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Marine Halogenated Natural Products of Environmental Relevance

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I. Introduction

Polyhalogenated compounds have been used for industrial and agricultural applications for some 50 years. Variations in the degree of halogenation can change their properties in almost any desired direction, so that their application fields were diverse and production rates were high. However, the other side of the coin provided evidence that the polyhalogenated xenobiotics are serious environmental contaminants. Their detection in the environment along with the linking of their presence to adverse affects observed in the living environment was an important step toward the recognition that there is a thorough need of environmental protection.

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The primary class of contaminants were DDT and its metabolites, as well as polychlorinated biphenyls (PCBs). These "classic" contaminants had a fellowship of related compounds including chloropesticides (HCH/lindane, chlordane, toxaphene, endrin, and related cyclodienes) and industrial chemicals, polychloronapthalenes (PCNs). Their toxic effects and environmental behavior led to their classification as persistent organic pollutants (POPs) and persistent bioaccumulative and toxic chemicals (PBTs). Some PBTs including most just mentioned were ranked as the "dirty dozen" whose production and use have been forbidden in a worldwide act following the Stockholm convention on POPs.

However, new environmental contaminants emerged in recent years including brominated flame retardants [polybrominated diphenyl ethers (PBDEs), polybrominated biphenyls (PBBs), hexabromocyclododecane (HBCD)], polychlorinated paraffins, polychlorinated diphenyl ethers, and the list could be continued. In appears that once a compound or compound class was forbidden or at least "in the news," a substitute with similar properties, good and bad, was created. Examples are polychlorinated terphenyls, which were substitutes for PCBs (without getting the same attention as PCBs), toxaphene, which emerged as a chloropesticide because of restrictions on DDT, and the currently widely discussed PBDEs which are subsequently substituted with more complex fire retardants. These compounds have been unintentionally released into environment in known and traceable manners. Thus, it takes shorter and shorter periods until environmental concerns are developed by researchers.

In addition to this mix of manmade halogenated pollutants, a rather new spectrum of compounds attracts currently more and more attention, i.e., naturally produced organohalogen compounds or halogenated natural products (HNPs). HNPs have a long history, and natural products chemists have thus far identified about 4,000 different compounds (Gribble 2004). Gordon W. Gribble, the late D. John Faulkner, and others have prepared outstanding review articles on this topic (Gribble 1998, 1999, 2000, 2004; Faulkner 1980, 2002; Naumann 1993, 1999; Field et al. 1995). The halogenated secondary metabolites are produced by such diverse organisms as algae, sponges, sea worms, and bacteria, with an increase of ~200 novel HNPs that are discovered annually (Gribble 2004). Relatively new, however, is their link with environmental issues, the topic of this review article. This connection means that the HNPs are detected in higher organisms that were not the natural sources but have accumulated the natural products. Their detection in top predators indicated that HNPs resembled some of the adverse properties of halogenated xenobiotics, i.e., persistency and the bioaccumulative character, and this in turn leads to the question of their (eco)toxicological relevance and thus their role as environmental contaminants.

In the late 1990s, three papers were published that carefully addressed this topic (Haglund et al. 1997; Tittlemier et al. 1999; Vetter et al. 1999a).

The careful announcements that HNPs were probably detected in higher organisms were necessary and justified because it sounded unbelievable. It has to be remembered that a major simplifying argument for the particular toxicity of anthropogenic POPs, that no analogue compounds are found in nature, had to be revised (see following). In the first days, environmental scientists had to face some irrational scepticism of other researchers on their results. Tittlemier et al. (1999) cite in their key article to the field that ". . . some types of synthetic compounds, including halogenated hydrocarbons such as PCB, are not found in nature." When we described a halogenated monoterpene as an abundant contaminant in fish and mammals, one of the anonymous reviewers commented on the chromatogram (Fig. 1) with the remark that the peak of the novel compounds must be an artifact. It was claimed that such an abundant compound would have been detected earlier.

Unexpectedly, the situation has changed within recent years since more and more evidence was provided on the natural origin of some abundant halogenated compounds in the gas chromatograms of various samples. Today, HNPs are recognized as possible contaminants of marine environmental samples and food. Ironically, the situation is now almost the opposite. Residues from unknown compounds in environmental samples are sometimes suggested to arise from HNPs without providing evidence. The natural origin of a halogenated compound is however not always easy to prove. In several cases, the natural producers are still unknown or ambiguous. Nevertheless, it is time for a first review on the environmental issue of the HNPs and a first balance after fewer than 10 years of dedicated research.

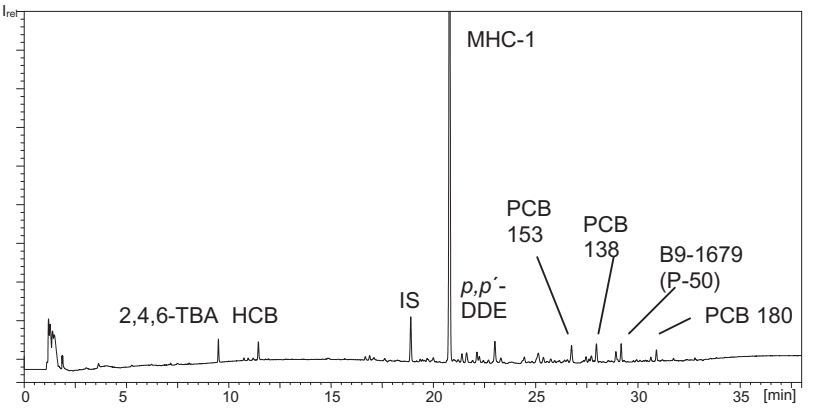


Fig. 1. GC/ECD chromatogram (HP-1) of the purified organohalogen extract of a Norwegian salmon (Adapted from Vetter et al. 2001b with permission from the American Chemical Society).

II. Mass Spectrometric Investigation of Halogenated Natural Products (HNPs)

HNPs elute in the same gas chromatography (GC) retention range and cover the same mass range as anthropogenic halogenated pollutants. The identification of HNPs in an environmental sample is thus not simple. Given the fact that chlorinated anthropogenic compounds are more abundant, more diverse, and more widely distributed in the environment as compared with organobromines, the identification of chlorinated HNPs is more difficult. However, the environmentally relevant HNPs identified to date are mostly brominated or at least contain bromine, whereas exclusively chlorinated, natural products are scarce. In fact, the so-called Q1 (see Section III. B) is currently the only important polychlorinated HNP identified whereas diverse abundant brominated and mixed HNPs have been described (see Section III).

A random worldwide comparision may allow us to estimate that all chlorinated compounds combined are rather two orders than one order of magnitude more abundant than anthropogenic brominated compounds, although there are exceptions. PCBs and chloropesticides are often found in the parts per million (ppm) range in marine mammals, and the detection of traces of HNPs in such samples will not be readily possible. If they occur, they were previously assigned to unknown minor compounds or metabolites of anthropogenic POPs. Consequently, the identification of brominated and mixed brominated-chlorinated HNPs is more likely, whereas chlorinated HNPs can only be identified under particular circumstances. The following scheme thus focuses more on the detection of bromine-containing compounds.

In the 1980s, gas chromatography in combination with electron-capture negative ion mass spectrometry (GC/ECNI-MS) was shown to be a promissing tool for the detection of brominated compounds (Crow et al. 1981). Under these conditions, the bromine atom attached to a carbon has relatively low energetic unoccupied molecular orbitals (LUMO), and the charge is well stabilized by the large bromine atom. Homolytic cleavage of the C-Br bond yields the bromide ion, which then (re-)achieves the Nobel gas configuration for which it strives, whereas a neutral (M-Br) radical is left. Thus, bromine atoms in halogenated compounds are prone to electron capture processes. Due to the equal natural abundance of the bromine isotopes, screening for the bromide ion with virtually equal peak heights of m/z 79 and m/z 81 is a sensitive method for the identification and determination of all organobromine compounds present in environmental and food samples (Buser 1985). Owing to this low selectivity for a particular compound (almost all organobromines respond to the bromide ion), some authors reported coelutions of diverse brominated compounds (Vetter and Jun 2003; Marsh et al. 2004a). It was thus recommended to use further low-mass ions for distinguishing between different classes of dibrominated

to polybrominated compounds. Br^- was found to be typical of all organobromines (Buser 1985), whereas Br_2^- is often found in the GC/ECNI-MS of nonaromatic organobromines with at least two Br substituents. Aromatic organobromines either form no additional low-mass fragments or an intense fragment ion at m/z 159 (HBr_2^-). In several studies, the relevance of this fragment ion was described more precisely (Vetter 2001; Vetter et al. 2002a; Vetter and Janussen 2005). Current knowledge suggests that m/z 161 is only abundant in diphenyl ether derivatives that bear at least one bromine substituent in the ortho position (Melcher et al. 2005a). This condition is fulfilled for anthropogenic BDEs and naturally produced methoxy-BDEs (MeO-BDEs), except the respective non-ortho congeners, which are rarely found. Other compounds forming m/z 159 are diMeO-BDEs.

Likewise, the chloride ion may be used for initial screening on chlorinated and mixed halogenated compounds (Asplund et al. 1999; Vetter et al. 2002a). However, the chloride ion is not necessarily abundant in the mass spectra of mixed halogenated compounds (Buser 1985). Although the electronegativity of chlorine is higher, the larger convalent radius of bromine is obviously favorable and the formation of the heavier halogenide is predominant.

Once all brominated compounds in a sample are detected in the SIM mode, GC/ECNI-MS full-scan analysis may accomplish the initial measurements. Unfortunately, the molecular ion (M^-) can be very low in abundance for polybrominated compounds. For selected compounds only, this can be improved by lowering the ion source temperature. However, the structural information obtained from GC/ECNI-MS full-scan measurements is usually low but often suitable to add the missing piece to a puzzle. Moreover, the information obtained from low-abundance M^- ions is often equivocal. Although brominated isotope patterns are very distinct, as are chlorine isotope patterns, some isotope patterns of mixed brominated and chlorinated/brominated compounds are almost identical (Fig. 2). Excellent mass spectra can be assigned unequivocally to the number and kind of isotopes present in an organohalogen compound, but this is not easy to obtain (compare the isotope patterns of the mass spectra in Section III with those in Fig. 2). Thus, it is unterstandable that misinterpretations may occur (Sinkkonen et al. 2004), and a thorough comparison of the isotope pattern in a sample with the theoretical abundances of the isotopic peaks should be carried out. Attention should be paid particularly to the low-abundance isotopic peaks to overcome erronous assignments of the halogenated patterns. For instance, the major isotopic peaks of heptachloro-, pentabromo-, and dichlorotetrabromo isotope pattern look very similar. However, only the latter two have a low abundant monoisotopic peak. These two can be distinguished by the very low abundant seventh line, which is not present in the pentabromo isotope pattern. Further examples for very similar isotope patterns are shown in Fig. 2.

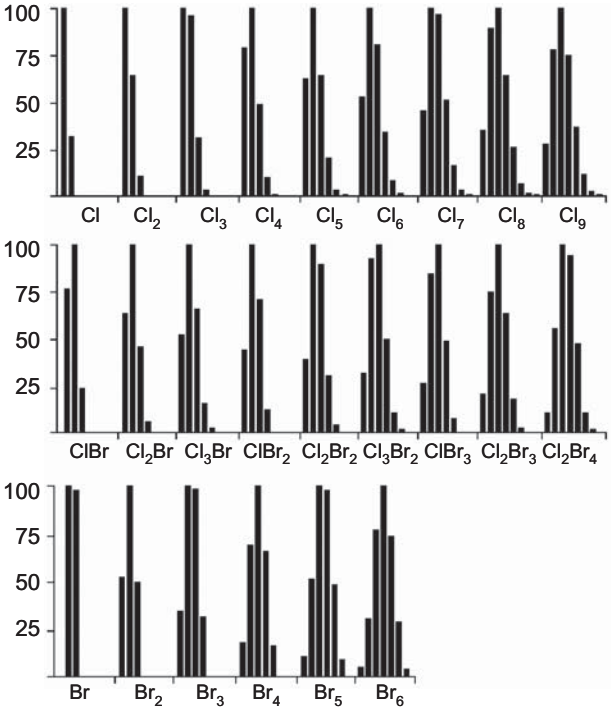


Fig. 2. Halogen isotope patterns.

Gas chromatography in combination with electron ionization (GC/EI-MS) is usually significantly less sensitive for the detection of polybrominated compounds than in GC/ECNI-MS. Another disadvantage of GC/EI-MS is the detection of the background from matrix remainders that are suppressed by GC/ECNI-MS and can reach very high abundance, particularly when GC/EI-MS full-scan analyses require concentrated solutions of sample extracts. Moreover, there are no fragment ions in the GC/EI-MS spectra that directly prove the presence of chlorine and bromine. Thus, distinguishing brominated for mixed halogenated compounds requires high-quality mass spectra (see foregoing). In addition, the more-sensitive SIM technique can hardly be performed in nontarget analysis. However, if high-quality spectra are obtained, GC/EI-MS is the method of choice for studying the fragmentation patterns. For instance, many brominated and mixed halogenated compounds show abundant $[M\text{-}Br]^+$ and $[M\text{-}2Br]^+$ fragment ions that are often very useful for structure information. For instance, the $[M\text{-}2Br]^+$ fragment ions of a pentabromo compound (Br_3 pattern) appear to be more easily distinguished from the respective dichlorotetrabromo compound (Cl_2Br_2 pattern). Furthermore, elimination of chlorine may provide clarity on the exact isotope pattern of a polyhalogenated com-

pound. Table 1 lists the relative abundances within halogen isotope patterns that should be virtually matched by the organohalogen compound being studied.

In many cases, high-resolution mass spectrometry (HRMS; usually in the EI mode) should be used to establish the elemental composition, as was carried out on several occasions (Tittlemier et al. 1999; Vetter et al. 1999a, 2001b; Teuten et al. 2005a). This is, however, much easier if the numbers and types of halogens are known. Given the fact that the hydrogen atom exceeds the nominal value (Table 2), the exact masses of different structural variants are usually the heavier the more hydrogens are found in the molecule. However, the exact masses are usually lower than the nominal masses because the halogens are lighter. The molecular ion can be scanned, or the exact masses of different elemental compositions that can be calculated from the low-resolution mass spectrum can be screened in the SIM

Table 1. Halogen isotope abundances of chlorinated, brominated, and mixed halogenated compounds.[a]

Halogen	X[b]	X + 2	X + 4	X + 6	X + 8	X + 10	X + 12	X + 14
Cl	100	32.0						
Cl_2	100	64.0	10.2					
Cl_3	100	96.0	30.7	3.3				
Cl_4	78.2	100	48.0	10.2	0.8			
Cl_5	62.5	100	64.0	20.5	3.3	0.2		
Cl_6	52.1	100	80.0	34.1	8.2	1.0		
Cl_7	44.7	100	95.9	51.1	16.4	3.1	0.3	
Cl_8	34.9	89.3	100	64.0	25.6	6.5	1.0	0.1
Cl_9	27.2	78.2	100	74.6	35.8	11.4	2.4	0.3
Br	100	97.9						
Br_2	51.1	100	49.0					
Br_3	34.0	100	98.0	32.0				
Br_4	17.4	68.1	100	65.3	16.0			
Br_5	10.4	51.1	100	97.9	47.9	9.4		
Br_6	5.3	31.3	76.6	100	73.4	28.8	4.7	
BrCl	77.0	100	24.1					
$BrCl_2$	61.8	100	45.0	6.2				
$BrCl_3$	51.6	100	64.2	17.2	1.7			
Br_2Cl	43.9	100	69.6	13.5				
Br_2Cl_2	38.5	100	89.0	31.3	3.8			
Br_2Cl_3	31.8	92.8	100	49.4	11.4	1.0		
Br_3Cl	26.2	85.4	100	48.7	7.9			
Br_3Cl_2	20.6	73.7	100	63.4	18.4	2.0		

[a]Only abundances >0.1% are listed.
[b]X = all-^{35}Cl and all-^{79}Br is appropriate.

Table 2. High-resolution mass spectrometry (HRMS) calculation of the monoisotopic peak.[a]

	^{12}C 12.00000	^{1}H 1.007825	^{35}Cl 34.968854	^{79}Br 78.918348	^{16}O 15.994915	^{14}N 14.003074	Calculated exact mass (nominal mass) (u)
DBP-Br_4Cl_2	10	6	2	4	—	2	539.664 (540)
Q1	9	3	7	—	—	2	383.812 (384)
6-MeO-BDE 47	13	8	—	4	2	—	511.726 (512)
MHC-1	10	13	3	2	—	—	395.845 (396)

[a]Other isotopes are ^{13}C (13.003354), ^{37}Cl (36.965896), ^{2}H (2.014012), ^{15}N (15.000108), ^{81}Br (80.916344).

mode. Once the elemental composition is known, the fragmentation pattern can provide valuable information as to the structure of organobromine and mixed halogenated compounds. Currently available data confirm that a wide range of HNPs exist that may end up in food or be accumulated in the environment. These compounds include brominated, mixed halogenated, and to a lesser degree chlorinated compounds with an aromatic, aliphatic, or heterocyclic backbone. Nitrogen and oxygen are frequently found on the HNPs discussed next. In fact, most of the HNPs discussed in this review bear at least one hetero atom in addition to halogens. These different possibilites should be kept in mind when an unknown compound is investigated.

III. Individual HNPs of Environmental Concern

For several decades, the research of environmental chemists on anthropogenic POPs and the research of natural products chemists on HNPs was conducted almost isolated in the respective research discipline. Very little if any overlap was observed at the end of the 20th Century. In retrospect, it is not always clear why there was not more exchange between the two groups. A recent study of sponges led to the detection of >100 HNPs but many of them were in very low abundance and would not have become the focus of natural products chemists (Vetter and Janussen 2005). Some of them could, however, be of environmental concern. Currently, research of natural products chemists is cited by environmental chemists and vice versa, and it appears that interests of both disciplines are becoming more mixed without losing their different directions or intents of research.

It was long thought that HNPs are neither persistent nor lipophilic and thus do not bioaccumulate. A prerequisite for the presence of such halogenated compounds in the lipids of the top predators in food chains is that they are lipophilic ($\log K_{ow} > 5$), persistent (nondegradable in the liver), and bioavailable (able to pass through membranes). Faulkner (1980) predicted that brominated phenols are probably the most stable HNPs and are therefore most likely to appear as contaminants in other analyses.

Although this is generally the case, recent work has identified the natural producer of two compounds previously detected with high concentrations in marine mammals (Vetter et al. 2002b). In addition to the few nonpolar HNPs with known bioproducers (Vetter et al. 2002b; Flodin and Whitfield 1999a; Asplund et al. 2001), the natural origin of several other common organohalogen compounds is no longer debated (Tittlemier et al. 1999; Vetter et al. 1999a, 2001a,b). The classification of compounds described in the following subsections as halogenated natural products is diverse.

A. Halogenated Dimethyl-2,2′-Bipyrroles (HDBPs)

This compound class summarizes halogenated components that share a 1,1′-dimethyl-2,2′-bipyrrole (DBP) spine. Five hexahalogenated congeners with

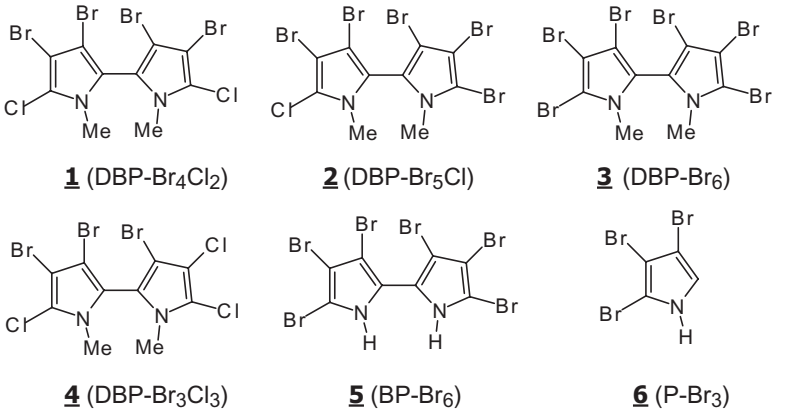


Fig. 3. Structures of halogenated dimethylbipyrroles and related compounds. **1**: 5,5′-dichloro-1,1′-dimethyl-3,3′,4,4′-tetrabromo-2,2′-bipyrrole; **2**: 5′-chloro-1,1′-dimethyl-3,3′,4,4′,5-pentabromo-2,2′-bipyrrole; **3**: 1,1′-dimethyl-3,3′,4,4′,5,5′-hexabromo-2,2′-bipyrrole; **4**: 1,1′-dimethyl-3,3′,4-tribromo-4′,5,5′-trichloro-2,2′-bipyrrole; **5**: 3,3′,4,4′,5,5′-hexabromo-2,2′-bipyrrole; **6**: 2,3,4-tribromopyrrole.

a bromine/chlorine distribution of 3/3 (two isomers), 4/2, 5/1, and 6/0 have been described (Fig. 3) (Tittlemier et al. 1999). Tittlemier et al. designated codes to the compounds based on the abbreviation DBP, separated by a hyphen following Br_x and Cl_y. The short term of the most abundant tetrabromodichloro-1,1′-dimethyl-2,2′-bipyrrole congener (**1**) is thus DBP-Br_4Cl_2. Structure elucidation was performed using isotope exchange, namely N-H→ N-D, and the proposed structures, when synthesized, fully agreed with the MS prediction (Tittlemier et al. 2002c).

Historic Data, Identification, and Linking to Known Natural Sources. In 1992, Elliot et al. (1992) described a relatively abundant compound in bird eggs from both the Canadian Pacific and Atlantic coasts. This compound, labeled UHC, was subsequently isolated from bald eagles (*Haliaeetus leucocephalus*) and studied by GC/MS (Fig. 4) (Tittlemier et al. 1999). Initially suspected to be a pentabromo compound (see Section II and Fig. 2; compare with Fig. 4b), the use of larger amounts (on isolation) and HRMS indicated that this novel compound carried four bromine and two chlorine atoms. Even more surprising, HRMS analysis demonstrated that the compound bore two nitrogens. The molecular formula was established as $C_{10}H_6Br_4Cl_2N_2$, and a possible structure was suggested to be 5,5′-dichloro-1,1′-dimethyl-3,3′,4,4′-tetrabromo-2,2′-bipyrrole (**1**) (Tittlemier et al. 1999). The simultaneous detection of a hexabromo (**3**), a chloropentabromo (**2**), and two tribromotrichloro homologues, one of which was (**4**), along with the related known hexabromo-2,2′-bipyrrole (**5**) previously identified by natural products chemists (Andersen et al. 1974), produced strong evidence

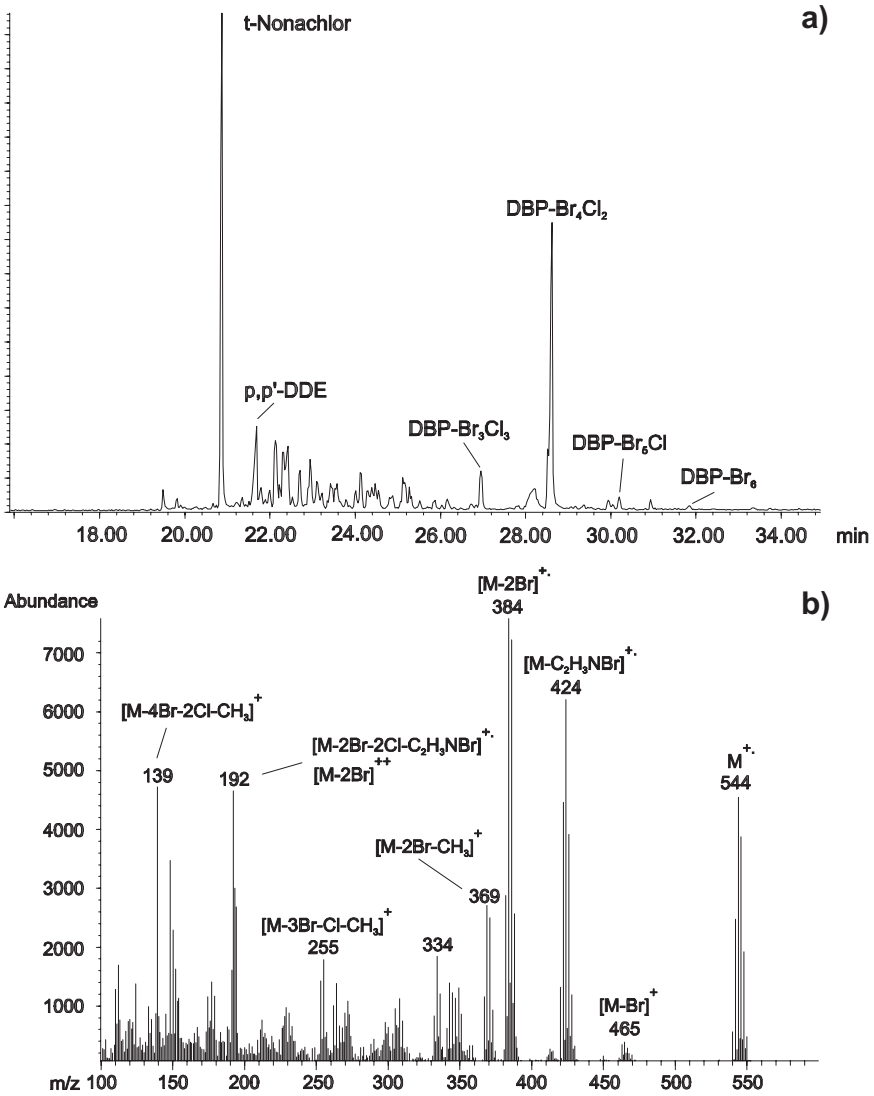


Fig. 4. GC/ECNI-MS total ion chromatogram of bald eagle liver extract (*top*) (a) and EI-MS of DBP-Br_4Cl_2 (*bottom*) (b) (Adapted from Tittlemier et al. 1999 with permission from the American Chemical Society).

for this structure (Tittlemier et al. 1999). In the same year, Gribble et al. (1999) synthesized the key compounds (and the hexabromo congener), and comparison of the synthesized standard and the isolate from bird eggs confirmed that the correct compound was synthesized and that the proposed structure was correct (see Fig. 3). Gribble et al. (1999) based their synthesis on the preparation of the backbone. The subsequent one-pot

halogenation reaction yielded a mixture that proved to be similar to the residue pattern found in seabirds. Thus, it was concluded that the natural halogenation was somewhat random (Gribble et al. 1999). It was suggested that first a chlorination with chloroperoxidase, followed by bromination with bromoperoxidase, had occurred (see Section IV) (Gribble et al. 1999). Monomeric tetrabromopyrrole had also been isolated from the marine bacterium *Chromobacterium* sp. (Andersen et al. 1974). It is noteworthy that tetrabromopyrrole and tribromopyrrole isomers (**6**) were found to be extremely unstable, especially when exposed to light and oxygen (Andersen et al. 1974; John et al. 2004). This finding is in sheer contrast to the recalcitrant HDBPs, the focus of this chapter, which have not been discovered by natural products chemists.

Investigation of samples from Australia led to the detection of several brominated compounds (see Sections III.C, III.D), whereof the one labeled BC-10 turned out to be DBP-Br_4Cl_2 (**1**, Fig. 3) (Vetter 2001; Vetter and Jun 2003). Reddy et al. (2004) isolated DBP-Br_4Cl_2 from marine mammal extracts and determined the $\Delta^{14}C$ value. The ^{14}C radioisotope has a half-life of 5,730 years, which excludes its determination in samples older than ~50,000 years. Because the anthropogenic POPs (exception, toxaphene) are produced from coal or oil sources dating back manifold years more, the detection of ^{14}C is an indirect proof of the natural source. Reddy et al. (2004) indeed determined ^{14}C in the isolate, but the depletion accounted for an age of ~5000 years of the compounds, which is rather unrealistic given the wide distribution in our time and typical half-lives in the environment that do not exceed ~10 years. Reddy et al. (2004) suggested three scenarios that could explain their results, i.e., (i) a mix of anthropogenic and natural sources, (ii) utilization of aged carbon during the biosynthesis, and (iii) biosynthesis in ancient years. The most plausible hypothesis was suggested to be utilization of aged carbon. However, other parameters may apply as well, which are the following: (iv) isotope fractioning during food web enrichment, (v) isotope fractioning during isolation, and (vi) interference from another coisolated compound. The natural producer of DBP-Br_4Cl_2 is unknown (in contrast to **5**), and it appears plausible that methylation of the nitrogens (e.g., the conversion of **5** into **3**) was performed by another organism (see Section III.C). Because radiocarbon measurements only deliver an average value for all carbons, some unequivocal data may arise from this point. Unfortunately, because of the uncertainties addressed in parameters (i) to (vi), the radiocarbon measurements, along with the more striking data obtained for MeO-BDEs, which are different from those determined for HDBPs, provide no unequivocal proof of their natural production; however, they support the HNP theory. Other important issues that clearly point toward a natural source for HDBPs are the high concentrations in marine environments and the virtual absence in industrial regions, as well as a distribution pattern different from that of classic anthropogenic contaminants (Tittlemier et al. 1999, 2002b). In addition, a mixed halogenated

pattern is relatively rare for industrial chemicals except for their formation during incineration (Tittlemier et al. 2002b). In the latter unintended case, however, we would expect a mixture of several DBP-Br_4Cl_2 isomers, which is in contrast to the unique DBP-Br_4Cl_2 isomer found abundantly in the environment.

Tittlemier et al. (2004) determined physicochemical (PC) parameters of five HDBPs (Table 3). As anticipated, the vapor pressures decreased with increasing number of bromine substituents that replaced chlorine atoms [$P°_{L,25}$ (7.55–191) 10^{-6} Pa], but the water solubility and octanol–water coefficient remained untouched from the pattern of halogens (Table 3). These values rank the HDBPs in the range of PCB congeners. For instance, PCB 101 showed comparable water solubility (0.98 10^{-6} g/L), and the log K_{OW} of penta- and hexachloro biphenyls was also comparable to the HDBPs. The PC parameters were used in a distribution model that indicated that >99% of HDBPs are located in sediments and soil (Tittlemier et al. 2004).

It is evident that demethylation of HDBPs will decrease the lipophilic character of the HDBPs, comparable to bromoindols/N-methylindoles (see Section III.G) or halogenated phenols/anisoles (see Section III.E). Monomeric tribromopyrrols, which obviously turned out to be stable, were recently synthesized by John et al. (2004). Pellets spiked with 2,3,4-tribromopyrrole (**6**) administered to predatory fish had a deterrent effect, and only 6 of 14 fish actually consumed the pellets. These fish were significantly larger than those that refused the pellets (John et al. 2004). Attempts to detect the bromopyrroles in fish tissue were not undertaken. Unfortunately, even the major HDBPs, DBP-Br_4Cl_2 and DBP-Br_6, are not commercially available, which hinders a more thorough worldwide study of their relevance.

Analytical Aspects. Standard sample cleanup methods suitable for the determination of POPs (PCBs, chloropesticides, PBDEs) can be applied to HDBPs. The most sensitive and suggested detection method is GC/ECNI-MS (Tittlemier et al. 1999, 2002b). The GC/ECNI-MS of DBP-Br_4Cl_2 is dominated by the molecular and bromide ions, both found in equal amounts (Tittlemier et al. 1999). Therefore, m/z 544 and m/z 546 are recommended for selective determination of DBP-Br_4Cl_2. The higher the degree of bromination, the higher the ratio of M^- to Br^- becomes (SA Tittlemier, personal communication 2005). Limits of detection (S/N > 3) were 0.2 pg (m/z 500/502, DBP-Br_3Cl_3), 0.25 pg (m/z 544/546, DBP-Br_4Cl_2), 0.01 pg (m/z 588/590, DBP-Br_5Cl), and 0.01 pg (m/z 632/634, DBP-Br_6) (SA Tittlemier, personal communication). Given the high response for the bromide ion, m/z 79 and m/z 81 have also been used for DBP-Br_4Cl_2 (Vetter 2001), but coelutions with other brominated compounds of natural or anthropogenic origin may occur. Thus, using the bromide ion is more suitable as a general screening method, whereas quantification should be performed by determining isotope masses of the molecular ion (see Section II); this appears to be

Table 3. Physicochemical parameters of selected halogenated natural products (HNPs).

Compound	Log K_{OW}	Water solubility $S_{W,25}$ (g/L)	Melting point	H_{25} (Pa m^3/mol)
Br_4-Cl_2-DBP (Tittlemier et al. 2004)	6.5 ± 0.3	(0.9 ± 0.1) 10^{-5}	209°–210°C	0.036 ± 0.004
Br_6-DBP	6.7 ± 0.3 (Tittlemier et al. 2004)	(1.4 ± 0.3) 10^{-5}	247°–248°C	0.0020 ± 0.0004
Q1	5.9–6.4 (Hackenberg et al. 2003; Vetter 2000)	0.46 10^{-5} (Vetter et al. 2004)	154°–155.5°C (Jun et al. 2002)	n.d.
TBA	4.44 (Pfeifer et al. 2001) 4.48 (Mackay 1982)	1,220 10^{-5} (Vetter et al. 2004)	87°–89°C (Vetter et al. 2004)	n.d.
2′-MeO-BDE 68	~6.85 (Teuten et al. 2005a)	n.d.	Oil, boiling point 120°C at 0.24 Torr (Vetter and Jun 2003)	n.d.
6-MeO-BDE 47	~6.85 (Teuten et al. 2005a)	n.d.	116.5°–117.5°C (Francesconi and Ghisalberti 1985)	n.d.

particularly necessary for the detection of DBP-Br_6. The major compound DBP-Br_4Cl_2 elutes in the last third between BDE 47 and BDE 100 from DB-5 as stationary phases (Vetter and Jun 2003). A heptachlorobiphenyl congener may coelute with DBP-Br_2Cl_4 on DB-5 columns (data not shown). More comprehensive retention data for all prominent HDBPs on different columns were also published by Tittlemier et al. (2002c).

Distribution and Concentrations of HDBPs in the Environment. In the first study, bird eggs from Pacific offshore surface feeders accumulated more than 10-fold-higher HDBP concentrations than Pacific offshore subsurface feeders and Atlantic bird eggs (see Fig. 4a for an example). By contrast, birds from the Great Lakes did not contain HDBPs, which is an additional clue for HDBPs being natural products (Tittlemier et al. 1999). Marine samples from Canada also confirmed this. Nitrogen stable isotope mass spectrometric (IRMS) analysis of tissue along with the quantification of HDBPs demonstrated that these HNPs biomagnified with trophic level from invertebrate → fish → seabird (Tittlemier et al. 2002a). A global study with marine mammals, which did not include samples from Africa, South America, and the Antarctic, was carried out by Tittlemier et al. (2002b). The highest concentrations of 9.8 mg/kg ΣHDBPs was determined in California sea lions (Table 4) (Tittlemier et al. 2002b). High concentrations (up to 4 ppm) were also found in bottlenose dolphins from Australia. Concentration and distribution of HDBPs did not correlate with PCBs (Tittlemier et al. 2002b). In pinnipeds, HDBPs are less abundant than in cetaceans, and they seem to be less persistent than PCB 153 (Tittlemier et al. 2002b). Surprisingly high concentrations were also determined in selected canned fish samples. DBP-Br_4Cl_2 was also identified in human milk from the Faeroe Islands (Vetter and Jun 2003). Sea eagle eggs from Norway contained traces of DBP-Br_4Cl_2 (Herzke et al. 2005). Pool samples of human milk from southern Canada contained 13–4,480 pg/g lipids, which was low compared to fish and seafood (Tittlemier et al. 2002d). Studies of marine birds of prey demonstrated that they bioaccumulate HDBPs in tissue, plasma, and liver. HDBPs also seem to be transported to yolk during egg development (Tittlemier et al. 2003).

Consequences. Although natural producers of HDBPs have not been identified, their assignment to natural sources is no longer debated. Therefore, the question mark at the end of the initial paper (Tittlemier et al. 1999) is no longer necessary. Marine birds and cetaceans appear to contain the highest burden, but fish are also known to be potentially contaminated with HDBPs. Reference standards are lacking, which hinders a thorough worldwide investigation. Toxicological investigation pointed to dioxin-like effects, albeit the bioactivity was much more moderate (Tittlemier et al. 2003). Even in environmental samples with the highest concentration, no toxic effects could be determined in the respective samples. The relatively high

Table 4. Concentrations (selection) of HDBPs (ng/g lipids) in the marine environment and food.

Species	Location	*N*	sumHDBPs	PCB 153	Source
Beluga	North America		14–18	540–8,000	Tittlemier et al. (2002b)
	Svalbard		2	1,270	Tittlemier et al. (2002b)
Dall's porpoise	NW North Pacific Ocean	5	2,540	1,240	Tittlemier et al. (2002b)
Hector's dolphin	New Zealand	5	48	76	Tittlemier et al. (2002b)
California sealion	California	5	93–9,800	910–90,800	Tittlemier et al. (2002b)
Harbour seals	Different locations	69	0.02–526	65–282,000	Tittlemier et al. (2002b)
Bottlenose dolphin	Australia	4	250–4,150[b]	230–8,800	Vetter et al. (2001a)
Green turtle	Australia	1	26[b]	70	Vetter et al. (2001a)
Marine fish[a]	Canada	62	<0.6–1,100		Tittlemier (2004)
Freshwater fish[a]	Canada	39	<0.6–220		Tittlemier (2004)
Canned fish[a]	Canada	86	25–6,660		Tittlemier (2004)
Shrimp[a]	Canada	33	<0.6–48		Tittlemier (2004)
Sediment	Canadian Arctic	2	~0.03		Tittlemier et al. (2002a)
Arctic cod[a]	Canadian Arctic	5	1.1		Tittlemier et al. (2002a)
Black guillemot[a]	Canadian Arctic	6	9.5		Tittlemier et al. (2002a)
Seal[a]	Canadian Arctic	10	~0.1		Tittlemier et al. (2002a)
Seabird eggs	Pacific, Canada	19	32–140	~70–100	Tittlemier et al. (1999)
Offshore surface feeders + seabird eggs[a]	Atlantic	40	1.7–4.8	>20	Tittlemier et al. (1999)

[a]Wet weight.
[b]Estimated from response factor of 2-MeO-BDE 68.

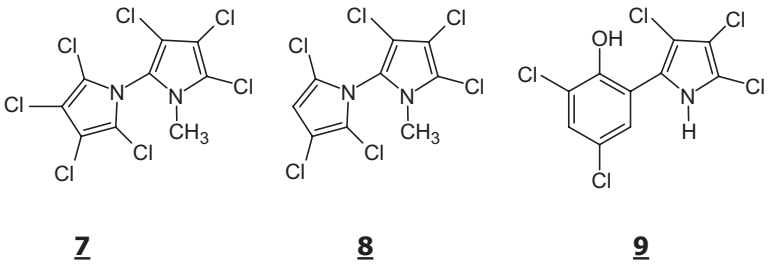
response in the AHR assay is somewhat surprising because it is expected that DBP-Br_4Cl_2 is not planar. In this case, the rotation about the central pyrrole–pyrrole bond would be hindered (this aspect is discussed more detail in Section III.B). An estimate of human exposure to bioaccumulative HNPs was presented by Tittlemier (2004).

B. Heptachloro-1′-Methyl-1,2′-Bipyrrole (Q1)

The trivial name "Q1" (an abbreviation for question 1) was originally assigned to the first prominent unknown compound detected in the GC/MS analysis of Antarctic seals. Unlike all other relevant marine natural products found at elevated concentrations in higher organisms discussed in this article, Q1 is the only exclusively chlorinated HNP, a feature that is obviously more characteristic for terrestrial samples. Of course, there may be many more, but their detection is more complicated than that of brominated natural products (see Section II). Very recently, indications for a chlorohexabromo- and the heptabromo congener of Q1 were presented by Teuten et al. (2005b).

First evidence for a natural origin of Q1 was produced in 1999 when the molecular formula of Q1 was determined by HRMS (Vetter et al. 1999a). The unique composition of $C_9H_3Cl_7N_2$ had never been reported in any scientific paper except as an unstable reaction intermediate that could definitely be ruled out (Findeisen and Wagner 1978; Vetter et al. 1999a). The follow-up synthesis of Q1 led to the chemical structure (**7**) shown in Fig. 5. This structure is spectacular because even the unsubstituted 1,2′-bipyrrole backbone is a chemical feature that was not described in organic chemistry or other disciplines of natural science before the synthesis of Q1 (Jun et al. 2002).

Neither Q1 nor closely related structures have been detected by natural products chemists, and according to present knowledge, no relevant lower chlorinated analogues or chlorinated homologues have been detected in environmental samples. In view of the fact that the chemical synthesis of



7 **8** **9**

Fig. 5. Structures of Q1 (2,3,3′,4,4′,5,5′-1′-methyl-1,2′-bipyrrole, **7**), Q1-hex (2,3′,4,4′,5,5′-1′-methyl-1,2′-bipyrrole, **8**), and pentachloro-2-pyrrolyl-2-phenol, **9**.

Q1 only provided a maximum yield of 3.6% (Jun et al. 2002), the biochemical formation of Q1 without any known by-products appears to be a real masterpiece of nature. In Q1, every third atom is chlorine, which accounts for 64% by weight (Vetter 2002). Moreover, the heptachlorinated natural product Q1 is the compound that brings together the highest number of halogens of all HNPs discussed in this review, and only a very few HNPs top this degree of halogenation.

Several further properties confirmed that Q1 is an HNP, although the natural producer is still unknown. The HNP that most closely resembles the structure Q1 is the pentachloro-2-pyrrolyl-2-phenol (**9**) isolated from the terrestrial bacterium *Actinoplanes* sp. ATCC 33002 (Cavalleri et al. 1978) (see Fig. 5). Pyrrolylphenols appear to be the condensation products of phenyl and pyrrole units and may thus represent the link between the halogenated bipyrroles and diphenyl ethers (see Section III.C) in which two of the same units are dimerized. It is currently unknown whether these compounds can be formed from the same enzymes, and will be a matter of conditions and substrate availibilities, or are products of specific enzymes and organisms.

However, a marine source of Q1 appears to be more plausible. The respective pentabromo isomer of *g*, pentabromopseudilin, also exists. Originally isolated from the marine bacterium *Alteromonas luteoviolaceus*, pentabromopseudilin is both antibiotic and cytotoxic (Burkholder et al. 1966; Laatsch and Pudleiner 1989). Except these examples and the 2,2′-bipyrroles (Section III.A), HNPs from marine bacteria are relatively scarce (Huth 1999). Bacterial HNPs are produced in the late logarithmic phase of growth or in the stationary phase (van Pée 1996). It is largely unknown why these compounds are produced by the bacteria, although they have been mostly discovered upon screening for antibiotics (van Pée 1996).

Historic Data, Identification, and Linking to Known Natural Sources. Given the high abundance of Q1 in various samples from Africa (Vetter et al. 1999b), the Antarctic (Vetter 2000), and Australia (Vetter et al. 2001a), it was likely that other researchers had detected Q1 as well. Using a donated standard of Q1, it was subsequently determined that Q1 is identical with an unknown compound originally labeled U3 in several papers published since the beginning of the 1980s (Hackenberg et al. 2001; Ballschmiter and Zell 1981). However, no attempts to elucidate the structure were made then. In 1996, Weber and Goerke (1996) described an abundant compound in Antarctic samples that showed the molecular ion at *m*/*z* 384. Owing to GC/EI-HRMS investigations the molecular formula $C_{10}H_3Cl_7O$ was assigned to the compound (Weber and Goerke 1996). However, the deviation in the HRMS measurements from the theoretical value of 383.800 of $C_{10}H_3Cl_7O$ was 15 mu (0.015 u) whereas the correct molecular formula of Q1 (383.812; see Table 2) differed only by 3 mu from the value measured by Weber and Goerke. Therefore, this apparent misinterpretation was not

a matter of an inadequacy of the analytical method but that nitrogen-containing compounds were not taken into account at that time. This structural feature would have meant that Q1 is a natural product, which appeared to be unthinkable in those days. Weber and Goerke also mentioned elimination of C_2HOCl and other fragment ions from the molecular ion, which must now be revised. The possibility that there is nitrogen in compounds bioaccumulated in higher organisms was ignored until the discovery of the HDBPs (Tittlemier et al. 1999) and Q1 (Vetter et al. 1999a). In 1997, van den Brink (1997) studied three less commonly known contaminants in Antarctic samples. A figure (7.2b) published in his article shows the GC/MS spectrum of a nonachlor isomer (*trans*-nonachlor or MC6, which was later identified as Q1 (Vetter et al. 2003b). Thus, Q1 was not a new discovery in gas chromatograms but a new interpretation of a compound detected for a long time, i.e., the addressing of its natural origin. Even though Vetter et al. determined the correct molecular formula, several misidentifications were circulated until the structure was determined by synthesis. The unique structural feature of the 1,2′-bipyrrole backbone (see Fig. 5) finally added the missing pieces to the Q1 puzzle (Jun et al. 2002).

PC parameters were estimated or determined for Q1 as well (see Table 3). The log K_{OW} clearly exceeds the target value of 5.0 for bioconcentration but was lower than the value determined for HDBPs while still in the range of pentachlorobiphenyls (Table 3). The lack of any hydrogen directly attached to the aromatic system can be related to some chemical stability. The water solubility is particularly low, and molecular modelling clarified that the Q1 molecule is not planar. The unique appearance of Q1 is striking, because very little evidence was produced for the presence of one of the additional 78 theoretically possible monochloro- to hexachloro homologues in environmental samples (Vetter et al. 2003b). Hackenberg et al. (2001) detected traces of hexachloro isomers whereas Vetter and Jun (2002) isolated and elucidated the structure of one of the hexachloro isomers (see Fig. 5). It is unclear if hexachloro isomers of Q1 would be original HNPs or transformation products of Q1. However, hints on mixed halogenated congeners and the brominated analogue of Q1 (Teuten et al. 2005b) may bring more light into the otherwise mysterious story of Q1.

Reference standards of Q1 are commercially available from LGC Promochem so that researchers from all over the world are able to determine Q1 in food and environmental samples.

Analytical Aspects. As for HDBPs, all standard sample cleanup procedures for POPs can be applied to the analysis of Q1. GC/ECNI-MS is the method that offers the highest selectivity and sensitivity for the detection of Q1 (Vetter et al. 2003b). For this purpose, the most abundant isotope masses of the molecular ion (m/z 386 and m/z 388) are recommended. The limit of detection for Q1 in the GC/ECNI-MS-SIM mode was ~0.13 pg (Vetter et al. 2000). Relative to *trans*-nonachlor, the GC/ECNI-MS response

was 4.5 times higher than the ECD response (Vetter et al. 2000). GC/EI-MS is about 1–2 orders of magnitude less sensitive for Q1 than GC/ECNI-MS. The suggested SIM masses in the GC/EI-MS mode are also *m*/*z* 386 and *m*/*z* 388, but the $[M\text{-}Cl]^+$ (SIM masses *m*/*z* 351 and *m*/*z* 353) and the $[M\text{-}2Cl]^+$ (SIM masses *m*/*z* 316 and *m*/*z* 314) are more abundant (Vetter 2000).

GC/ECD is only suitable for samples in which Q1 is relatively abundant, which appears to be the case only in environmental samples from the Southern Hemisphere because of their lower contamination with man-made POPs. Q1 elutes from DB-5-like columns before *p,p′*-DDE and may coelute with *trans*-nonachlor (Vetter et al. 1999b). Thus, the use of the ECD for Q1 determination is only possible after a thorough testing of the GC oven program with potential interfering compounds. Irrespectively, coelution with Q1 may cause overestimation of nonachlor concentrations in environmental and food samples when GC/ECD is used. This problem can be partly solved by separation of aromatic and aliphatic organochlorine compounds. Under such conditions, Q1 is usually found in the PCB fraction while nonachlor elutes into the chloropesticide fraction (Vetter 2002), although under certain circumstances partial elution into the more-polar chloropesticide fraction has initially been reported (Vetter et al. 1999b). The nonpolar behavior of Q1 during sample cleanup indicates that the nonbinding *n*-electron pair on the nitrogen is fully in possession of the pyrrole ring system to create the aromatic character. Likewise, it was not possible to protonate the pyrrole nitrogen.

A high-selectivity method for Q1 is obtained when GC is used in combination with a phosphorus-nitrogen detector (PND) (Melcher and Vetter 2004). Nitrogen-containing polyhalogenated compounds are hardly found in the anthropogenic "POP"-fraction and Q1 and the HDBPs almost exclusively give response in the PND owing to the two nitrogens (Fig. 6). The detection limit for Q1 in the PND (~20 pg) is relatively high because nitrogen amounts only for ~7.3% of the molecular weight. However, this detector may be useful for the identification of further N-containing HNPs (Melcher and Vetter 2004).

Hackenberg et al. (2003) developed a technique that allows estimation of PC parameters including water solubility and log K_{OW} alone from GC retention data. The suitability was tested with Q1, and this method may be used for other HNPs with unknown structure as well.

Distribution and Concentrations of Q1 In the Environment. Q1 was a very prominent peak in the GC/ECD chromatograms of marine mammals from Africa, the Antarctic, and Australia. The highest Q1 level reported to date (14,000 ng/g) were found in samples from Oceania (Vetter et al. 2003b); this also marks the highest concentration of a HNP determined in environmental samples to date (Table 5). Q1 was detected in the brain of Antarctic skuas (Vetter et al. 2000), in the blubber and brain of Antarctic fur seals

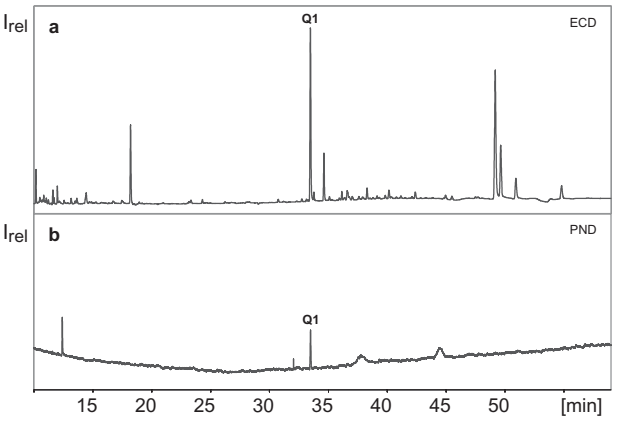


Fig. 6. GC/ECD (*top*) and GC/PND (*bottom*) chromatograms of a dolphin from Australia. The PND chromatogram underscores the high selectivity of this detector (Melcher and Vetter 2004).

(Vetter 2000, 2002), and also in human milk (Vetter et al. 2000). However, Q1 was virtually not present in ringed seals from Spitsbergen or the Canadian Arctic, as well as Baltic seals (Vetter et al. 2000; Vetter 2002). Q1 was detected neither in fish from Hongkong nor in seals from Lake Baikal (Vetter 2002). Low concentrations (~0.1% of *trans*-nonachlor) were determined in beluga from Canada (Vetter 2002). Four tissues of Antarctic fur seal contained Q1 and *p,p′*-DDE in virtually the same amounts, so that a similar body distribution and bioaccumulative behavior was suggested (Vetter 2000). However, cetaceans contained much higher Q1 concentrations than seals from the same habitat (Vetter et al. 2000; Vetter and Jun 2003).

Three fish species from the Antarctic resulted in lowest concentrations in the bottom invertebrate feeder than in the other fish-feeding species where Q1 was the third most abundant compound, after HCB and *p,p′*-DDE (Weber and Goerke 2003). Thus, it was suggested that the natural source may be in the upper water column and there most likely in the euphotic zone (Weber and Goerke 2003). However, Hackenberg et al. (2001) determined highest concentrations in fish from the South Atlantic and deep-sea fish from the North Atlantic. Moreover, Q1 was detected in Mediterranean deep-sea fish (Vetter 2002). Q1 was detected in a commercial fish-oil capsule and in cod livers canned in 1948 (Vetter and Stoll 2002). In addition, Q1 was detected in fish food and in fish fed with the respective food (Vetter and Stoll 2002). Traces were also detected in an omega-3 egg (Vetter and Stoll 2002).

Concentrations in Antarctic air (Signy Island, 60°72′S, 45°60′W) were relatively low; however, no other compound than Q1 was more abundant

Table 5. Concentrations (selection) of Q1 (ng/g lipids) in the marine environment and food.

Species	Location	Q1	PCB 153	Ratio	Source
South African fur seal (*Arctocephalus pusillus*) ($n = 11$)	Cape Cross (Namibia, Africa)	43–540[a,b]	2–273[a]		Vetter et al. (1999b) Vetter (2000)
South polar and mixed pair Skua eggs ($n = 7$)	Potter Peninsula (Antarctic)	3–110[b,c]	1.5–60		Weichbrodt et al. (1999)
Brown skua ($n = 4$)	Potter Peninsula (Antarctic)	40–194[b,c]	17–147		Weichbrodt et al. (1999)
Antarctic air ($n = 3$)	Signy Island (Antarctic)	1.1–1.4[b] fg/m^3	n.d.		Vetter et al. (2000)
Human milk ($n = 4$)	(Faeroe Islands)	12–230 ng/g			Vetter et al. (2000)
Bottlenose dolphins	Queensland, Australia	690–14,000[b]	230–8,800		Vetter et al. (2001a)
Common dolphin	Queensland, Australia	2,090[b]	175		Vetter et al. (2001a)
Green turtle	Queensland, Australia	15[b]	70		Vetter et al. (2001a)
Dugongs	Queensland	n.d.–246[b]	19–170		Vetter et al. (2001a)
Monk seal ($n = 14$)	Mauretania	9–117[b]			Vetter (2002)
African fur seal ($n = 1$) (blubber/ kidney/liver/lung)	Namibia	323/62/11/2.1)[b]	n.d.		Vetter (2002)
Air	Lista, southern Norway	detected			Vetter et al. (2002a)
White-tailed sea eagle	Norway	3–4 ng[c]			Herzke et al. (2005)
Fish liver *Gobionotothen gibberifrons* (1987/1996)	Antarctic	0.4/0.6 (mean)	1.1/2.1		Weber and Goerke (2003)
Fish liver *Champsocephalus gunnari* (1987/1996)	Antarctic	2.5/3.2	0.4/0.5		Weber and Goerke (2003)
Liver					
Fish liver *Chaenocephalus aceratus* (1987/1996)	Antarctic	4.0/4.9	0.9/1.8		Weber and Goerke (2003)

[a]Highest values in different samples; cocorrelation between the concentrations of Q1 and PCB 153.

[b]Concentrations corrected relative to estimations in the original papers where quantification was carried out using the response factor of *trans*-nonachlor, which turned out to be higher than that of Q1 (Vetter et al. 2003b).

[c]Wet weight.

in Antarctic than in Arctic air (Vetter et al. 2000). It should also be noted that Q1 concentrations, and that of anthropogenic POPs, in the Weddell Sea (which is more than 1,000 km south of Signy Island) were significantly higher than those close to the Antarctic convergence. Thus, it may be possible that the air in the Weddell Sea could contain higher concentrations of Q1 despite the lower air temperatures, which alter the air–water equilibrium in favor of the water phase.

Conclusions (Q1). Molecular modeling led to the observation that the planar pyrrole units have to undergo pyramidal configuration to surmount the barrier of the interannular N-pyrrole–C-pyrrole bond. Thus, Q1 cannot be planar, and this feature was made responsible for the very little or absent activity in the AHR assay (Vetter et al. 2004). Note that this is different from HDBPs, which were moderately toxic in the AHR test although estimated not to be planar as well. Q1 itself is symmetrical and thus a nonchiral molecule so that the hindered rotation about the interannular bond does not lead to atropisomers. However, an isolated Q1-Hex congener (**8**) was nonsymmetrical, and both features, hindered rotation and chirality due to the nonsymmetrical substitution pattern, lead to the formation of atropisomers, a feature than can be investigated by enantioselective gas chromatography. In fact, enantioselective analysis elucidated the structure in this particular case (Vetter and Jun 2002). The question for a natural product is this: Would Q1-Hex be naturally formed enantiopure or as a racemate? Because Q1 is not chiral and Q1-Hex was only chemically synthesized, and thereby of course was formed in racemic composition, this compound cannot be used to be studied in detail. However, several HDBPs are chiral and would be suitable for studying this problem. Unfortunately, any efforts undertaken to date to enantioresolve HDBP astropisomers failed thus far (Vetter et al., unpublished results, 2005). Although Q1 was detected in marine samples from all six continents (see Table 5), the highest concentrations in the ppm range are found in the Southern Hemisphere and particularly in samples from Australia.

C. Brominated Phenoxyanisoles (MeO-BDEs)

Brominated phenoxyanisoles are also named as brominated methoxydiphenyl ethers. The second name refers to the structural similarity with the man-made (poly)brominated diphenyl ethers (PBDEs), which are in wide use as flame retardants (see also Section III.C.). The currently widely applied nomenclature does not use IUPAC rules but "treats" the respective compounds as metabolites of BDEs. The resulting names have their starting point in the plain BDE, without a methoxy group. The IUPAC number valid for the same substitution pattern as for PCBs is assigned to this substructure. In a second step, the additional position of the methoxy group is determined. In this way, 2′-MeO-BDE 68 is the short name for

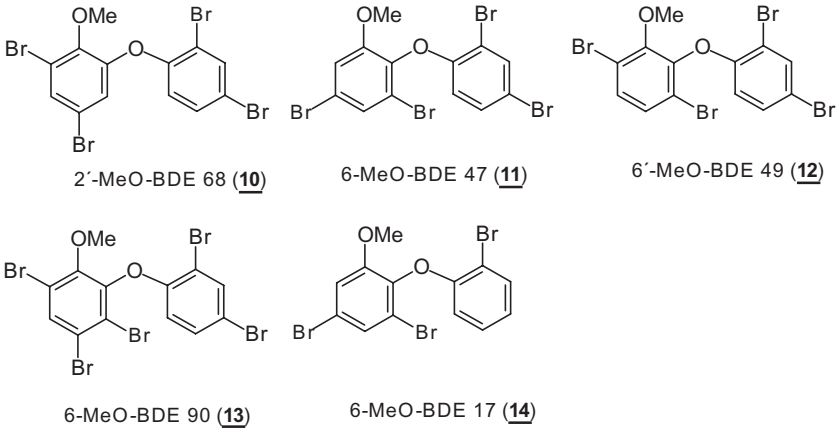


Fig. 7. Structures of relevant tetrabromophenoxyanisoles (**10,11,12**), as well as examples of a penta- and a tribromophenoxyanisole detected in marine fish and mammals (**13,14**).

2′-methoxy-2,3′,4,5′-tetrabromodiphenyl ether or 4,6-dibromo-2-(2′,4′-dibromo)phenoxyanisole (Fig. 7, **10**).

Since the initial paper by Sharma and Vig (1972), natural products chemists have discovered a wide range of tri- to hexabromophenoxyanisoles (or MeO-triBDEs to MeO-hexaBDEs), primarily in sponges but also in algae and cyanobacteria (Carté and Faulkner 1981; Fu et al. 1995; Elyakov et al. 1991; Kuniyoshi et al. 1985; Capon et al. 1981; Asplund et al. 2001; Cameron et al. 2000; Salva and Faulkner 1990; Handayani et al. 1997; Bowden et al. 2000; Agrawal and Bowden 2005; Malmvärn et al. 2005b). It is known that MeO-BDEs exist together with their unmethylated OH-BDE homologues. In the red alga *Ceramium tenuicorne*, for instance, OH-BDEs were about two orders of magnitude more abundant than MeO-BDEs (Asplund et al. 2001). 6-MeO-BDE 47 (**11**) and related compounds were also isolated from the tropical mollusk *Asteronotus cespitosus*, which feeds on sponges (Vetter et al. 2002b). Note, however, that the mollusk accumulated rather than synthesized the MeO-BDEs.

It would be curious if sponges, a species largely without lipids, were to produce highly lipophilic compounds such as MeO-BDEs. In fact, it was shown that cyanobacteria living on the sponge produce brominated secondary metabolites and that *Oscillatoria*, which has a high lipid content, is the original producer of MeO-BDEs (Unson et al. 1994; Moore et al. 2002). It has been suggested that sponges produce the brominated phenoxyphenols and that the cyanobacteria produce the methylether (anisole), which would partly explain the varying proportions between OH- and MeO-BDEs (Vetter and Jun 2003). Malmvärn et al. (2005a) indicated that cyanobacteria may be sources of OH-BDEs, MeO-BDEs, and PBDDs.

These observations may indicate time, or season-dependent variations but also complex varying patterns between individuals. The ecological and biological background on the natural formation of MeO-BDEs is beyond the topic of this article, but it should be borne in mind that variations in the HNP content within one or different populations may be more pronounced than for anthropogenic compounds that have been described with diverse models.

Historical Data, Identification, and Linking to Known Natural Sources. In 1997, Haglund et al. (1997) screened marine samples from the Baltic Sea for brominated aromatic compounds. They detected anthropogenic polybrominated diphenyl ethers (BDEs) including the dominated BDE 47 and three methoxy derivatives of tetrabromo diphenyl ethers (MeO-tetraBDEs), whereof the most abundant accounted for ~50% of the BDEs. Similarly, Asplund et al. (1999) found MeO-BDEs on one level with BDEs in Baltic salmon muscle and egg. The initial dilemma was, as discussed in these papers, that it could not be unequivocally clarified if these compounds were metabolites of BDEs or bioaccumulated HNPs (Haglund et al. 1997; Asplund et al. 1999). However, this uncertainty toward the origin of the MeO-BDEs in both papers stimulated an intense research program in Sweden that soon produced evidence for a natural origin of MeO-BDEs. Today it is known that the initially detected three MeO-tetraBDEs are 2′-MeO-BDE 68 (**10**), 6-MeO-BDE 47 (**11**), and 6′-MeO-BDE 49 (**12**), 6′-MeO-BDE 49 being the first eluting and least relevant congener (Asplund et al. 1999; Malmvärn et al. 2005a). In addition, 2′-MeO-BDE 68 and 6-MeO-BDE 47 were recently isolated from whale blubber and investigated by spectroscopic methods (Teuten et al. 2006).

As seen from the structural code, 6-MeO-BDE 47 differs from BDE 47, a key compound in products used as brominated flame retardants (BFRs), only in the MeO-substituent in the *ortho*-position (Asplund et al. 1999). Reports on residues of BFRs in the environment have increased of late (de Boer et al. 2000), and it seems, for lack of plausible other sources, that the unknown brominated compounds detected in marine organisms may be metabolites of BFRs. A study of the contamination of marine mammals in Australia led to the detection of a series of nonpolar brominated compounds (originally labeled BC-2, BC-1, BC-3, BC-10, and BC-11) including two MeO-tetraBDEs later identified as 2′-MeO-BDE 68 (BC-2) and 6-MeO-BDE 47 (Vetter et al. 2001a; Vetter 2001; Melcher et al. 2004). The concentrations of anthropogenic BFRs were very low, and a natural source was plausible. Shortly after, sponges (*Dysidea* sp.) from the same region were identified as the producers of 2′-MeO-BDE 68 and 2′,6-diMeO-BDE 68 (BC-11, see Section III.D) (Vetter et al. 2002b; Cameron et al. 2000); this was eventually the first direct identification of a bioaccumulated natural organohalogen in wildlife. Teuten et al. isolated 2′-MeO-BDE 68 and 6-MeO-BDE 47 from whale blubber and determined both $\Delta^{14}C$ and $\delta^{13}C$

ratios (Teuten et al. 2005a). Particularly, radiocarbon measurements confirmed the natural origin of these MeO-BDEs because the $\Delta^{14}C$ ratio of about +100 ppm was in range of new inorganic carbon in the Atlantic Ocean whereas technical BDEs, PCBs, and DDT had $\Delta^{14}C$ values of about –998 ppm (Teuten et al. 2005a).

Malmvärn et al. (2005a) identified the red alga *Ceramium tenuicorne* from the Baltic Sea as a producer of phenoxyphenols and phenoxyanisoles. Moreover, fish from the proximity of the algae also contained many of these HNPs. The algae not only contained the major MeO-tetraBDEs but also homologues. However, the MeO-BDE pattern in algae and fish was not identical (Malmvärn et al. 2005a). Varying concentrations and ratios of MeO-BDEs in individuals may be the result of different bioavailability, uptake, elimination, metabolism, and selective retention of the HNPs but also different ages, feeding behavior, and distribution of the investigated species; however, they may also indicate the presence of different natural producers found in different habitats that enter the food web in a different way (Melcher et al. 2005a).

Although different mechanisms may change the ratios of the dominating MeO-BDEs, 2′-MeO-BDE 68, and 6-MeO-BDE 47 on the way from the natural producer to high-trophic biota, it appears that marine mammals that received the MeO-BDEs from sponges, or associated organisms, are more abundant in 2′-MeO-BDE 68, whereas those originating from algae, or associated organisms, are dominated by 6-MeO-BDE 47. However, this hypothesis needs further clarification (see also below). In addition to 2′-MeO-BDE 68 and 6-MeO-BDE 47, several tri- to hexa-MeO-BDEs (see Fig. 7; **13** and **14** for examples) and even mixed halogenated phenoxyphenols and phenoxyanisoles were identified in higher marine biota (Asplund et al. 1999; Sinkkonen et al. 2004; Marsh et al. 2004a; Malmvärn et al. 2005a; Melcher et al. 2005a). Mixed halogenated (one Cl, several Br) phenoxyphenols and phenoxyanisoles point more toward algae being the natural producers, as mixed halogenated compounds in sponges are rare. This observation also produced evidence that OH-BDEs co-occur with MeO-BDEs (Fig. 8).

The biosynthesis of brominated phenoxyanisoles is not known in detail, but the natural MeO-BDEs known to date share the presence of the methoxy group in the *ortho*-position relative to the O-bridge. Thus, it was concluded that the phenol- or methoxy group occurs exclusively in the *ortho*-position. However, natural products chemists have isolated related HNPs with hydroxyl groups in the *para*-position (see Section III.D) (Higa et al. 1979).

Note that both 2′-MeO-BDE 68 and 6-MeO-BDE 47 have previously been mislabeled, and this has caused inaccurate citations in the initial phase of research (Asplund et al. 1999; Vetter et al. 2001a; Sinkkonen et al. 2004). The highest concentrations of MeO-BDEs detected in marine mammals are on one level with the highest concentrations of BFRs determined in environmental samples (Vetter et al. 2002b; de Boer et al. 2000).

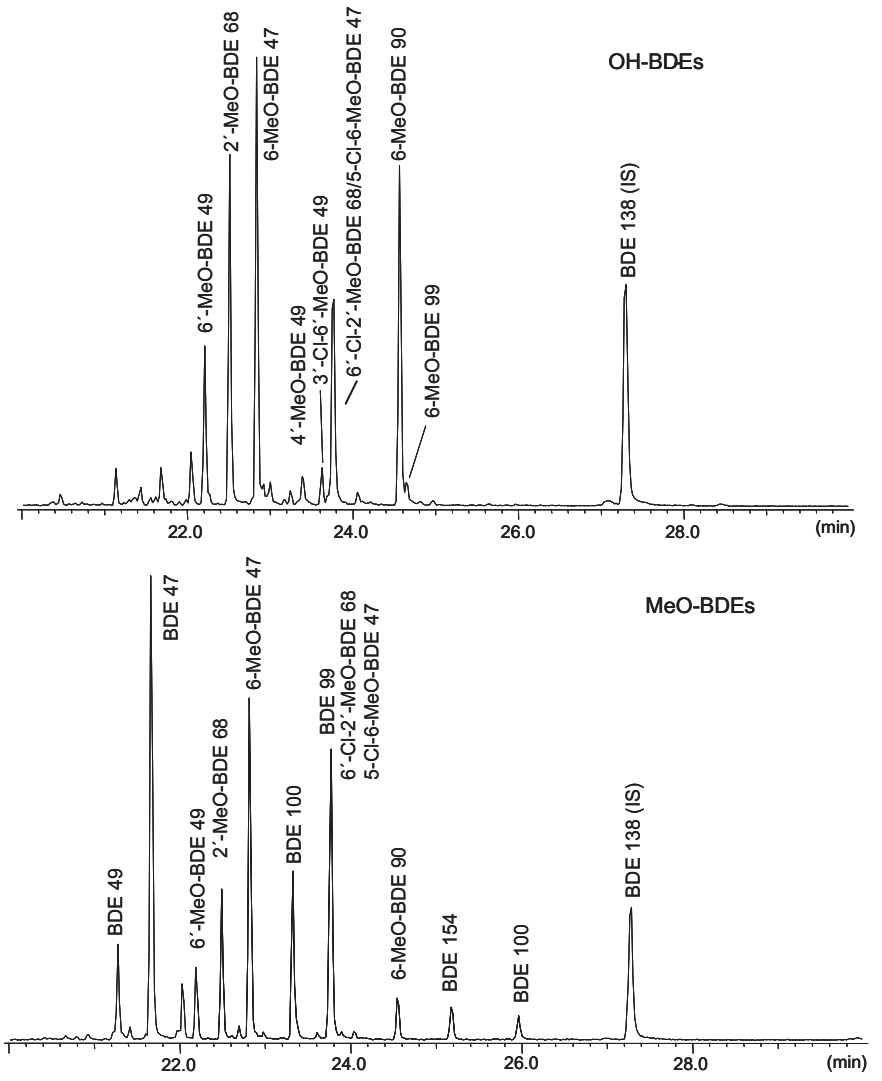


Fig. 8. GC/MS ion chromatogram (CP-Sil 8, *m*/*z* 79 and 81) of Baltic salmon blood. *Top*: OH-BDEs after conversion into the respective anisoles; *bottom*: the MeO-BDE fraction that was separated from the phenols before analysis (*bottom*).
Related MeO-BDEs of most OH-BDEs were detected in the sample (Marsh et al. 2004b).

Authentic MeO-BDE reference standards have been synthesized by several groups (Marsh et al. 1999, 2003, 2004a; Vetter and Jun 2003; Nikiforov et al. 2003), and 6-MeO-BDE 68 is commercially available from LGC Promochem whereas a range of both OH-BDE and MeO-BDE standards is available from CIL.

Analytical Aspects. A sensitive detection for MeO-BDEs is obtained with GC/ECNI-MS in the SIM mode using *m*/*z* 79 and 81 (Haglund et al. 1997; Vetter et al. 2001a). However, several potential coelutions with other brominated compounds in samples have been detected (Marsh et al. 2004a; Vetter et al. 2003b). Therefore, additional confirmation is recommended, which becomes more important if not only the two major MeO-tetraBDEs are analyzed but also tri-, penta-, and hexabromo phenoxyanisoles. Additional confirmation can be obtained by the determination of *m*/*z* 159 and *m*/*z* 161 ($[HBr_2]^-$), which is only abundant in GC/ECNI-MS spectra of BDEs with a bromine subsitutent in the *ortho*-position (see Section II) (Melcher et al. 2005a). This structure is fulfilled for all known naturally produced MeO-BDEs, but reductive debromination may lead to metabolites of natural MeO-BDE that do not contain any Br in the *ortho*-position (Melcher et al. 2005a).

GC/EI-MS is less sensitive, but the molecular ion is abundant and provides a much higher selectivity for MeO-BDEs (Pettersson et al. 2004). GC/EI-MS was shown to distinguish *ortho*- (these form $[M\text{-}94]^+$ fragment ions) from *para*- (these form $[M\text{-}15]^+$ fragment ions) and from *meta*-substituted MeO-BDEs (these do not show the respective fragment ions observed in the EI-MS of the isomers) (Marsh et al. 2003, 2004b). With this information, the unknown MeO-tetraBDE by-product of the synthesis of 2′-MeO-BDE 68 (Vetter and Jun 2003) is also an *ortho*-MeO-tetraBDE.

It became evident that, besides MeO-BDEs, occurrence of OH-BDEs should be explored as well. Although the latter are usually detected in blood but not in blubber, the co-occurrence of both classes is an important feature, and it is still known where the transfer of the phenols into the anisoles actually takes place. Because the halogenated phenoxyphenols have to be methylated before GC analysis, a preseparation step of OH-BDEs and MeO-BDEs is necessary, which allows the individual determination of both classes of compounds (see Fig. 8). Several methods have been developed, for instance, for the determination of OH-PCBs, and are mostly based on KOH partitioning as used by Malmvärn et al. (2005a). Verrevault et al. (2005) reported the presence of 3-MeO-BDE 47 in glaucous gulls (*Larus hyperboreus*) from the Norwegian Arctic, which, owing to the methoxy-group in the *meta*-position, would not support a natural source for this compound. However, it was mentioned by the authors that due to the known coelution with 2′-MeO-BDE 66 (Marsh et al. 2004a), it may also be this congener or a mixture or both (Verreault et al. 2005). In these samples, both MeO-BDEs and OH-BDEs were detected along with high concentrations of anthropogenic BDEs (Verreault et al. 2005). Thus, these samples were suspected of containing MeO-BDEs and OH-BDEs of both natural and anthropogenic origin. In this study, 2′-MeO-BDE 68 was not detected at noticable concentrations; however, 6-MeO-BDE 47 was (Verreault et al. 2005). Moreover, 2′-MeO-BDE 68 was not detected in polar bears from Svalbard (Nikiforov et al. 2003). These examples indicate that determina-

tion of the natural source of MeO-BDEs is complex. Nevertheless, it is unequivocally clear that natural sources do exist (Vetter et al. 2002b; Teuten et al. 2005a). In the laboratory, OH-BDEs can be prepared from MeO-BDEs by treatment with BBr_3 in 1,2-dichloroethane (Francesconi and Ghisalberti 1985; Vetter and Jun 2003).

Distribution and Concentrations of MeO-BDEs in the Environment. Table 6 lists selected concentrations determined in environmental samples. In the first article on MeO-BDEs, they were not detected in human adipose tissue (Haglund et al. 1997). Fish samples from different locations, except Arctic guillemot and freshwater fish, contained MeO-BDEs (Sinkkonen et al. 2004). MeOBDEs up to several mg/kg were detected in dolphins from Australia (Vetter et al. 2001a; Melcher et al. 2005a). Except for the Australian cetaceans, 6-MeO-BDE 47 is usually more abundant than 2′-MeO-BDE 68 (see Table 6).

Concentrations of 2′-MeO-BDE 47 in blood of nestlings of white-tailed sea eagles from the Swedish Baltic coast increased from May to June, i.e., the period at which the potential bioproduction (green algae blooms) occurs. Such an increase was not observed for anthropogenic POPs (Olsson et al. 2000). In four locations from the Baltic Sea, MeO-BDEs were ~20 fold more concentrated than in the Kattegatt (North Sea) (Asplund et al. 2004).

Malmvärn et al. (2005a) investigated the presence of OH-BDEs and MeO-BDEs in algae and fish living in the same habitat. Although many compounds were found in both species, some were not. For instance 6′-OH-BDE 49 and 4′-OH-BDE 49, which was suspected to be a metabolite of anthropogenic PBDEs, were not found in the algae. However, there are too many parameters that remained unknown to put these findings in the right light. For instance, the investigated algae may not necessarily be the only natural producer of OH- and MeO-BDEs in the habitat.

Kierkegaard et al. (1999) studied BDE 47 and 6-MeO-BDE 47 in pike taken between 1968 and 1996 in Lake Bolmen (Sweden). Although the anthropogenic BDE 47 showed an increasing trend, the MeO-BDE decreased in the same period. If 6-MeO-BDE 47, was the metabolite of BDE 47, one could have expected an increasing trend for 6-MeO-BDE 47 as well. This finding supports the thinking that the bulk of the MeO-BDE goes back to natural production (Kierkegaard et al. 1999). However, an increasing trend of euthrophication has been observed in the Lake since the 1970s (Kierkegaard et al. 1999), which suggests that the natural producer of 6-MeO-BDE 47 has been driven out by organisms that found favorable life conditions in the changed ecosystem.

It is noteworthy that Asplund et al. (1999) detected both MeO-BDEs and OH-BDEs in salmon blood. Already in this review it was proposed that both classes of compounds have the same origin. The levels of the OH-BDEs were estimated to account for ~30% of the MeO-BDEs. Hovander

Table 6. Concentrations (selection) of MeO-BDEs (ng/g lipids) in the marine environment and food.

Species	Location	2′-MeO-BDE 68 (ng/g)	6-MeO-BDE 47 (ng/g)	Sum MeO-BDEs (ng/g)	Source
Salmon blood plasma (lw)	Baltic Sea	~60[a]	~170[a]	~270 ng/g	Asplund et al. (1999)
Herring	Baltic Sea			7.4–34	Haglund et al. (1997)
Grey and ringed seal blubber (liver)	Baltic Sea	8.5–40 (0.2–1.0)[a]	95–160 (1.5–1.8)[a]	121.5–220 (2–3.7)	Haglund et al. (1997)
Fish oil		0.1–8.7[a]	0.3–28[a]	0.4–30	Haglund et al. (1997)
Salmon muscle		8[a]	28[a]	30	Haglund et al. (1997)
Dolphins Different species	Mediterrrane an Sea	<1–167[a]	<1–628[a]	<3–808	Petterson et al. (2004)
Arctic cod	Arctic, Norway			0.3–17[a]	Sinkkonen et al. (2004)
Salmon	Atlantic			3.5–6.8	
Salmon	Baltic Sea				
Cetaceans	Australia	1,200–11,200	790–1,910	100–1,530	Melcher et al. (2005a)
Crocodile eggs	Australia	57–69	200–240	Not determined	Melcher et al. (2005a)

[a]Peak assignment by the present author derived from data published in meantime.

et al. (2002) detected 6-OH-BDE 47 in human plasma (peak 39) but its source could not be identified.

Another interesting finding was addressed by Melcher et al. (2005a) on their detection of eight MeO-triBDE congeners structurally related to 2′-MeO-BDE 68 and 6-MeO-BDE 47 in samples from Australia. The lack of identification of several MeO-triBDEs by natural products chemists indi-

cated that, at least to some degree, metabolism of 2′-MeO-BDE 68 and 6-MeO-BDE 47 has played an important role in the formation of MeO-triBDEs. Their formation, however, may not have occurred in dolphins but could also have occurred during transfer from the natural producer to marine mammals via the food chain (Melcher et al. 2005a). This report appears to be one of the first hints in the literature that not only HNPs itself but also their transformation products can be found in the environment.

Conclusions. It has to be repeated that evaluation of the natural contribution to the environmental load of OH- and MeO-BDEs is still not possible. With respect to anthropogenic sources there are open questions as well. For instance, hydroxylation of BDEs is known to occur in a similar manner as hydroxylation of PCBs, for instance, in marine mammals and in fish. However, the methylation step is somewhat strange because MeO-PCBs have not been described in the same context. The ecological role of the natural MeO-BDEs appears to be chemical protection (Gribble 1999). 6-MeO-BDE 47 exhibited antibacterial activity against *Escherichia coli* and other microorganisms (Carté and Faulkner 1981; Kuniyoshi et al. 1985) and acted as enzyme inhibitors (Fu et al. 1995). In another study, 2′-OH-BDE 68 was inactive in two biotests (Lui et al. 2004).

D. Compounds Related to Brominated Phenoxyanisoles

Compounds related to MeO-BDEs are MeO-BDDs, diMeO-BDEs, and diMeO-BBs (Fig. 9, **15**–**19**) all of which have been identified by natural product chemists and/or in higher organisms. These structures are of particular interest because they resemble very closely those of anthropogenic or toxic compounds. In 1981, Carté and Faulkner suggested the presence of brominated dibenzo-*p*-dioxins in sponges but could not confirm the presence of 2,4,7-tribromodibenzo-*p*-dioxin (2,4,7-triBDD) in the poriferans (Carté and Faulkner 1981). However, some 20 yr later Utkina et al. (2001, 2002) identified 4-MeO-1,2,6,8-tetraBDD (**15**), 5-MeO-1,3,6,7-tetraBDD (**16**), and 5-MeO-1,3,7-triBDD (**17**) and the respective OH-PBDDs in sponges (*Dysidea herbacea*) from Northwest Australia. Although the chemical names of the three dibenzo-*p*-dioxin derivatives may give the impression that the compounds have completely different structures, there is actually only one Br substituent that is different between (**15**) and (**16**), and in (**17**) only the varying Br is missing (see Fig. 9). The differences arise due to the chemical naming system based on the unsubstituted PBDD, which receives different labeling for (**15**) and (**16**). So far, the MeO-PBDDs have not yet been found accumulated at a higher trophic level. Vetter and Wu (2003) used charcoal chromatography and found no evidence for the presence of an abundant PBDD derivative in the investigated dolphins. Very recently, however, unsubstituted di- and tribromodibenzo-*p*-dioxins of yet unknown isomer structure were determined by Haglund et al. (2005) in pike

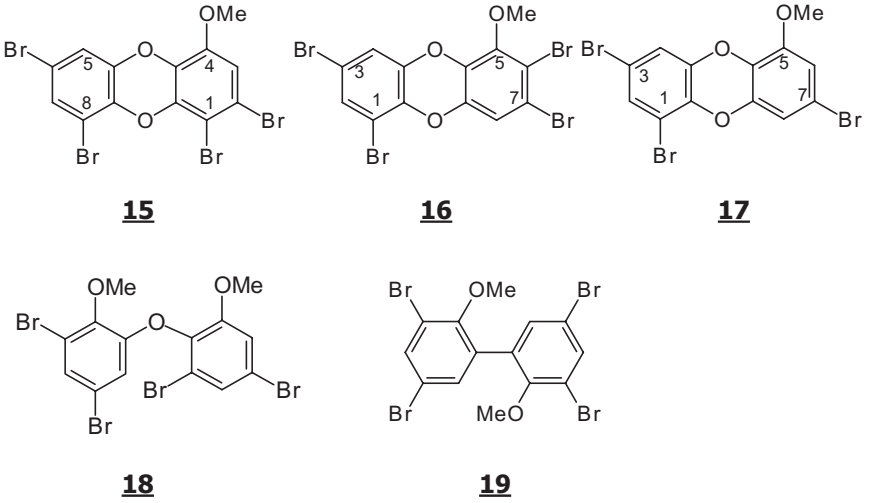


Fig. 9. Structures of methoxylated tri- and tetrabromodibenzo-*p*-dioxins (**15**,**16**,**17**), 2′,6-dimethoxy-2,3′,4,5′-tetrabromodiphenyl ether (BC-10, **18**), and 2,2′-dimethoxy-3,3′,5,5′-tetrabromobiphenyl (2,2′-diMeO-BB 80, **19**).

from Kvädöfjärden (Baltic Sea). Owing to the unique presence at this site, it was proposed that the PBDDs may be of natural origin. However, this hypothesis requires a more thorough investigation, which is surely in process (see also next paragraph).

By contrast, dimethoxylated BDEs were unequivocally identified in marine mammals and food samples (Vetter et al. 2002b). Although diverse congeners including dihydroxy and hydroxylmethoxy derivatives were identified by natural product chemists (Norton and Wells 1980; Carté and Faulkner 1981; Utkina et al. 1987; Elyakov et al. 1991; Fu et al. 1995; Cameron et al. 2000; Voinov et al. 1991; Vetter et al. 2002b; Lui et al. 2004), only 2,6′-diMeO-BDE 68 (the molecular ion is found at *m*/*z* 542) have been described in marine mammals to date (Vetter et al. 2002b; Vetter and Jun 2003). 2,6′-diMeO-BDE 68 concentrations ranging from 0.2 to 49 ng/g were determined in marine mammals from the Pacific Ocean (Marsh et al. 2004b). It was reported that 2,6′-diMeO-BDE 68 and BDE 99 may coelute on DB-5-like columns (Vetter and Jun 2003). Because halogenated MeO-BDEs have also been detected at the retention time of BDE 99 (see Fig. 8b), the peak purity at the respective retention time should be checked, irrespective of the compounds to be analyzed. Fig. 10 illustrates that 6-OH-2′-MeO-BDE 68 (**20**) may be a precursor of both 2,6′-diMeO-BDE 68 (**18**) and the hypothetic 4-MeO-1,3,5,7-tetraBDD (**21**) (Vetter and Jun 2003). Note that reaction of the desmethoxy derivative of **20** to **21** would lead to plain PBDDs, as were descibed by Haglund et al. (2005). 2,6′-diMeO-BDE 68 and isomers did not show inhibitory activity in two bioassays (micro-

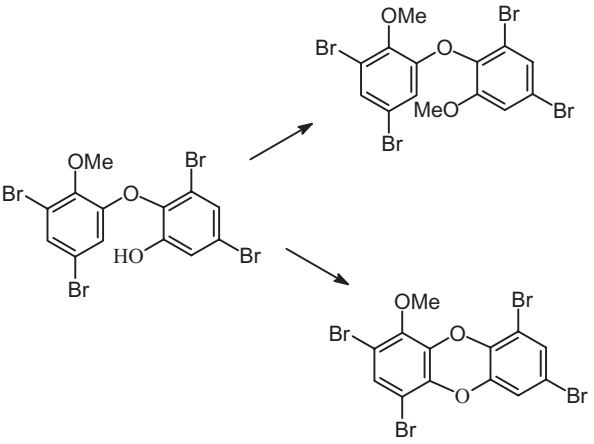


Fig. 10. Proposed mechanism of the formation of 2′,6-dimethoxy-2,3′,4,5′-tetrabromodiphenyl ether (BC-10, **18**) and 4-methoxy-1,3,5,7-tetrabromodibenzo-*p*-dioxin (21) from 6-hydroxy-2′-methoxy-2,3′,4,5′-tetrabromodiphenyl ether (**20**) (Adapted from Vetter and Jun 2003 with permission from Elsevier).

tubule proteins and meiotic maturation of starfish oocytes). The same was found for hydroxymethoxy derivatives whereas diOH-BDEs were active (Lui et al. 2004). Obviously, one hydroxyl group only or two methoxy groups reduced the bioactivity.

Marsh et al. (2004b) synthesized 2,2′-diMeO-BB 80 (**19**) and determined that this compound was identical with one previously detected in marine mammals from Australia (molecular ion *m*/*z* 526) (Vetter et al. 2001a). The abundance in Australian samples along with nondetectable PBBs and the identification of other HNPs in the samples (Fig. 11) clearly supports the natural source of 2,2′-diMeO-BB 80 (Vetter et al. 2001a). However, natural products chemists have not yet detected the compound. Note that peak assignment of BC-1 (2,2′-diMeO-BB 80) and BC-2 (2′-MeO-BDE 68) was switched in the initial work by Vetter et al. (2001a, 2002b) because both compounds eluted in reversed order from DB-5 and β-BSCD GC columns. Thus, the 2,2′-diMeO-BB 80 concentrations in Australian samples (erroneously labeled BC-2 in table 3 of Vetter et al. 2001a) were 250–4.100 ng/g in dolphins, 26 ng/g in a green turtle, and l03 ng/g in a dugong sample (Vetter et al. 2001a), whereas cetacean samples from the Pacific Ocean contained 12–800 ng/g of lipid weight (Marsh et al. 2004b).

Higa et al. (1979) found brominated hydroquinones and substituted diphenylethers and triphenylethers (Fig. 12; **22**–**26**) in worms. These compounds may stem from the dimerization of the respective hydroquinones also detected in the samples. It is noteworthy that these dimers and trimers have hydroxyl groups in the *para*-position (Higa et al. 1979). Therefore, it cannot be excluded that natural brominated 4-phenoxyphenols also exist.

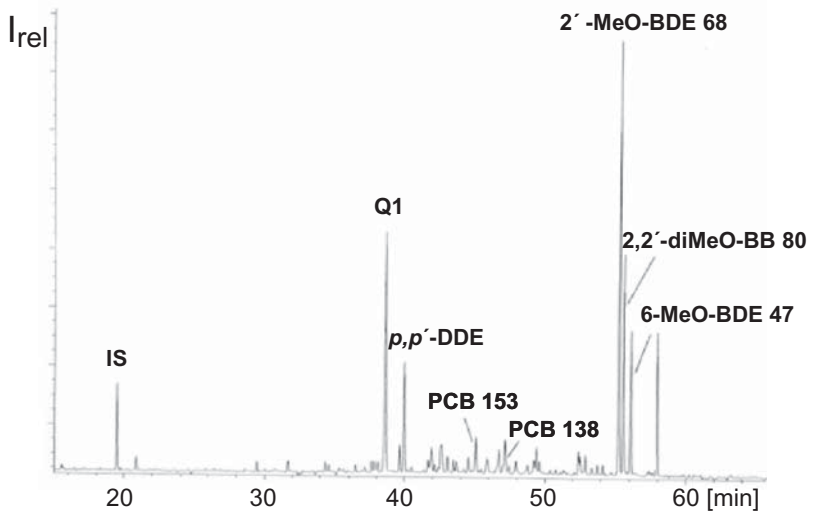


Fig. 11. GC/ECD chromatogram of common dolphin (*Delphinus delphis*) from Australia (Adapted from Vetter et al. 2001a with permission from Springer). Note that the peak assignment was corrected.

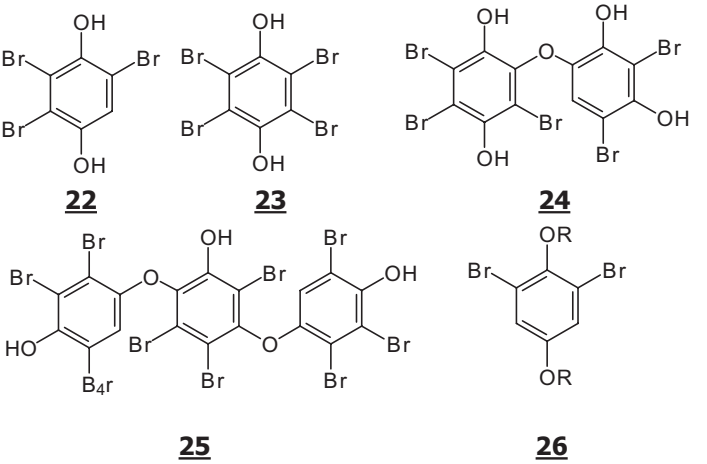
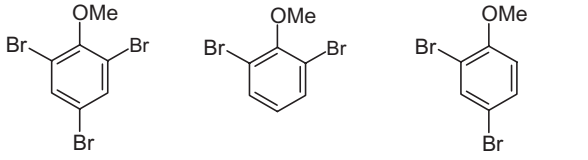


Fig. 12. Structures of bromoquinones and related compounds (**22**–**26**) (Illustrating Higa et al. 1979).

E. Brominated Phenols and Anisoles

2,4,6-Tribromoanisole (Fig. 13; **27**), structurally related bromo- and dibromoanisoles (**28**, **29**), and the corresponding bromophenols are regularly detected in marine fish by food control laboratories. However, publications in this field are relatively scarce (Rimkus and Wolf 1991; Miyazaki et al.



2,4,6-tribromoanisole **(27)** 2,6-dibromoanisole **(28)** 2,4-dibromoanisole **(29)**

Fig. 13. Structures of the most prominent natural bromophenols. 2,4,6-tribromoanisole (2,4,6-TBA or TBA, **27**), 2,6-dibromoanisole (2,6-DBA, **28**), and 2,4-dibromoanisole (2,4-DBA, **29**).

1981; Watanabe et al. 1983; Whitfield et al. 1998; Vetter et al. 2001b; Vetter and Stoll 2002). Bromoanisoles are also known as brominated methoxybenzenes.

As shown in Section III.C, brominated phenols and anisoles are linked to each other, so that when looking for sources, that which is said about anisoles is also valid for phenols and vice versa. Anisoles are readily produced from the respective phenols by microorganisms (Neilson et al. 1983) and fungi (Whitfield et al. 1997c). Brominated phenols and anisoles have both anthropogenic and natural sources. Owing to the low molecular weight and the volatility, dibromo- and tribromoanisoles were made responsible for a musty, corked off-flavor in wine (Chatonnet et al. 2004) and the acknowledged seafood flavor in fish and shrimps (Whitfield et al. 1998; Ma et al. 2005). The first one is most likely going back to anthropogenic sources whereas the second one is attributed to naturally produced compounds. Whitfield et al. (1998) studied a wide range of seafish and found bromophenols abundant in marine fish except for carnivorous pelagic species. Apart from these examples, the differentiation of the two potential sources is often ambiguous, and their abundance in coastal samples may in fact resemble a mixture of both sources. However, the only relevant anthropogenic sources for 2,4,6-TBP are its use (i) as a flame retardant in epoxy, polyurethane, plastics, paper, and textiles and (ii) as an important intermediate for the production of other commercial high-molecular weight flame retardants and fire extinguishing media (Eriksson et al. 2004; Hakk et al. 2004). Furthermore, 2,4,6-TBP is used as an impregnating agent by the wood industry (Mardones et al. 2003). These industrial application rates of TBP could never explain the high amounts and structural diversity of bromophenols and bromoanisoles detected in the environment.

Naturally occurring bromophenols and-anisoles are produced by marine worms (Higa et al. 1979; Gribble 2000), algae (Flodin and Whitfield 1999a), and probably also sponges (Vetter and Janussen 2005). It was demonstrated that the green algae *Ulva lactuca* contains a bromoperoxidase able to

convert phenol, 4-hydroxybenzoic acid, and 4-hydroxybenzylalcohol into bromophenols but failed using L-tyrosine as substrate (Flodin and Whitfield 1999a,b). In this process, only bromophenols with substituents in *ortho*- and *para*-positions were obtained (Flodin and Whitfield 1999a).

Given their relevance as flavor compounds, they are volatile and so it is no surprise that bromoanisoles have been detected in air samples from different locations including the Arctic and the Antarctic (Wittlinger and Ballschmiter 1990; Pfeifer et al. 2001; Führer et al. 1996; Führer and Ballschmiter 1998; Vetter et al. 2002a; Melcher et al. 2005b). Bromoanisoles are readily phototransformed; thus, it was concluded that the occurrence of bromoanisoles in air nearly excludes any long range transport (Wittlinger and Ballschmiter 1990). High concentrations in air from polar regions confirms the prediction that the majority of the compounds found in the environment are of natural origin. It was shown that weekly concentrations of TBA and 2,4-DBA in air do not follow the pattern of HCHs with similar volatility, thus supported their natural origin (Melcher et al. 2005b). The annual profile, low concentrations in spring, and high concentrations in summer that remained high until the end of the year, was virtually identical to the AOBr concentrations determined in surface waters with bioproduction of organobromines (Putschew et al. 2003).

When bromophenol-producing algae were added to fish food, the marine flavor was transferred to the fish by uptake of the bromophenols (Ma et al. 2005). Bromophenols are known compounds in blood of mammals and man, and recently TBP was detected in the blubber of seals, albeit at low concentrations (Vetter and Janussen 2005). Führer et al. (1996) determined that in theory there are 19 bromoanisoles (bromophenols), 19 chlorophenols, and 96 mixed halogenated anisoles (phenols). The PC parameters (see Table 3) indicate that bromoanisoles can evaporate into the atmosphere, are soluble in water, and can be accumulated by organisms in the aquatic environment (Pfeifer et al. 2001).

Historic Data, Identification, and Linking to Known Natural Sources. As mentioned, there are several organisms that can convert halogenated phenols into their respective anisoles. In addition, both compound classes can stem from anthropogenic and natural sources. On a first approximation, 2,4-DBA, 2,6-DBA, 2,4,6-TBA, 2,4-Br-6-Cl-THA, and 2,6-Br-4-Cl-THA originate from natural sources, whereas 2,4,6-TCA, 2,3,4,6-TeCA, and PCA originate mostly from natural sources (Ballschmiter 2003). The most relevant bromophenol and bromoanisole standards (**27**–**29** and others) can be purchased from different suppliers.

Analytical Aspects. The brominated phenols can be analyzed with all common standard cleanup techniques, but solvent concentration steps have to be carried out with care due to the high volatility of bromoanisoles. Therefore, SPME/headspace and stir-bar sorptive extraction techniques

have been developed for the determination of 2,4,6-TBA in aqueous samples and wine (Alzaga et al. 2003; Benanou et al. 2003). Comprehensive data of GC retention times of diverse halogenated anisoles were published by Pfeifer et al. (2001). For instance, bromophenols and bromoanisoles elute before HCH isomers from DB-5-like GC columns (Vetter and Janussen 2005). It is also noteworthy that TBP can be analyzed by GC without derivatization, which is not possible for OH-BDEs. Another peculiarity of bromophenols is the high proportion of the molecular ion (TBP, *m*/*z* 328; DBP, *m*/*z* 250), whereas the bromide ion is lower.

Distribution and Concentrations of Bromoanisoles and Bromophenols in the Environment. In freshwater fish and mussels from the North Sea, 2,4,6-TBA concentrations were usually 10–20 ng/g lw (lipid weight) but could reach up to 300 ng/g lw; under these conditions 2,4,6-TBA was the dominating peak in the chromatograms (Rimkus and Wolf 1991). On the other hand, the absence of 2,4,6-TBA in seal blubber and gull eggs from the same region indicated a low potential for bioaccumulation. It was suggested that the residues were from natural sources (Rimkus and Wolf 1991).

Air samples from the 1980s give rise to the presence of chloroanisoles and 2,4,6-TBA (Atlas et al. 1986; Wittlinger and Ballschmiter 1990). Concentration of 2,4,6-TBA was 18 ± 5 pg/m^3 in air from New Zealand (July–August 1985), 19 ± 10 pg/m^3 in air from American Samoa (August 1981), and 55 ± 24 pg/m^3 in air from the Gulf of Mexico (August 1981) (Atlas et al. 1986). Air from the coast of southern Norway contained ~35 pg/m^3 of 2,4,6-TBA in May 1999, which was on the same level as both α-HCH and lindane (Vetter et al. 2002a). Similar concentrations were determined for these three compounds (Melcher et al. 2005a). However, although the anthropogenic HCH isomers showed a decreasing trend in autumn and winter, 2,4,6-TBA concentrations remained much higher. This trend was even more pronounced for 2,4-DBA, which pointed to different sources for HCH isomers and bromoanisoles, with the latter most likely arising predominantly from natural production (Melcher et al. 2005a). Air from the lower troposphere of the southern Indian Ocean at Réunion (March 1986) contained 8–30 pg/m^3 bromoanisoles (Wittlinger and Ballschmiter 1990), suggesting a widespread distribution in the marine environment. During a cruise through the East Atlantic Ocean, relatively low haloanisole concentrations were detected, except at one site close to Cape Verde Islands, where 17 of the possible bromoanisoles were detected at concentrations that were in the range of 1 ng/m^3 and above (Führer et al. 1997). Halogenated anisoles in real air samples could not be trapped quantitatively on Envi-Carb/silica gel, most likely because of the higher air temperature in comparision to the method development (Führer et al. 1996). Highest concentrations were found for 3,4,5-TCA (99,000 pg/m^3), 2,3,4,5-TeCA (15,100 pg/m^3), and 2,4-DBA (2,310 pg/m^3), whereas 2,4,6-TBA accounted only for 170 pg/m^3 (Führer et al. 1996). The bromoanisole con-

centrations by far exceeded those in terrestrial air above a wastewater treatment plant in Germany (Führer et al. 1996). The high reactivity of bromoanisoles with hydroxyl radicals or transformation by photolysis makes any transport from the Northern to the Southern Hemisphere unlikely (Führer and Ballschmiter 1998). Consequently, the bulk of the marine environmental contamination with bromophenols and bromoanisoles likely originates from biogenic sources (Führer and Ballschmiter 1998). Although chlorinated anisoles in marine air and water more likely stem from anthropogenic processes (Pfeifer and Ballschmiter 2002), mixed halogenated anisoles can also be formed by light-induced Br → Cl exchange (Müller and Crosby 1983).

Pacific salmon from saltwater contained 6–35 ng/g bromophenols whereas freshwater fish from North America contained virtually no bromophenols (Boyle et al. 1992), which illustrates that the natural producers, if this is the source, are not ubiquitously distributed. Bromophenol content (sum of the concentration of 2-BP, 4-BP, 2,4-DBP, 2,6-DBP, and 2,4,6-TBP) in Australian prawns was 10–580 ng/g (*Penaeus plebejus*), 13–166 ng/g (*P. esculentus*), 36–1,100 ng/g (*P. latissulcatus*), and 12–570 ng/g in five other wild-harvested species, whereas commercially pond-raised animals contained no bromophenols (Whitfield et al. 1997a). Heads, including gut, of prawns contained higher bromophenol content than tails (range, 1.3–36 ng/g; mean, 6.8 ng/g) (Whitfield et al. 1997a). Each of the bromophenol congeners was dominant in at least 2 of 30 samples, but in 15 samples 2,4-DBP (**30**) was dominant (Whitfield et al. 1997a).

Owing to their lower bromophenol content (0.3–1.3 ng/g), cultivated prawns lack the desired seafood flavor, which results in a lower taste quality of farmed prawns (Whitfield et al. 1997b). However, a feeding study with cultivated prawns resulted only in a limited uptake of the bromophenols by the prawns, which was attributed to the chemical form of application (Whitfield et al. 2002).

Fish (benthic carnivores) contained up to 2,400 ng/g bromophenols (up to 2,300 ng/g arising from 4-bromophenol). The concentrations in 87 species of algae from East Australia, the potential source for bromophenols, was 0.9–2,590 ng/g ww (Whitfield et al. 1999). In the algae, the highest contribution was from 2,4,6-TBP when the bromophenol content was higher than 250 ng/g ww but could arise from other bromophenols when total bromophenol content was lower (Whitfield et al. 1999).

Recent investigations confirm that sponges and other marine organisms contain a wide range of bromophenols and other brominated compounds, which seem to originate from biogenic sources (Flodin and Whitfield 2000; Kotterman et al. 2003; Whitfield et al. 1997b; Shoeib et al. 2004; Vetter and Janussen 2005).

Conclusions. Despite the varied distribution of bromophenols and bromoanisoles in the marine ecosystem, 2,4,6-TBA appears to be the major

congener that has reached fish. The reason may be a higher lipophilicity and the higher persistence due to the lack of vicinal hydrogens (a feature that simplifies metabolism). Recently, a bacterial strain was isolated from estuarine sediments that is able to dehalogenate 2,4,6-TBA and related substrates into phenol (Boyle et al. 1999). Interestingly, the predominant *ortho*- and *para*-substituted bromoanisoles were all dehalogenated whereas the unusual 3-BP and 2,3-DBP were not transformed by the bacterium (Boyle et al. 1999). Similar results were also found during incubation of bromophenols with anaerobic sediments (Ronen and Abeliovich 2000).

Halogenated phenols and anisoles are widespread in nature. It was suggested that they may be the precursors of more complex HNPs. A plausible formation pathway would be the dimerization of bromophenols (oxidative, **30** + **30**, or via HBr elimination, **30** + **31**) to give the phenoxyphenol **32** followed by methylation to give the phenoxyanisole **10** (Fig. 14). This oxidative reaction scheme is similar to the intramolecular dibenzo-*p*-dioxin formation as discussed for Fig. 10. Similarly, 2,2′-MeO-BB 80 (**19**) may be formed as an artifact from two 2,4-DBP units. This reaction, in turn, may be the same that leads from simple pyrroles to bipyrroles.

In contrast to marine HNPs, little information on terrestrial HNPs and their environmental relevance is currently available. However, it was noted that the bacterium *Alicyclobacillus acidoterrestris* is able to produce 2,6-DBP and 2,6-DBP in shelf-stable juices (mixed-fruit drinks), adding to them a recognizable disinfectant taint (Jensen and Whitfield 2003). Phenolic compounds in the juices were the substrates. The taste threshold of 2,6-DBP in water is 0.5 ng/L. Particularly when present at lower concentrations, HNPs may reach the consumer in this manner.

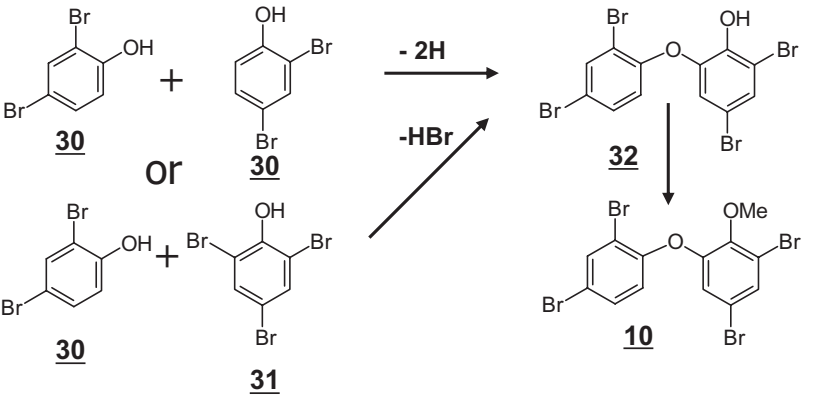
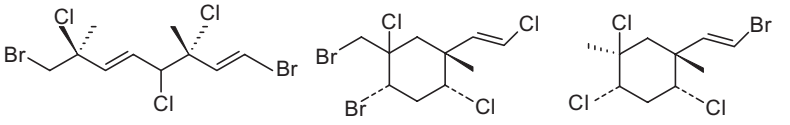


Fig. 14. Suggested formation of phenoxyphenols **32** from 2,4-dibromophenol (2,4-DBP, **30**) and 2,4,6-tribromophenol (2,4,6-TBP, **31**).

F. Mixed Halogenated Monoterpenes (MHC-1)

The key chromatogram that led to the investigation of the so-called mixed halogenated compound 1 (MHC-1) has already been shown (see Fig. 1). The elemental composition was determined using HRMS to be $C_{10}H_{13}Br_2Cl_3$ (Vetter et al. 2001b), which is met by halogenated monoterpenes with two double bond equivalents. Interestingly, MHC-1 is the only HNP discussed in this review without hetero atoms other than halogens. Gribble's very valuable review articles on halogenated natural products indicate that this composition equals that of two secondary metabolites **33** and **34** isolated from marine algae (Gribble 2000; Higgs et al. 1977; Stierle and Sims 1979; Jongaramruong and Blackman 2000). These algae are widespread so that bioproduction of MHC-1 is likely to occur at different places (Vetter et al. 2001b). The detection of MHC-1 correlated with the habitats of the red alga *Plocamium cartilagineum*, a known producer of related halogenated monoterpenes (Higgs et al. 1977; Stierle and Sims 1979; Vetter and Jun 2003). Thus, analysis of this seaweed may be suitable for identifying the natural producer and subsequently the exact isomer structure of MHC-1. One of the potential structures of MHC-1 (**34**) is similar to telfairine (**35**), which is 100% lethal to mosquito larvae at 10 ppm (Higgs et al. 1977; Stierle and Sims 1979). In addition, (**33**) and four other structurally similar halogenated monoterpenes from *Plocamium cartilagineum* were mutagenic in the Ames test (Leary et al. 1979) (Fig. 15).

Historical Data, Identification, and Linking to Known Natural Sources. MHC-1 was initially identified as an abundant peak in the gas chromatograms obtained from commercial fish samples under food control routine inspection. Fish is regularly controlled by food laboratories, and under such a study, a compound previously not detected in the GC/ECD chromatograms of comparable samples was detected (J. Hiebl, personal communication, 1998). Owing to similar GC retention times, it was first suspected to be Q1 (see Section III.B) but GC/MS analysis confirmed the presence of both Br and Cl. A mass spectrometric study gave evidence of a mixed halogenated monoterpene (Vetter et al. 2001b). The exact isomeric structure of MHC-1 is still unknown. Given the several asymmetrical



33 **34** **35**

Fig. 15. Halogenated natural products that are isomeric to MHC-1 (**33**,**34**) as well as the related monoterpene telfarine (**35**), a known natural pesticide.

carbons on halogenated monoterpenes, a total synthesis of MHC-1 cannot be expected in the near future. Therefore, efforts should be undertaken to isolate sufficient amounts from the natural producer.

Analytical Aspects. MHC-1 eluted from DB-1 and DB-5 columns between *trans*- and *cis*-chlordane and slightly before Q1 (Vetter et al. 2001b). Abundant fragment ions are present neither in the GC/EI-MS nor in the GC/ECNI-MS spectra (Vetter et al. 2001b). The molecular ion at *m*/*z* 396 is very small in GC/ECNI-MS but is detectable in GC/EI-MS and displays a dibromo-trichloro isotope pattern.

GC/ECNI-MS identification of MHC-1 in sample extracts is possible using *m*/*z* 158/160 (95%) and *m*/*z* 114/116 (50%), along with *m*/*z* 79/81 (100%) (Vetter 2001). Screening for both the bromide ion and the $[BrCl]^-$ ion together with the retention time range above enables an unequivocal determination of MHC-1 (Vetter et al. 2001b; Vetter 2001; Vetter and Janussen 2005). MHC-1 is stable against H_2SO_4 and is usually found in one fraction together with the chloropesticides (hexachlorocyclohexanes, chlordane, toxaphene) (Vetter et al. 2001b). Because no reference standard is available, *trans*-chlordane has been used for semiquantitative purposes (Vetter et al. 2001b).

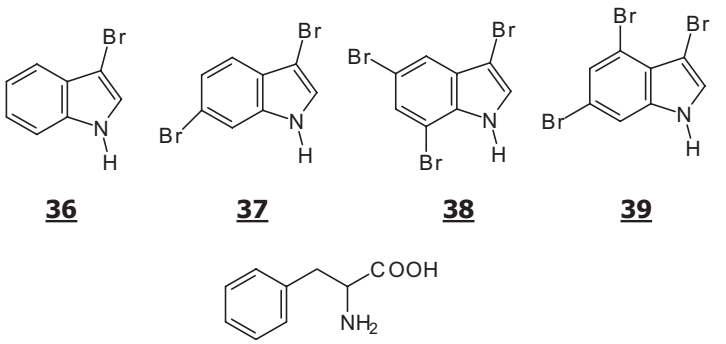
Distribution and Concentrations of MHC-1 in the Environment. MHC-1 was abundant in pollack from Denmark (940 ng/g lw) but also in selected samples of farmed salmon from Norway (7–700 ng/g). In the latter samples, MHC-1 at least accounted for 20% of PCB 153 but could surmount this most prominent PCB congener in fish as well (Vetter et al. 2001b). In the pollack sample, MHC-1 was the dominant peak (see Fig. 1). In seals, highest concentrations were determined in hooded seals from Jan Mayen (58 and 59 ng/g) (Vetter et al. 2001b). MHC-1 was also detected in freshwater fish fed with food produced with marine fish (Vetter et al. 2001b; Vetter and Stoll 2002). The commercial fish food contained MHC-1 half as abundant as PCB 153 (Vetter et al. 2001b; Vetter and Stoll 2002). Fish fed 3 mon with MHC-1-containing food contained MHC-1, but the concentrations were relatively constant. The increase in concentrations was virtually balanced out by the increase in size of the fish (Vetter and Stoll 2002).

MHC-1 was also detected in air samples from the North Sea coast in the south of Norway (Vetter et al. 2002a) as well as in a fish oil capsule and cod livers canned in 1948 (Vetter and Stoll 2002). Human milk samples from the Faeroe Islands that contained Q1, 2′-MeO-BDE 68, 6-MeO-BDE 47, Br_4Cl_2-DBP, 2′,6-diMeo-BDE 47, and diMeO-BB 80 also contained MHC-1 (Vetter and Jun 2003). Finally, MHC-1 was detected in a sponge sample collected in the Antarctic (Vetter and Janussen 2005). It could not be established if this sponge was the natural producer or only accumulated MHC-1 (Vetter and Janussen 2005).

Conclusions. MHC-1 is regularly detected in fish analyzed by official German food control authorities, albeit the concentrations are relatively low (J. Hiebl, personal communication, 2005). The high concentrations in the original paper on MHC-1 (Vetter et al. 2001b) might thus be a consequence of a particular ecological or environmental condition. Many related compounds have shown bioactivity, and a more thorough investigation of MHC-1 should follow in the future.

G. Bromoindoles

The most famous bromoindole derivative is surely tyrian purple, which is produced by marine mollusks. However, simple bromoindoles are biosynthesized by acorn worms and add an iodoformic flavor to these species (Higa et al. 1979). Several mono- to tribromoindoles were determined in such worms from the tropical Indo-Pacific (Fig. 16; **36**–**39**) (Higa et al. 1979). However, due to the N–H bond, the polarity is increased. Consequently, the log K_{OW} of 5-bromoindole was reported to be 2.97 (Mackay 1982), so that bioaccumulation cannot be expected. Nevertheless, Maruya identified three dominating peaks in the GC/ECD chromatograms of oyster from Georgia (USA) (Fig. 17) that did not match the retention times of known anthropogenic contaminants. GC/MS analysis led to the discovery of three bromoindoles (Maruya 2003). The molecular ion provides the known feature of brominated compounds, i.e., an odd molecular mass (dibromoindole m/z 273, tribromoindole m/z 351 (Maruya 2003)). Samples collected in November had much higher bromoindole content (sum of the three bromoindoles was estimated at ~120 ng/g) than those in March and August (estimated concentrations, 1–10 ng/g) (Maruya 2003). The sources are not fully understood. 2,6-Dibromoindole was detected in marine infauna samples from South Carolina (Fielman et al. 1999) whereas 3,6-dibromindole was detected in ascidians from Palau (Qureshi and Faulkner 1999). Others have detected more complex bromoindoles including brominated indole aldehydes.



36 **37** **38** **39**

Fig. 16. Structures of known natural bromoindoles (**36**–**39**) and the known precursor phenylalanin (**40**). Tyrosin may be a substate as well.

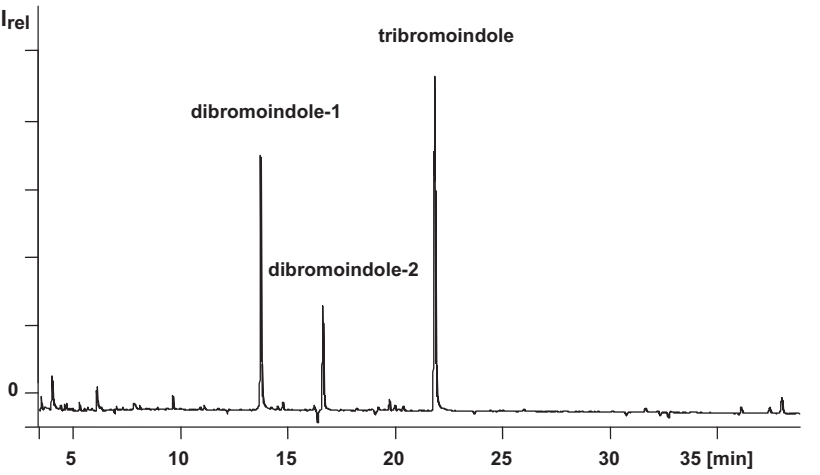


Fig. 17. GC/ECD chromatogram of the detection of three bromoindoles in common oyster (Adapted from Maruya 2003 with permission from Elsevier).

The oyster species feeds exclusively on suspended particulates, which may be the source for the bromoindoles. Another option would be that they are transfromation products of more complex bromoindoles synthesized by algae or other organisms (Maruya 2003). Liu and Gribble (2002) have synthesized the most relevant *N*-methyl-indoles (2,3,6-tribromo- and 2,3,5,6-tetrabromo-1-methylindole) detected in algae and brittle star, which are now available for toxicological testing. Surprisingly, only the bromoindoles and not the more lipophilic *N*-methyl-bromoindoles have been detected in biota samples.

H. HNPs That Resemble Structures of Chlorinated Anthropogenic POPs

Recent research has also elucidated the occurrence of naturally produced chlorinated dioxins (Gaus et al. 2000, 2001; Hoekstra et al. 2000), which may be formed in a similar way as shown in Fig. 10. In addition to being present in sediments, the PCDDs are also found in relatively higher concentrations in higher organisms (Moore et al. 2002; Jiminez et al. 2000; McLachlan et al. 2001).

New cytotoxins were isolated from toxic mussels from the Adriatic Sea. These compounds are most likely produced by dinoflagellates and taken up by the mussels (*Mytilus galloprovincialis*) via filtration of contaminated water. The compounds related to the diarrhetic shellfish poisoning (DSP) toxins to date have only been described in edible shellfish from the Mediterranean Sea. Interestingly, one of the isolates, 2,3,5,6,7,15-hexachloro-4-sulfoxy-14-pentadecen (Ciminiello et al. 2001), is closely related to the anthropogenic medium-chain chloroparaffins.

I. Unknown Compounds and Potential HNPs

In the early work of Ballschmiter and coworkers, several abundant unknown compounds were labeled. U3 was shown to be Q1 (Section III.B), but the structures of the other "U's" suggest some of these may be HNPs of unknown structure and origin. Kuehl et al. (1991) discovered an abundant major mixed halogenated compound in the blubber of dolphins collected during a mass mortality along the U.S. Atlantic coast in 1987–1988. The structure of the compound remains unknown.

Pettersson et al. (2004) detected several brominated compounds by using GC/ECNI-MS in the SIM mode. One unknown organobromine was coeluting with BDE 138. It was reported that this and other unknown compounds could lead to an overestimation of BDEs when GC/ECNI-MS determination is based on the bromide ion only (Pettersson et al. 2004).

A compound labeled UBC-1 has been described in the European Arctic by Vetter (2001). The molecule formed no molecular ion in GC/ECNI-MS. m/z 160 suggested, at least partly, a saturated backbone with a molecular mass of 526 u. UBC-1 eluted slightly ahead of BDE 47 (Vetter 2001). The same retention range was recently described for pentabromophenylpropylether (Hackenberg et al. 2003), which indeed has a molecular mass of 526 Da, which makes it possible that UBC-1 is no HNP but an anthropogenic flame retardant.

A series of brominated compounds was detected in Arctic and Antarctic air and Antarctic sponges (Vetter and Janussen 2005; Vetter et al. 2002a). Some of them were related to TBA but the structure of others, partly mixed halogenated compounds, is still unknown (Vetter 2002). However, the compounds described in this section do not necessarily originate from HNPs, but a careful study of their origin and relevance appears to be warranted.

IV. Biosynthesis of HNPs

An extensive discussion of this topic is beyond the scope of this review. Many papers have been published in this field that have been summarized in many valuable review articles which should be consulted in case of more interest in this field (van Pée 1996; Butler and Walker 1993; Moore 1999).

Ocean water contains ~0.5 mol/L chloride and 0.001 mol/L bromide. This rich source is utilized by many marine organisms in the formation of metabolites (Butler and Walker 1993). A major pathway toward HNPs is the reaction of activated hydrogens by haloperoxidases. These enzymes occur widely in nature, including bacteria (Neidleman and Geigert 1986; Jensen and Whitfield 2003). Interestingly, microbial haloperoxidases differ from similar enzymes in animals and plants in that they need neither metal ions nor cofactors to catalyze reactions (Picard et al. 1997; Jensen and Whitfield 2003).

The principal reaction of organic substrates with haloperoxidases is the following (Butler and Walker 1993):

$$\text{Org-H} + \text{Hal}^- + \text{H}_2\text{O}_2 + \text{H}^+ \rightarrow \text{Org-Hal} + 2\ \text{H}_2\text{O} \quad (1)$$

Haloperoxidases are classified in the following way: chloroperoxidases utilize Cl^-, Br^-, and I^-, bromoperoxidases utilize bromide and iodide, and iodoperoxidases utilize iodide to produce HNPs (Ballschmiter 2003). If chloride is added to a bromoperoxidase, no chlorinated products are formed (Flodin et al. 1999). A common bromoperoxidase contains vanadium (V) as a prosthetic group. The vanadium bromoperoxidases (V-BrPO) are acidic proteins. Other important peroxidases are Fe-Heme containing (protoporphyrin as the prosthetic group) (van Pée 1996). Because peroxidases are commercially available or can be easily gained from natural sources, the halogenation can be reproduced in the lab.

In this way, Walter and Ballschmiter (1991) showed that incubation of anisole, H_2O_2, bromide, or chloride with different peroxidases yielded a wide range of halogenated anisoles. When only 200 ppm bromide was present in the chloride source (NaCl), mixed halogenated compounds were produced (Walter and Ballschmiter 1991). Flodin et al. isolated a bromoperoxidase from the green algae *U. lactuca* and studied the production of bromophenols. It is noteworthy that both bromophenol content and bromoperoxidase activity underlay extreme seasonal variation, with high values in summer and low values in winter (Fig. 18) (Flodin et al. 1999).

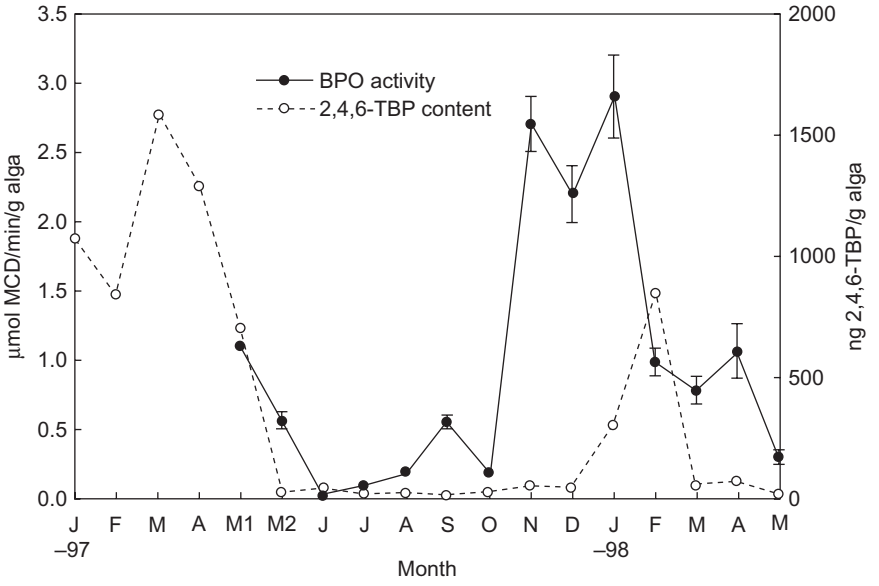


Fig. 18. Annual cycle (January 1997–May 1998) of 2,4,6-tribromophenol content and bromoperoxydase activity in algae from Australia (Adapted from Flodin et al. 1999 with permission from Elsevier).

Highest concentrations in algae were determined in late spring/early summer (in the Southern Hemisphere in November to February) (Flodin et al. 1999). During winter, the bromoperoxidase activity was very low. Therefore, the date of harvesting algae and sponges for the isolation of HNPs is of great importance (Flodin et al. 1999).

V. Perspectives

Given the emerging importance of HNPs, these compounds have to be considered as environmental chemicals once they reach higher organisms. This case applies when the halogenated secondary metabolites, whose natural functions are, among others, chemical defense against a direct predator, are reaching higher organisms that may even be found in different habitats. In line with this description, Ballschmiter distinguished five classes of polyhalogenated compounds (Ballschmiter 2003): (i) biogenic polyhalogenated compounds (including chloromethane and the HNPs described in this work), (ii) natural/geogenic (probably specific halogenated dioxins in clay), (iii) nonhalogenated precursors (e.g., phenols that are halogenated in the environment), (iv) halogenated precursors (halophenols that are converted into haloanisoles), and (v) anthropogenic polyhalogenated compounds (chloropesticides, PCBs).

As pointed out previously, we operate in a "data-poor" environment when dealing with our understanding of HNPs (Moore et al. 2002). Furthermore, there is a need for the development of models that adequately describe the transfer of HNPs to specific environmental compartments (Moore et al. 2002). Current distribution models for HNPs are based on anthropogenic pollutants (Tittlemier et al. 2004; Hackenberg et al. 2003; Vetter et al. 2004), but because of seasonal variations in their production, a less predictable distribution pattern may exist.

It should be noted that HNPs might have a more important impact on anthropogenic compounds than currently understood. For instance, it was concluded that the same microorganisms that transformed 2,4,6-TBA were also able to transform the anthropogenic fire-retardant tetrabromobisphenol A (TBBPA) (Ronen and Abeliovich 2000). Thus, evolutionary pollution of sediments with natural bromophenols and other HNPs may have caused development of specialized microorganisms that now are able to transform these compounds. Without such breakdown mechanisms for HNPs, the environment would have become contaminated with HNPs steadily produced for millions of years.

Given the structural similarity of some anthropogenic POPs with the HNPs, it is not surprising that microorganisms can use some anthropogenic POPs as substrates. In this context, the selective halogenation pattern of anthopogenic POPs appears to be important. Because not all substitution patterns on aromatic compounds are naturally produced, some anthro-

pogenic POPs with unique isomer structures may be transformed to a lesser degree whereas others that closely resemble the structure and pattern of HNPs may be metabolized more easily. Consequently, differences in the transformation rate may go back to the limited feasibility of the natural enzymes for the POPs. Therefore, a thorough knowledge of HNPs will support the understanding of the environmental fate of man-made POPs. This knowledge requires a closer inspection of the structure-dependent transformation of HNPs, which may be a key in the understanding of transformation of anthropogenic POPs.

In other studies it was shown that AOBr formation in sediments that received wastewater was biotic. Indeed, a wide range of HNPs are found in marine infauna (Fielman et al. 1999). It was also found that a low content of nutrients favors formation of organobromine compounds (Putschew et al. 2003). In such media, however, the co-occurrence of natural and anthropogenic compounds does not always allow assigning the proportions of the respective sources. For instance, a high number of brominated compounds, partly of unknown sources, was recently detected in sediments of River Havel and Spree (Berlin, Germany) (Schwarzbauer et al. 2001).

In the 1970s, chemical stress by anthropogenic POPs (PCBs and DDT) was made responsible for such threatening effects as eggshell thinning and reproductive failure in seals. In connection with the latter, 50–70 ppm PCBs has been defined as the critical concentration where reproductive failure begins (Helle et al. 1980). The highest concentration of all HNPs determined in a single sample was ~25 ppm. Given the low number of samples analyzed for HNPs to date, this seems to be remarkable.

Summary

A wide range and steadily increasing number of halogenated natural products (HNPs) is detected in marine organisms that are not the natural source of these compounds but which have accumulated these HNPs in a similar way as known to occur with anthropogenic halogenated pollutants such as PCBs and DDT. The HNPs have aromatic, aliphatic, and heterocyclic spines and are brominated, chlorinated, or mixed halogenated (Cl and Br). The exact isomer structures of HNPs are often closely related to the anthropogenic POPs, and for some compounds both natural and anthropogenic sources are likely to exist. Some of the HNPs are nonpolar, persistent, and can thus be found even in marine mammals and birds of prey. The most important HNPs detected in top predators are halogenated 1,1′-dimethyl-2,2′-bipyrroles (HDBPs), the heptachloro-1′-methyl-1,2′-bipyrrole Q1, the tetrabromophenoxyanisole isomers 6-MeO-BDE 47 and 2′-MeO-BDE 68, and related compounds. Each of these compounds has been detected in higher trophic biota with concentrations exceeding 1 mg/kg.

Acknowledgments

I particularly wish to thank Gordon W. Gribble for his outstanding review articles on naturally produced organohalogen compounds, which delivered a steadily inspiring source of information. I am also grateful to my coworkers and my research partners for their valuable input.

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