from 14 grey seals (Chapter 4) were collected by personnel from the Swedish Museum of Natural History upon autopsies of the seals. The samples were collected from seals that were found drowned in the Baltic proper, between 1995 and 2006.

3.1.6 Sediment

Sediment core samples were collected in 2005 (Paper II) using core samplers and were sectioned on board the sampling vessels. Top sediment was used in the study.

3.2 Extraction methods

Organic environmental contaminants of concern are primarily lipophilic and thus the extraction methods have been optimized for extraction of lipids and lipid soluble compounds. Historically, Soxhlet extraction [115,116] and batch extractions [117-119] were used. The liquid-liquid extraction method developed by Blight and Dyer employing a solvent mixture of methanol and chloroform is commonly used for lipid extraction. Jensen and co-workers developed a method of equal lipid extraction efficiency for fatty aquatic organisms without using halogenated solvents [119]. This method was later modified to give good lipid extraction also for lean matrices by substituting acetone for 2-propanol [120]. The improvement was probably due to better extraction of phospholipids. Further work lead to the most recent method that was optimized for extraction of phenolic analytes in fish and blue mussels [121], changing the ratio of n-hexane to diethyl ether from (9:1) to (3:1).

All biological samples within this thesis were extracted according to Jensen et al. [119] (Paper I and II) or Jensen et al. [121] (Paper III and IV), with some minor alterations. For example the diethyl ether was replaced by methyl-tert-butyl ether in Paper IV. In addition, the n-hexane was replaced with c-hexane in Paper III and IV to reduce the risk of the analytical procedure.

The phenolic compounds analysed are not strictly lipophilic. Relatively few methods have been optimized for simultaneous analysis of phenolic and neutral compounds in tissue samples [115,121-124]. Methods developed for extraction of phenolic and neutral compounds are e.g. liquid-liquid extraction [117,121,125] and pressurized liquid extraction (PLE) [123,124]. PLE, also
called accelerated solvent extraction (ASE), use conventional solvents under enhanced temperature and pressure. Anhydrous sodium sulfate or Hydromatrix are often used as dehydrating agents for extraction of tissue samples using PLE. When using anhydrous sodium sulfate low recovery for phenolic compounds such as OH-PCBs and OH-PBDEs were reported in some studies [123,124].

The liquid-liquid extractions used in Paper I, II and IV were not evaluated for dioxin analysis, in particular, within this thesis. However, since the dioxins are neutral lipophilic compounds and resembles the structures of e.g. PCBs and DDTs, it may be concluded that the same extraction methods can be used. The extraction method used was chosen to be the same for PBDD/F as for the OH-PBDE and MeO-PBDE analysis. Commonly, dioxin analyses of biotic samples are carried out by mixing the tissues with sodium sulfate for dehydration, placing the mixture in a glass column and extracting the analytes with hexane and dichloromethane [126] or by Soxhlet extraction [127]. Similar concentrations of PBDD/F in biota were reported in studies using the sodium sulphate method [80] as in the Papers presented herein (Paper I, II and IV).

The samples for PBDD/F analysis were extracted in parallel to the samples extracted for MeO-PBDEs and OH-PBDEs, instead of using the same samples. This has been done to ensure that the PBDDs found are not artefacts formed during the potassium hydroxide partitioning (see chapter 3.5.1).

3.3 Determination of extractable material and carbon content

Analytical data require some sort of normalisation to make comparison of data possible and correct. Usually, lipophilic compounds are presented on lipid weight (l.w.) or on wet weight (w.w.) basis. Lipid weight basis allows a better inter-species comparison than fresh weight, especially when comparing biomagnification. Wet weight and lipid weight have been determined gravimetrically in all Papers. Due to the samples composition it is difficult to compare matrices like cyanobacteria, algae, mussels and fish. These matrices contain very different amounts of water and lipids that make comparison on a wet weight basis particularly problematic. Further, the species analysed have very different lipid composition (Table 3.1), implying difficulties in doing the comparisons on a lipid basis, as well. The data in Paper II is presented on lipid weight basis, while Paper I, III and IV is normalized on extractable organic matter (EOM). EOM is the equivalent to lipid weight, but not only lipids are extracted from e.g. algae. EOM includes all compounds hydrophobic enough to be extracted with the solvents used, e.g. some pigments. Still, it needs to be pointed out that it is difficult to determine EOM gravimetrically in e.g. algal samples since the total weights are low. Therefore, the data presented in Paper IV were normalized in relation to the organic carbon (OC) content in the
samples. Carbon content was determined by measuring the oxidation of carbon to carbon dioxide under combustion of freeze dried samples, with elemental analysis. Finally, it seems that the results are in general comparable for all normalization methods used (Paper IV).

3.4 Lipid removal

Lipids were removed, in all samples (Paper I-IV), by treatment with concentrated sulfuric acid and silica gel columns treated with sulfuric acid. The sulfuric acid treatment is a destructive method and accordingly not suitable for all analytes, but PCBs, PBDEs, MeO-PBDEs and PBDDs/PBDFs are not affected, allowing sulfuric acid to be used for clean-up of these analytes. For analysis of analytes sensitive to sulfuric acid or more lipid containing matrices a non-destructive method such as gel permeation chromatography (GPC) [28,122,124] or acetonitrile partitioning [128] may be recommended. GPC is separating molecules on size, but the solvent used as well as the polarity and planarity of the analytes also affect the separations [129].

Acetonitrile partitioning is used for lipid reduction, by dissolving aromatic analytes like PCBs to a higher extent than lipids. The partitioning is explained by $\pi$-electrons interactions between the aromatic compounds and nitrile group in the acetonitrile. The more lipophilic the aromatic analytes are the less soluble they are in acetonitrile, thus to insure a good recovery of the most lipophilic analytes the partitioning is often repeated three times. Every treatment will solve approximately 10% of the total lipids, resulting in a 70% lipid reduction. However, the lipid composition will affect the effectiveness of the reduction.

The sulfuric acid clean-up procedure was originally developed for removal of fat in biological tissue containing e.g. triglycerides. Several of the samples included in the studies within this thesis have a slight different lipid composition compared to e.g. tissue from fish (Table 3.1). The presence of lipid soluble pigments (Chlorophyll) may affect the EOM determination. During the present work it became clear that the clean-up processes applied for some of the species/matrices were not sufficient, for example for certain algae samples. Problems were observed as drifts in retention time and sometimes as a broad “fat peak” in the chromatograms. The lipid composition varies slightly depending on e.g. species, season, feed, salinity, etcetera [130-136]. Thus, the examples of lipid compositions of a few analysed species in this thesis, presented in Table 3.1, are generalization over time and species. It is obvious that lipids will not behave in the same manner during extraction and clean-up, due to their differences in chemical structure (Figure 3.2). In the future,
improved clean-up and lipid removal methods are required to target the lipids within the matrices, in particular for plants like the algae samples.

Analysis of phenols (usually simple phenols) in algae samples have in many cases been based on extraction by polar solvents such as methanol or ethyl acetate [137,138]. These methods did not extract lipids to a major extent. Malmvärn tried to remove the algae matrix using GPC, and although it worked in principal, the matrix proved hard to elute resulting in re-conditioning difficulties and thus, the GPC is not a useful tool in algae clean-up [17].

![Figure 3.2. Chemical structures of some common lipids discussed within this thesis.](image)

Figure 3.2. Chemical structures of some common lipids discussed within this thesis.
Table 3.1. Examples of lipid content composition in cyanobacteria, brown and green algae, mussels, fish muscle and plasma from rat and fish.

<table>
<thead>
<tr>
<th></th>
<th>Cyanobacteria</th>
<th>Brown Algae</th>
<th>Green Algae</th>
<th>Mussels</th>
<th>Fish muscle</th>
<th>Rat</th>
<th>Plasma</th>
<th>Fish</th>
</tr>
</thead>
<tbody>
<tr>
<td>References:</td>
<td>[131,139,140]</td>
<td>[130]</td>
<td>[141]</td>
<td>[132]</td>
<td>[134,135,142]</td>
<td>[136]</td>
<td>[143]</td>
<td></td>
</tr>
<tr>
<td>Free fatty acids</td>
<td>+</td>
<td>V</td>
<td>III</td>
<td>IV</td>
<td>n.a.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triacylglycerols</td>
<td></td>
<td>III</td>
<td>II</td>
<td>II</td>
<td>I</td>
<td>I</td>
<td>II</td>
<td></td>
</tr>
<tr>
<td>Waxes</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pigments&lt;sup&gt;a&lt;/sup&gt;</td>
<td>II</td>
<td>n.a.</td>
<td>I (+)</td>
<td></td>
<td></td>
<td></td>
<td>n.a.</td>
<td></td>
</tr>
<tr>
<td>Phospholipids</td>
<td>III</td>
<td>II</td>
<td>IV</td>
<td>I</td>
<td>II</td>
<td>IV</td>
<td>I</td>
<td></td>
</tr>
<tr>
<td>Glycolipids</td>
<td>I</td>
<td>I</td>
<td>III</td>
<td></td>
<td>II</td>
<td></td>
<td>n.a.</td>
<td></td>
</tr>
<tr>
<td>Sterols</td>
<td>+</td>
<td>IV</td>
<td>V</td>
<td>IV</td>
<td>III</td>
<td>III</td>
<td>III</td>
<td></td>
</tr>
</tbody>
</table>

The relative composition is given as roman numbers corresponding to the relative amounts found of each lipid group, I being in highest abundance. A repeated number describes an equal contribution and "+" indicates presence. Only lipids present in >1% of the total lipid content are presented.

<sup>a</sup> including: carotene and chlorophyll
3.5 Separation of substance groups

Separation of substance groups is done to ensure minimum co-elution of individual compounds upon instrumental analysis. It also facilitates the possibility of treating different substance groups with the appropriate clean-up procedure. A schematic description of the methods used (Paper I-IV) is shown in Figure 3.3.

Figure 3.3. Scheme for sample clean-up for analysis of OH-PBDEs and MeO-PBDEs (left) and PBDDs (right).

3.5.1 Separation of neutral and phenolic compounds

Neutral and phenolic compound are separated using potassium hydroxide partitioning [144] (Paper I-IV). The phenolic compounds are re-extracted from the potassium hydroxide phase after acidification. It has been shown that the partitioning of phenols was not complete [121], and thus the potassium hydroxide partitioning was repeated twice in Paper III and IV. The potassium hydroxide may theoretically promote ring closure of ortho-OH-PBDEs forming PBDDs. This is only possible when there is a bromine substituent in the non-hydroxylated ring, according to predioxin reactions, as reported for the chlorinated counterparts by Jensen et al. [145]. A separate extraction was thus carried out for PBDD/F analysis to minimize the risk of artefacts.
Separations of neutral and phenolic compounds can also be achieved on Florisil® columns [122], silica gel SPE cartridges [124] or silica gel columns with acidified mobile phases [121].

3.5.1 Separation of non-planar and planar compounds

To enable clean extracts during dioxin analysis, co-planar compounds are separated from non-planar. Separation can either be achieved by a 2-(1-pyrenyl)-ethyldimethylsilylated silica column [146, 147] or as utilized in Paper I, II and IV, by using a column with activated charcoal mixed with celite. The non-planar compounds were washed from the charcoal column and the planar compounds were recovered through back-flush of the column. The planar compounds were cleaned-up using a silica gel column impregnated with sulfuric acid. No clean-up was done of the non-planar fractions (Figure 3.3).

3.6 Derivatisation

Phenolic compounds were derivatised to ensure improved gas chromatographic (GC) behaviour at analysis (Paper I, III and IV). Untreated phenolic compounds will substantially interact with the stationary phase in the GC column, leading to misshaped and wide peaks or in worst case no visible peaks at all. The derivatisation was achieved through methylation using diazomethane, synthesised in house from N-methyl-N-nitroso-p-toluene sulfonamide [148] and dissolved in diethyl ether. Diazomethane is carcinogenic and explosive and thus laboratory work requires a permit. The use of diazomethane has been approved by the Swedish work environment authority. The derivatisation of the commonly found natural OH-PBDEs using standards were investigated and found to be almost complete (unpublished data).

Methylation is a common way of derivatisation leading to the formation of stable methyl ethers. Other groups have used methyl iodine [149] or methyl chloroformate [122], finding these as adequate, and even indicate to give a better recovery than diazomethane. Methoxy-derivates are very stable and easy to analyse and tolerate destructive clean-up methods (e.g. concentrated sulfuric acid). In contrast, acetylation e.g. pentafluorobenzoyl chloride (PFBCl) [121] and silylation [150] are unstable and instrumental analyses have to be carried out fast. In addition, these derivatisation agents increase the molecular weight of the analytes to a larger extent than methylation, prolonging the retention time and also complicating identification for heavier analytes by approaching the maximum mass range of the low resolution mass spectrometer (LRMS) instruments in use (m/z <1000).
3.7 Instrumental analysis

Most analyses were carried out by gas chromatography/mass spectrometry (GC/MS). PBDDs and PBDFs were analysed using electron ionization (EI) and high resolution mass spectrometry (HRMS; R >10000). MeO-PBDEs were analysed using MS in the electron capture negative ion chemical ionization (ECNI) mode. All quantifications were done in ECNI mode employing selected ion monitoring (SIM), scanning for the bromine ions m/z 79 and 81. Thus, the method is not compound selective and identification depends solely on the retention time and may therefore result in identification problems with possible co-elutions. However, the retention time, elution order and the mass spectra of MeO-PBDEs have been thoroughly investigated [21,151]. In addition, ECNI fullscan was employed on mussel samples in Paper I (unpublished) to ensure the correct identifications.

3.8 Quality Assurance/Quality Control

Solvent blank samples were analysed in parallel to the samples. In Paper I, III and IV small amounts of PBDEs were detected in the blank samples and were probably associated with PBDE in laboratory dust. When blank contamination was an issue, the sample concentrations were adjusted for the blank values as in Paper I. In Paper III and IV the obvious contamination was not deducted from the samples but affect the limit of quantification (LOQ) (See below within this paragraph).

Surrogate standards were used to control the recovery. Generally the recoveries of the analyses were high for the biological tissues analysed (e.g. blue mussels and fish) in this thesis work. Lately however, problems with low recovery of phenolic compounds in some matrices occurred. In blood samples from fish and seals the recoveries were low, a problem that was not observed when the same extraction method was used for human blood samples. The recoveries of the surrogate standards were 28 ± 20% for 4-OH-DE121 in herring plasma [152], chapter 4.4) and 15 ± 14 % and 7 ± 9 % for 4-OH-BDE121 and 2’-OH-BDE28 in seal blood coagulate (Chapter 4.4). The recoveries of the neutral surrogate standards were satisfactory in both in the seal blood and the herring plasma. The recoveries were; 4-MeO-BDE121 (78 ± 4 % and 77 ± 12 %) and BDE138 or BDE77 (77 ± 12 % and 89 ± 16 %), respectively. Hence there was a difference between the slightly acidic OH-PBDEs and the neutral compounds.

A laboratory reference material, consisting of a composite sample of blue mussel tissue from the west coast of Sweden bought in a supermarket, was used for Paper IV to ensure precision in the analysis.
Limit of detection (LOD) and LOQ are important in trace analysis. In general, the LOD is not critical for the OH-PBDEs and the MeO-PBDEs in Baltic biota, since the levels of these compounds are so high. The LOD was defined as the quantity giving rise to a signal with a signal-to-noise (S/N) ratio of 3. The LOQ for phenols and anisoles was defined as a signal 10-fold greater than the standard deviation of the S/N ratio. If the blank samples were contaminated, the LOQ was defined as 3-fold greater than the background signal. For the PBDD analyses LOQ was set equal to the LOD, since there were no interferences and a high signal quality.
4. Additional results

In this chapter some unpublished data are presented to complete the picture of the research done within this thesis. Hence OH-PBDE, MeO-PBDE and PBDD concentrations as determined in flounder from the east and west coast of Sweden are presented below. Further, levels of the PBDDs are presented in food webs from the Baltic Sea. Analytical data of OH-PBDE, MeO-PBDE and PBDD in herring and seal blood from the Baltic Sea are presented as well as in material from the south-western Pacific Ocean around New Zealand.

4.1 Flounders

Flounders from Kvädöfjärden in the Baltic proper and west coast of Sweden, Fladen and Väderöarna were analysed for MeO-PBDEs and PBDDs. The analyses were done according to the methods described in Paper I.

The flounders from Baltic Sea have approximately ten times higher concentrations of $\sum$MeO-PBDEs (59 ng/g EOM) than in flounders from Fladen (0.59 ng/g EOM) and Väderöarna (0.54 ng/g EOM), respectively. The MeO-PBDE congener composition presented in Figure 4.1 is similar for flounders in Kvädöfjärden and Fladen, but the flounders from Väderöarna have a higher contribution of 2’-MeO-BDE68. The $\sum$PBDDs concentration in flounders from Kvädöfjärden was 0.025 ng/g EOM, while no PBDDs were detected in flounders from the west coast.

![Figure 4.1. MeO-PBDE congener specific contributions in flounders from the Baltic Sea (Kvädöfjärden) and from the west coast of Sweden (Fladen and Väderöarna).](image-url)
Figure 4.2. PBDDs concentrations in species sampled from the Askö area during 2006 and 2007. The concentrations are presented in ng/g EOM (top), ng/g carbon content (middle) and ng/g d.w. (bottom).
4.2 PBDD concentration in Askö samples taken at different trophic levels

The presences of PBDDs were studied in the cyanobacteria (*Aphanizomenon flos-aquae*), baltic clam, blue mussels, flounder and perch from Askö. The results are, presented in Figure 4.2. It is concluded that PBDDs are present in all species, with highest concentration in the cyanobacteria. The results are presented on extractable organic matter, carbon content and dry weight to make comparisons as good as possible. Irrespective of the manner of normalization, the concentrations decrease with increasing trophic level.

4.3 Samples from New Zealand

Australian marine waters have high levels of NOCs in e.g. algae [55-57]. Thus, it is likely that the New Zealand waters also have a high potential for natural production. This was investigated in a food web study, including biota from the south-west Pacific Ocean at New Zealand. The study was conducted as a comparative study to the Baltic Sea location.

The food web study from New Zealand includes analysis of fish muscle and liver tissue and filter feeders, i.e. diloma, green lip mussels and oysters. The samples were freeze dried and ASE extracted with dichloromethane. The sampling and extraction was done by the National Institute of Water and Atmospheric Research in New Zealand before shipping the samples to Sweden. On arrival, surrogate standards were added and the samples were partitioned with aqueous potassium hydroxide. Further clean-up and analysis of OH-PBDEs and MeO-PBDEs, PBDD was done as described in Chapter 3.

The results of OH-PBDE, MeO-PBDE and PBDD levels are presented in Figure 4.3. The concentrations of OH-PBDEs are similar in fish liver and in mussels and oysters. The OH-PBDE conger patterns are however very different with 6-OH-BDE47 and 2’-OH-BDE68 dominating in the fish liver, while the filter feeders contain penta- and hexabrominated OH-PBDEs as well. The MeO-PBDEs are present at similar levels and similar patterns in the fish, mussels and oysters. The PBDD concentrations are much higher in the filter feeding species than in the fish. The pattern though, is similar in fish compared to the mussel and oyster samples. The diloma samples differ from the others for all the three substance groups. It has very high levels of OH-PBDEs compared to the other species and a different congener pattern of MeO-PBDEs and PBDDs compared to the other samples.
Figure 4.3. OH-PBDEs (top), MeO-PBDEs (middle) and PBDDs (bottom) patterns and levels in ng/g d.w. (± standard deviation) in New Zealand food web samples. Note the different scales on the y-axis.
4.4 Herring and seal blood concentrations

Individual blood plasma samples from 21 herring from the Askö area from 2007 were analysed. The most abundant brominated compounds within each substance group are shown in Figure 4.4. The detected OH-PBDEs and MeO-PBDEs congeners are presented in Table 4.1. Figure 4.4 show high concentrations of OH-PBDEs compared to neutral substances like PBDE, MeO-PBDE and PCB. However, since the recovery of the surrogate standard in the herring blood was low (chapter 3.8), it may be misleading with recovery corrected data. The mean concentration of 6-OH-BDE47 is 320 ng/g l.w. when no consideration to recovery is made. The concentration of this OH-PBDE is however still as high as commonly found in e.g. blue mussels from the Baltic Sea.

Twelve individual seal blood coagulate samples, collected from Baltic Seal grey seals between 1995 and 2006, were also analysed. Phenolic compounds and PCBs dominate the seal blood as depicted in Figure 4.5. A larger number of OH-PBDE and MeO-PBDE congeners were detected in seal blood compared to herring blood (Table 4.1).

<table>
<thead>
<tr>
<th>OH-PBDEs</th>
<th>MeO-PBDE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herring**</td>
<td>Seal**</td>
</tr>
<tr>
<td>Herring*</td>
<td>Seal**</td>
</tr>
<tr>
<td>6-OH-BDE47</td>
<td>6-OH-BDE47</td>
</tr>
<tr>
<td>2'-OH-BDE68</td>
<td>2'-OH-BDE68</td>
</tr>
<tr>
<td>6-OH-BDE85</td>
<td>6-MeO-BDE85</td>
</tr>
<tr>
<td>6-OH-BDE90</td>
<td>6-MeO-BDE90</td>
</tr>
<tr>
<td>6-OH-BDE99</td>
<td>6-OH-BDE99</td>
</tr>
<tr>
<td>2-OH-BDE123</td>
<td>2-MeO-BDE123</td>
</tr>
<tr>
<td>6-OH-BDE137</td>
<td>6-MeO-BDE137</td>
</tr>
</tbody>
</table>

* Herring samples from Askö 2007, ** seal samples from the Baltic proper, 1995-2006
Figure 4.4. Concentrations (ng/g l.w.) of 2,4,6-triBP, 6-OH-BDE47, 6-MeO-BDE47, BDE-47 and CB-153 in herring plasma.

Figure 4.5. Concentrations (ng/g l.w.) of 2,4,6-triBP, 6-OH-BDE47, 6-MeO-BDE47, BDE-47 and CB-153 in grey seal blood coagulates.
5. Discussion

5.1 Data normalization

Concentrations of POPs in biota are usually presented on wet weight (w.w.) [31,153] or lipid weight (l.w.) [62,154] basis, while POPs concentrations in sediment are presented on dry weight basis (d.w.) and/or extractable organic carbon (OC) [155,156]. The OC is considered a good normalization for sediment since hydrophobic pollutants adsorbs to the organic carbon [156]. Bierman suggested that the OC corrected data in sediment corresponds to the lipid weight in animals [157]. Organic carbon has been indicated to be the optimal data normalization in cyanobacteria [158]. In the algae presented in Paper IV an evaluation of the data normalized on wet weight, extractable organic matter (EOM), dry weight and extractable organic carbon content was conducted. Generally, the data was comparable on EOM, d.w. and OC basis. However, the alga species have different cell structures compared to each other and, thus there are some differences to be noted. For example, Furcellaria and Fucus have a more robust and hard cell structure than the other species analysed, i.e. Ceramium, Cladophora, Pilayella, Polysiphonia and Enteromorpha. Furcellaria and Fucus contain much less water and EOM but have a higher d.w. and OC content. This will affect the inter-species comparison. In addition, the cell structure of Furcellaria and Fucus may be better at adsorption of OHCs. In conclusion, I have chosen to present the algae species comparisons on a OC basis.

5.2 Trends

5.2.1 Temporal variations

In Paper II the time trends of MeO-PBDEs and PBDDs are investigated in perch from the Baltic proper (Kvädöfjärden). The temporal variations do not indicate any clear trends, instead the levels fluctuate from year to year (Figure 5.1). The fluctuations in concentration over time may possibly relate to the primary production, i.e. with phytoplankton and algae, and thus indirectly with temperature. A weak, but not statistically significant, correlation was indicated between the levels of MeO-PBDEs and PBDDs and the water temperature, depth visibility and inorganic nutrient concentrations (Paper II). This may imply a correlation with primary production.
5.2.2 Seasonal variations

Variation of OH-PBDEs, MeO-PBDEs and PBDDs are also seen during the summer season. The variations in concentrations of OH-PBDEs and MeO-PBDEs and the indication of seasonal variation of triBDDs, presented in *Paper III*, show an increasing concentration of the OH-PBDEs, MeO-PBDEs and PBDDs from May to June and a decrease in their concentration in August (Figure 5.2).

![Figure 5.1. Temporal variations of concentrations (ng/g EOM) of ∑PBDDs (top) and ∑MeO-PBDEs (bottom) in perch from Kvådöfjärden, sampled in the years 1990 to 2005 (Paper II).](image)

![Figure 5.2. Seasonal variation of ∑OH-PBDEs, ∑MeO-PBDEs and 1,3,7-/1,3,8-triBDD in blue mussels sampled in 2008 from the Baltic proper (Askö). Note the different y-scales for phenolic (left) and neutral (right) compounds (Paper III).](image)
The seasonal variation indicated for 1,3,7-triBDD/1,3,8-triBDD in the blue mussels (Paper III) has been validated by GC-HRMS analysis (Figure 5.3). The estimated concentrations of triBDD as presented in Paper III were carried out by GC-LRMS and were pseudo quantified against the 2’-MeO-BDE68. The estimated level of triBDD was 230 ng/g EOM in the mussels sampled in June. The corresponding levels determined by GC-HRMS (Figure 5.3) were 120 ng/g EOM and 250 ng/g EOM for 1,3,7-triBDD and 1,3,8-triBDD respectively, resulting in a total concentration of 370 ng/g EOM. It is notable that the quantifications by GC-LRMS and GC-HRMS are highly comparable.

All the PBDDs show a seasonal variation and the extent of the variation increases with the number of bromines in the molecule (monoBDDs-tetraBDDs). PBDFs were also found in the samples but in considerably lower concentrations than the PBDDs (Figure 5.3). A seasonal variation was also observed for the PBDFs particularly for the triBDFs and to a lesser extent (a slight increase) for the tetraBDFs. No variation was observed for the monoBDFs.

![Figure 5.3.](image)

**Figure 5.3.** The seasonal variation in concentration (ng/g l.w.) of PBDFs (left) and PBDDs (right) in blue mussels from Askö sampled in 2008. The total concentrations are given in the graph. Note the different scales in the two graphs.

The seasonal variations of OH-PBDEs, MeO-PBDEs and PBDDs are based on few data points as a result of bad weather during the sampling season. It is thus not possible to establish whether the highest concentrations have been found.

A seasonal variation of OH-PBDEs, MeO-PBDEs were also found in algae simultaneously sampled with the mussel samples at Askö (Paper IV). Indications of elevated levels of PBDDs in June were also observed. The seasonal variation observed for both red (Ceramium tenuicorne) and green
(Cladophora glomerata) macroalgae (Paper IV) are shown in Figure 5.4 and follow the same seasonal variations as presented for the blue mussels (Paper III). The levels correlate well between the algae and mussels (Paper IV).

Figure 5.4. Seasonal variation in concentrations (ng/g OC) of ΣOH-PBDEs (top) and ΣMeO-PBDEs (bottom) in macroalgae from Askö (Paper IV). The species analysed are specified under the bars.
Both the temporal and seasonal trend studies indicate that the production of OH-PBDEs, MeO-PBDEs and PBDDs vary. The production of these substances seems to be highly correlated with temperature and thus the life-cycles of some algae and/or cyanobacteria. The peak in the seasonal variation, together with the concentrations found in the algae (Paper IV) seem to be mostly related to the growth season of some green, red and brown algae [159]. This is indicating *Pilayella, Ceramium* and/or *Cladophora* as the major producers of OH-PBDEs and PBDDs. The large cyanobacteria blooms found in the Baltic proper are usually most abundant in July and August [7].

5.2.3 Geographical distribution

Higher levels of OH-PBDEs, MeO-PBDEs and PBDDs are found in Baltic Sea biota compared to biota from Swedish west coast waters (Paper I and IV, Chapter 4.1), i.e. in algae, blue mussels and flounder. The levels of OH-PBDEs, MeO-PBDEs and PBDDs in blue mussels presented in Paper I are much higher in the Baltic proper compared to the west coast of Sweden (Figure 5.5). The difference in concentration between the Baltic proper and the west coast is far greater than the small variations that can be seen for PCBs and PBDEs in blue mussels from the same locations [160]. The levels of OH-PBDEs, MeO-PBDEs and PBDDs may reflect the difference in algae or cyanobacteria abundance or species composition in the two coastal waters. The abundance of red filamentous macroalgae and cyanobacteria is higher in the Baltic proper [6,7,161] as a result of eutrophication. It may also be a result of a higher production of natural compounds in the Baltic. Paper IV shows the presence of OH-PBDEs, MeO-PBDEs and PBDDs in all species with no obvious differences between red, brown and green algae (autumn samples). In addition, these compounds were observed to have lower concentration at the west coast than the east coast in all species sampled, i.e. *Cladophora, Polysiphonia* and *Ceramium* (Paper IV). Thus, the difference in concentrations of OH-PBDEs, MeO-PBDEs and PBDDs found in the mussels (Paper I) and algae (Paper IV) between the east and west coast of Sweden is more likely related to a higher production of NOCs in the Baltic Sea.

The comparison of the levels of OH-PBDEs, MeO-PBDEs and PBDDs in the Baltic Sea to the worldwide geographic distribution is hard to assess. Very few studies reports data of OH-PBDEs, MeO-PBDEs and PBDDs in the same sample (Paper I, Paper IV, [20]). There are also very few studies of these compounds in low trophic level organisms [59,60,79,87].
OH-PBDEs have been analysed in mussels from Hudson bay, but were not detected [60]. OH-PBDEs have also been reported in fish blood from the Detroit river, showing concentration in the low pg/g w.w. [30]. The concentrations of OH-PBDEs in herring plasma from the Baltic Sea (chapter 4.4.) are approximately two orders of magnitude higher than the reported concentration from Detroit River. Routti et al. report $\sum$OH-PBDE in ringed seal blood from the Baltic Sea and Svalbard with a factor 2.5 higher concentrations in the Baltic [31]. The concentrations in the ringed seal from the Baltic [31] were three times lower than detected in the grey seal blood in this thesis (chapter 4.4).

Similar or lower levels of MeO-PBDEs than in the Baltic Sea have been reported, at comparable trophic levels elsewhere, e.g. in mussels from Hudson bay and Liaodong bay (China) and in fish from Liaodong bay [59,60].

Haglund and co-workers have reported PBDDs in bivalve and fish from the Baltic Sea and the west coast of Sweden, generally showing higher PBDD concentrations in the Baltic [79].

The high levels of PBDDs found in filter feeders from New Zealand (Chapter 4.3) are much higher than what is found in blue mussels from the Baltic region.
(Paper I). The levels of OH-PBDEs and MeO-PBDEs in the New Zealand mussel samples (Figure 4.3) are similar, while the blue mussels from the Baltic Sea are dominated by the MeO-PBDEs (Figure 5.5). The data in Chapter 4.3 together with the literature cited indicates that the world-wide distribution of these compounds varies. The differences in composition probably reflect differences in producers between areas. Far more research efforts need to be done in this area, not the least to expand the assessments to all oceans on the globe.

5.3 Food web distribution

Brown algae (Fucus vesiculosus and Dictyosiphon foenicolaceus) (Paper I, IV), blue mussels (Paper I), flounder (muscle) (Chapter 4.1) and perch (muscle) (Paper II) from Kvädöfjärden in the Baltic Sea were compared for their content of OH-PBDEs, MeO-PBDEs and PBDDs. The analyses were done according to the methods described in Paper I and IV. The results of this interspecies comparison are depicted in Figure 5.6. The levels of OH-PBDEs are highest in algae, followed by the filter feeding blue mussel. The pattern is reversed for the MeO-PBDEs and PBDDs, showing bioaccumulation to the filter feeding blue mussel. The fish species however, contain low concentrations of both MeO-PBDEs and PBDDs. The presence of PBDDs were further studied in the cyanobacteria, Aphanizomenon flos-aquae, baltic clam, blue mussels, flounder and perch from Askö, presented in Figure 4.2. The study showed presence of PBDDs in all species, with highest concentration in the cyanobacteria. Both comparisons conclude that although OH-PBDEs, MeO-PBDEs and PBDDs are bioaccumulative, but they do not seem to biomagnify. The rapid decrease in concentrations of the analytes found in blue mussels (Paper III and Figure 5.3) supports the limited retention of these compounds.

Also, Paper II shows variations in retention of PBDDs and MeO-PBDEs in perch, possibly partially explained by the metabolic stability or discriminations in uptake of higher brominated congeners.

The bioaccumulation of MeO-PBDEs have been studied in e.g. blue fin tuna, harbour seals, harbour porpoises, ringed seal and polar bears [32,63,64] and a few studies describe the biomagnification potential [32,64,162]. The trophic magnification factor (TMF) in a marine food web study from Australia indicated biomagnification, but the TMF was lower for MeO-PBDEs than for PBDEs [162]. Weijs et al. found the biomagnification factor (BMF) for 2′-MeO-BDE 68 and 6-MeO-BDE 47 to vary between 0.1 and 5 and 0.1 and 23, respectively [64]. Letcher and co-workers reported bioaccumulation in polar bears, but no biomagnification from ringed seal [32].
Figure 5.6. MeO-PBDEs (top), OH-PBDEs (middle) and PBDDs (bottom) patterns and levels in ng/g EOM (± Standard deviation) in biota from Kvädöfjärden. Note the different scales on the y-axis.
Halogenated phenolic compounds are primarily associated with wildlife and human blood and not with muscle or lipid tissue. A comparison is made, and shown in Figure 5.7, between algae, blue mussels, herring plasma and seal blood (Chapter 4.4) from the Baltic proper. All species are sampled in the waters around Askö, except for the grey seals. The herring plasma is shown both as recovery corrected data and as non-recovery corrected data. The retention of OH-PBDEs in herring and seal blood is notably high. The levels of 6-OH-BDE47 are much higher in the blood compare to the algae (*Ceramium tenuicorne*) and mussel sample. However, when comparing the total OH-PBDE concentration the levels are similar. This is due to a different congener pattern in the algae samples. In the herring and seal blood, 6-OH-BDE47 is the dominant congener, while the algae contain several congeners and mostly 6-OH-BDE137. It should be noted that the algae sample was not taken the same year as the mussel and herring blood. Also, the seal blood was taken from seals, sampled 1995-2006, individuals collected from different locations in the Baltic proper.

![Figure 5.7. Comparison of OH-PBDEs (ng/g EOM ± Stddev) in four Baltic Sea species, sampled around Askö. Note that the time of sampling differs.](image)

### 5.4. Exposure and uptake

The route of exposure of OHCs for the mussels, fish and seal presented herein are direct uptake from the water via the gills, or by their diet. The difference observed in congener pattern in e.g. the herring blood compared to mussels and seal blood may indicate their route of exposure via the gills. Considering the pKₐ values (Table 2.2) at least 6-OH-BDE137 and 6-OH-BDE99 are predicted to be ionised at natural pH, and thus not likely to be taken up via the gills.
For the MeO-PBDEs, exposure via diet is more likely. In the perch (Paper II), two of the congeners were not detected; 2-MeO-BDE123 and 6-MeO-BDE137. This may possibly be explained by debromination, as seen both in vitro and in vivo for the PBDEs in fish [163-166]. Indications of debromination processes were also found in the seasonal variation study (Paper III). The higher brominated MeO-PBDE congeners have a more rapid decline according to this study, while the lower brominated, i.e. 6-MeO-BDE47 and 2’-MeO-BDE68, are stable in their concentrations and do not decrease from June and onwards over the sampling period. This may possibly be explained by debromination of hexa- and pentabrominated methoxylated diphenylethers leading to e.g. 6-MeO-BDE47 and 2’-MeO-BDE68.

5.5 Origin

It is evident, based on the high levels of OH-PBDEs, MeO-PBDEs and PBDDs found in the Baltic Sea biota, that these compounds are preferentially natural products. As presented herein (Paper I, III and IV), the levels of OH-PBDEs and MeO-PBDEs, in the Baltic proper biota, far exceed that of the possible metabolic or abiotic transformation precursors, PBDEs (Paper III and IV) [160]. The variation in concentration of these substances (Paper II, III and IV) gives further support for natural formation thereof. The difference in PBDF and PBDD concentrations (Paper I and IV and Figure 5.3) also support natural formation of PBDDs.

The possible producers suggested within this thesis and by Malmärn [17] are firsthand filamentous macroalgae and/or cyanobacteria. The high levels of OH-PBDEs and PBDDs in both algae (Paper I and IV, [20,21]) and in cyanobacteria (Paper I, [20]) from the Baltic Sea support the production of these compounds. As discussed above the Pilayella, Ceramium and/or Cladophora (chapter 5.2.2) seem to be the most likely producers of OH-PBDEs and PBDDs.

The producers of MeO-PBDEs however, are not as easily deduced. Although the pattern is similar in algae and mussels (Paper IV), the levels found in algae and cyanobacteria are fairly low. One possible explanation may be that the MeO-PBDEs are methylated by bacteria outside the algae itself, as described for other phenolic compounds [49-54]. It would thus be of interest to study algae free from microorganisms.

No single species of algae has been determined as a major producer of the compounds discussed herein. The difference in concentration within the same species from the same locations again indicates that the life-cycle is important.
Also, studies have shown that algae under stress produce higher levels of other brominated compounds such as PBPs [167-169]. The stress may for example be a result of grazing or ecological changes leading to reduction or increase in sunlight [170], e.g. by change in water level.

5.6 Ecological perspective

Several worrying effects are reported for Baltic Sea biota, e.g. the large-scale changes in biodiversity [2], decreases in body weight and/or blubber thickness in seal [112] and Baltic herring [171], a high mortality in fish eggs [172], as well as a massive bird death attributed to a neurological disease like thiamine deficiency [173] and the thiamine deficiency in salmon called M74 [174], where the salmon fry only live a few days. In addition, the constant chemical exposure of both anthropogenic and natural compounds may add to the already stressed ecosystem.

Although the substances discussed within this thesis do not seem to biomagnify, the levels may still reach high levels e.g. during summer time. Animals feeding on mussels or algae may be highly exposed to chemicals with toxic effects i.e. the OH-PBDEs and the PBDDs during these periods. Eider duck, long-tailed duck and flounder largely feed on blue mussels in the Baltic Sea. Their daily exposure may be considerable resulting in potential ecotoxicological effects.
6. Future perspectives

The high levels of the polybrominated chemicals discussed in this thesis seem to be linked to primary producers, such as algae and cyanobacteria, in the Baltic Sea. Further, the occurrence and levels of these brominated chemicals may be affected by Eutrophication. Accordingly it would be of interest to look closer into other marine and freshwater ecosystems with a similar degree of eutrophication as the Baltic Sea, or worse. Such environments may be e.g. the Black Sea, the North Sea and Wadden Sea, Chesapeake Bay and the northern Gulf of Mexico and Taihu Lake, close to Shanghai.

PBDD concentrations are presented in this thesis for two cyanobacteria species, *Aphanisomenon* and *Nodularia*. The PBDD levels in the *Aphanisomenon* were high, while the *Nodularia* was substantially lower. It is obviously impossible to determine if this is a result of the different species, sampling location or time of sampling. Studies including analysis of several samples of the two species, sampled in the proximity of each other, will throw further light on formation and sources of the PBDDs. In addition, there are still a few species of primary producers that has yet to be analysed, e.g. diatoms and dinoflagellates. Further research is required to lay out a more complete picture of PBDD, as well as OH-PBDE and MeO-PBDE producers.

To enable the determination of algal producers and the route of exposure, water samples must most likely be studied, preferably from locations close to algae growth. Possibly this can be done under laboratory conditions. Also, studies correlating production of natural OHCs and stressors like grazing is required.

Lastly, there is need of (eco)toxicity studies of foremost OH-PBDEs and PBDDs. If possible correlations between observed effects in Baltic wildlife and exposure levels to these compounds should be prioritized, still not omitting the potential impact anthropogenic chemicals may have.

This thesis is stressing the fact that natural halogenated products play a role in the Baltic Sea ecosystems.
7. Acknowledgements


Tack till mina medförfattare, Peter, Dennis, Sören, Lena, Anders och Anna M för allt kunnande och fantastisk hjälp, and Xitao - thank you for your kind contributions.

Anita – du har varit en klippa när det gäller allt krängligt som ekonomi och blanketter och har alltid tid att byta ett par ord.

Hrönn, examensarbetshandledare, rumskompis och vän – jag saknar dig och fåglarna fortfarande. Linda, du är en aldeles speciell vän och reskmrat, jag kommer saknar dig, Anna S, we’ll always have Canada, Johan F, tack för att du alltid ställer upp vare sig det är på lab eller med ett skratt, Jessica, bästa rumskompisen både borta och hemma. Emelie, tack för alla pratstunder, allt godis och uppmuntran, Ioannis, vår underbara GC-guru vad du har fått slita. Anna-Karin, du har ett fantastiskt humör och sätt – lycka till. Maria A, jag saknar dig, Anna V och Hans, de må ha varit kortvarigt men gött, Hitesh, your next. Lotta, Per, Margareta, Birgit, Maria S, Lisa, Andreas, Göran, Johan E tack för alla trevliga stunder och till de ”nya” ansiktena; Cecilia och Dennis lycka till. Yin, thank you for your good work and good luck with your PhD. Ett stort tack även till alla gamla miljökemister, ingen nämnd ingen glömd, för att ni gjort detta till en helt fantastisk arbetsmiljö.

Jag är lyckligt lottad att ha så bra vänner som stått vid min sida i vått och torrt under alla år. Ni har alltid funnits där även när tiden trott, Jenny, bästaste vänner 4-ever, Anna O, tack för alla tokigheter vi har gjort, Sara, underbara, Lina, du som förstår, och alla ni andra som gjort dessa år lättare och så njutbara, Linda, Jocke, Andreas, Susanne, Brian, Kajs, Sandra, Helene, m.fl.

Mamma och pappa, tack för allt stöd genom åren, inte minst nu under dessa konstiga månader. Ni är underbara.

Stefan, min kärlek till dig kan inte vara större. Tack för att du stått ut med mig under dessa år med övertid och stress och alltid mött mig med ett leende och mat på bordet. Ser fram emot att kunna återgälda allt. Nu väntar andra äventyr...

This thesis was financially supported by the Swedish environmental protection agency through the Swedish environmental monitoring program on contaminants, and by the Swedish research council FORMAS. Financial support was also received from the Stockholm University's strategic marine environmental research funds through the Baltic ecosystem adaptive management (BEAM) program and from Ångpanneföreningen (ÅF). A grant from the Stockholm University marine research center (SMF) have been received for sampling at Askö.
8. References


metabolites of BDE-47 affects their potency to release calcium from intracellular stores in PC12 cells. *Environmental Health Perspectives*, 118, 519-525.


123. Lacorte S., Ikonomou M.G., Fischer M. (2010). A comprehensive gas chromatography coupled to high resolution mass spectrometry based method for the determination of polybrominated diphenyl ethers and their hydroxylated


Wloclawski reservoir (central Poland). *Archives of Polish fisheries*, 16, 213-220.


